Characterizing the Coagulopathy Associated With Resuscitation Following Cardiac Arrest

By

Thomas Michael Glenn III

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Approved by:

Paul McDonagh

Paul McDonagh, Ph.D.
Department of Surgery and Sarver Heart Center

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DEDICATION

This honors thesis is dedicated to my friends and family who supported my pursuit of a degree in Physiology these past four years. I would also like to extend that gratitude to my thesis advisor Dr. McDonagh for the opportunity to conduct research in his lab and for all of the mentoring he has provided me these past few years.

ABSTRACT

It appears that a Post-Resuscitation Injury (PRI) contributes to the poor recovery in patients following cardiac arrest. One component of PRI may be a hypercoagulable condition. The purpose of this study was to determine if blood coagulability is increased in our laboratory model early in recovery following resuscitation from cardiac arrest. Adult Sprague Dawley rats were anesthetized and subjected to a cardiac arrest and resuscitation (A/R) protocol. During the study, arterial blood gases, heart rate, and blood pressure were monitored prior to cardiac arrest (PRE) and for two hours following resuscitation. Blood samples were taken at PRE, R60 and R120 to assess whole blood coagulability using thromboelastography (TEG). Twenty six A/R experiments were conducted. Five animals could not be resuscitated and two others did not survive the two hour recovery period. For the survivors, most (13/19) demonstrated a significant increase in coagulability (Coagulation Index, CI) during recovery. Surprisingly, the other survivors (6/19) demonstrated a hypocoagulable response during recovery in which marked intestinal hemorrhage, abdominal fluid accumulation, and hemodilution were often observed. It is believed that both the hypercoagulable and hypocoagulable responses may significantly complicate recovery in the early hours following successful resuscitation.

STATEMENT OF PURPOSE

The purpose of this study was to examine and characterize the coagulopathy that is believed to occur following cardiac arrest and resuscitation in a newly established laboratory animal model. It is suspected that a coagulopathy contributes to the poor recovery and high mortality observed in patients who have been successfully resuscitated following cardiac arrest. By identifying a quantifiable coagulopathy in this model, it could lead to further studies of mechanisms underlying the pathobiology of post resuscitation injury. Ultimately, future clinically-based studies may produce changes in the way patients are managed following resuscitation.

INTRODUCTION

Each year in the United States, there are more than approximately 300,000 cardiac arrests. Of those patients suffering from cardiac arrests, 30% are successfully resuscitated and have the opportunity to be admitted to a hospital. However, following admission, nearly 70% of those patients do not survive their hospitalization. Therefore, only a mere 9% of patients that have an out-of-hospital cardiac arrest survive to leave the hospital.[1] As evident from these statistics, sudden cardiac arrest is the leading cause of death worldwide.[2] Cardiac arrest is described as an event in which the heart suddenly beats abnormally, if at all. In many cases, ventricular fibrillation is the arrhythmia that leads to the cardiac arrest.[3] Typically during cardiac arrest, the inability of the heart to contract properly causes blood pressure to decrease and many vital organs stop receiving normal blood flow. As a result, they are deprived of oxygen and nutrients. This period of decreased tissue perfusion is referred to as ischemia.

In order for the patient to survive, clearly resuscitation must occur. The ultimate goal of the resuscitation process is to re-establish proper cardiopulmonary function. Throughout the resuscitation process, drugs are often administered that aim to restore proper cardiac function so that it may properly deliver blood to the working tissues. Epinephrine, lidocaine, amiodarone, and calcium chloride are some common medications given to patients following cardiac arrest.[4] Epinephrine, along with calcium chloride, act as positive inotropic agents, increasing the strength of cardiac muscle contraction.[4] Epinephrine, generally the first agent to be administered, is given to enhance cardiac contraction and cause systemic vasoconstriction to ensure that the blood remains near the vital organs.[4] Lidocaine and amiodarone are commonly given to deal with arrhythmias that either cause or result from cardiac arrest; specifically ventricular fibrillation and tachycardia.[4]

In nearly all cases, chest compressions accompany the medications that are given to provide a mechanical systole that ensures that blood flow still occurs throughout the resuscitation process. Following these interventions, once the return of spontaneous circulation (ROSC) has occurred and the patient is able to pump blood at a mean arterial pressure of 50 mmHg, they are considered resuscitated.

Research has revealed that following cardiac arrest, there is a post-cardiac arrest syndrome that occurs contributing to the high mortality of patients following successful resuscitation. During the post-resuscitation period, as it is commonly referred to, there are a series of physiologic alterations occurring that contribute to the alarming statistics listed earlier. Many of these pathological changes that are often seen following cardiac arrest contribute to the overall deterioration of health seen in resuscitated patients. Although the etiology of post-resuscitation injury is not yet clear, there are several theories. Some, but not all, of the major post-resuscitation injuries are explained below.

Free Radical Formation

For the patients that are resuscitated and admitted to a hospital following an out-of hospital cardiac arrest, the leading cause of death is brain injury.[5] One of the major reasons for the prevalence of brain injury is the formation of free radicals. Free radicals are atoms with unpaired electrons that are highly reactive with one another and alter cell function.[6] Free radicals certainly cause significant injury to the brain, but they also cause damage to other organs. It is believed that polymorphonuclear leukocytes, or neutrophils, the first responders to major injuries, are thought to contribute significantly to the formation of free radicals during ischemia-reperfusion injury situations.[6] Research has found that depletion or inhibition of neutrophil activity serves as a protective mechanism against free radical formation following ischemia.[6] In one such study by Matsuo et al., it was shown that in animals where an initial neutrophil count was seen above 225 cells/mm^3 before hemorrhagic shock, a small percentage

of the animals survived. However, in other animals with lower neutrophil counts, a higher percentage of animals survived.[6, 18, 19]

Often when severe trauma occurs and tissues are significantly injured, the cellular damage leads to rupturing of cells.[7] The cells that rupture then release their contents into their surroundings.[7] Neutrophils, phagocytes, and mitochondria are thought to be some of the largest cellular contributors to the production of free radicals during tissue injury.[7] During an inflammatory response, phagocytes are activated and throughout this event, a process known as an "oxidative burst" occurs.[7] Catalyzed by NADPH oxidase, this burst leads to the rapid production of reactive oxygen species in order to aid in the destruction of the foreign microbes that originally initiated this response.[7] This process however, while helpful to fighting foreign pathogens, can lead to the exposure of free radicals to neighboring tissues and cause cell lysis.[7]

Neutrophils, the prime type of phagocytic blood cell, are believed to contribute to the high level of oxygen-deprived free radicals that are observed. Neutrophils produce free radicals during an intracellular oxidative burst.[7] This reaction is initiated by the enzyme myeloperoxidase which converts intracellular hydrogen peroxide to hypohalous and hypochlorous acids.[7] These acids are extremely reactive and help aid in inflammation and immune processes.[7] Neutrophils also have the NADPH oxidase complex which it uses to reduce oxygen to superoxide, another potentially harmful chemical.[8] Superoxide is then further converted to hydrogen peroxide, hydroxyl radicals, or to N-chloroamines; all of which can be extremely harmful to native tissues.[8]

There are many mechanisms by which free radical products are toxic to the cells. The first process that occurs which leads to detrimental intracellular damages is associated with lipids. For any given cell, lipids are essential and provide vital structural and functional roles. The cell membrane is the primary cellular component composed of lipids that is involved with many processes such as cell attachment, ion conductivity, cell signaling, and protective mechanisms. Lipid peroxidation is the

primary mechanism by which cellular lipids are disrupted.[7] In essence, when exposed to lipids, an oxidation reaction occurs and the free radical compounds steal electrons from the lipids.[7] This peroxidation leads to extreme damages because the cell membrane, which is comprised mostly of lipids, is now destroyed. Without a cell membrane that is intact, many cellular processes are upset because proper chemical and electrical gradients are disrupted.[7] In addition, it becomes easier for further damage to be done to the cell.[7]

Proteins are another essential compound that nearly all cellular processes require for proper regulation. Similar to the oxidation of lipids seen with the cell membrane, proteins may become oxidized when exposed to free radicals.[7] In most cases, the oxidation of proteins leads to the loss of important sulfhydryl groups and the increase in production of carbonyl groups.[7] Oxidized proteins are much more susceptible to proteolysis and as protein degeneration occurs, cellular functions will be interrupted.[7]

Another form of cellular injury that occurs due to free radical exposure is with deoxyribonucleic acid, or DNA. In ways that are not completely understood, DNA structure can also be disrupted by free radicals and thus will need to be repaired by polymerase.[7] During the DNA reparation process, polymerase requires large amounts of NAD.[7] By choosing to use cellular stores of NAD for the DNA repair process, it is believed that this process will lead to significant decreases in the ability of the cell to produce ATP.[7] This energy deficiency ultimately is believed to lead to cell death and thus contributes significantly to post-resuscitation injuries.

Leukocyte Adhesion

Adhesion of leukocytes to blood vessel walls is a common phenomenon and it is absolutely necessary to sequester PMNs to muster a proper inflammatory response. Leukocytes initially adhere to the lumen of blood vessels. This process is required for PMN sequestration, migration, phagocytosis,

emigration, and other activities that are seen at the site of injury during an inflammatory response.[8] For neutrophils and monocytes, the glycoproteins that dictate adhesion are integrins and they consist of a variety of associated antigens that allow for adhesion. Integrins, specifically Mac-1, CR3, and LFA-1, bind to selectins that are expressed on endothelial cells. The process of leukocyte adhesion is necessary to fight infection but in several ischemic scenarios, such as cardiac arrest, problems can arise.

One example of the adhesion promoting molecules is known as P-selectin.[9] P-selectin is a membrane glycoprotein that is found in platelets and endothelial cells.[9] Upon cell activation, it is expressed on the cell membrane.[9] The purpose of the P-selectin molecule is to promote adhesion to the microvessels upon which it is expressed, a process known as adhesion.[9] The primary ligand for P-selectin is P-selectin glycoprotein ligand-1 (PSGL-1), which is present on neutrophils and monocytes. It therefore provides a binding place for inflammatory cells on the activated endothelial cells expressing P-selectin.[9] Following initial adhesion, it is believed that other leukocyte adhesion molecules, specifically CD11b and CD18, increase the strength of the leukocyte-endothelium complex by binding to their endothelial ligand, ICAM-1.[10]

In research studies, leukocyte adhesion and sequestration have been observed in many cases associated with ischemia and shock. It has also been observed in cases following cerebral stroke. In one study by Ritter et al., arterial leukocyte adhesion increased during reperfusion.[10] This same study reported that in the venules, there was also a 25-fold increase in leukocyte accumulation 1 hour following reperfusion.[10] Based on these studies performed in ischemia-reperfusion models and in stroke patients, it is believed that the same adhesion of leukocytes may occur during cardiac arrest-resuscitation. This would indicate that the period of ischemia, which occurs in each situation listed above, is the mechanism activating the adhesion process.

Although the adherence of leukocytes to the endothelium is not a significant problem in a normal individual, during an extreme injury such as stroke or cardiac arrest, it may cause problems.

When too much adherence occurs, the blood vessel lumen is narrowed and thus perfusion to the tissues will decrease. As more and more adherence occurs, it has the potential to lead to a complete occlusion in which no blood may pass through the blood vessel.

In addition to leukocyte adhesion occurring throughout the vasculature, it is believed that obstruction is occurring that is posing further microcirculation threats. During stroke or following cardiac arrest, the pressure drop across capillaries and other blood vessels is significantly reduced.[8] The large drop in driving pressure contributes to the trapping of leukocytes throughout the circulation. The larger of the leukocytes, notably neutrophils and monocytes, have been observed to obstruct capillaries throughout the heart muscle, brain, skeletal muscle, the lung, and intestines.[8] One such study by Hallenbeck et al. measured the accumulation of granulocytes in the right hemisphere following brain ischemia. This study found that at 60 minutes following reperfusion, as compared to the control, there was a statistically significant increase in granulocyte accumulation in the brain.[11] Similar increases were seen in the liver but were not statistically significant.[11]

Ultimately, this accumulation and obstruction of capillaries can impact the tissues significantly. One immediate problem that obstruction leads to is decreased perfusion. With a blocked capillary, the corresponding tissues will not receive proper blood flow and thus, will become hypoxic and be further damaged.[8] Another consequence of the obstruction is a lack of a true immunological response. Because the entrapped leukocytes cannot reach their proper destination, they cannot carry out their normal immunological functions.[8]

Blood Coagulation

One of the tightly regulated features associated with our blood is coagulation, or the pathway by which blood clots. In addition to aiding in recovery from injuries, it is also suspected that the coagulation pathway plays an important role following resuscitation and causes yet another form of

post-resuscitation injury.[12] Few studies have been conducted to gather information on whether some form of coagulopathy occurs following cardiac arrest. One such study, performed by White et al., looked at eighteen male swine that were subjected to eleven minutes of ventricular fibrillation.[12] For the eight swine (44%) that were successfully resuscitated, the results suggested that a coagulopathy occurred.[12] They made this conclusion based on many different analytic characteristics that were found during their tests for coagulation (clot strength, speed of clot formation, platelet concentration, and others).[12]

A strong belief among many researchers is that the coagulopathy associated with resuscitation is a hypercoagulable one. Hypercoagulability is described as an increase in the tendency of blood to clot.[13] The reasons for this increase in coagulability are not yet known however there are many theories as to why hypercoagulability may be one form of post-resuscitation injury.

As stated before, there is an inflammatory response that is observed following cardiac arrest and resuscitation. During this period of time, which may last for a few days, proinflammatory cytokines such as: IL-1B, IL-6, MCP-1, and TNFB, are upregulated.[9] The upregulation of these inflammatory mediators are believed to lead to greater expression of proteins that cause coagulation.[9] Tissue factor (TF), coagulation factor III, and CD142 are some of the proteins that regulate the activation of the extrinsic pathway for coagulation.[9] One of these proteins, tissue factor, is the primary initiator of the extrinsic pathway that acts by binding to soluble factor VIIa to initiate a response which ultimately produces thrombin and fibrin.[9] In addition to creating a meshwork for blood clots in the vasculature, thrombin and fibrin also have other actions that promote coagulation. For example, thrombin plays a pivotal role in the process of platelet aggregation.[9] During the inflammatory response, monocytes and endothelial cells respond to circulating levels of the cytokines listed above by increasing the expression of tissue factor on their cell surfaces. Studies have been conducted, in a sepsis model, which use

antagonists of tissue factor or factor VII to block their hypercoagulable actions.[9] Animals in which this study was done had improved survival rates and were shown to exhibit less of a coagulopathy.[9]

Another mechanism by which a hypercoagulable response may occur in patients following cardiac arrest is associated with neutrophils. Neutrophils can promote procoagulation activity, due to their expression of binding sites for the prothrombinase complex.[14] The binding sites for factor Va and factor Xa are located on the cell membrane of neutrophils.[14] Binding of these factors to their receptors on the neutrophils will lead to the production of thrombin and thus, induce a hypercoagulable state. It is also important to note that some studies have shown that neutrophils may also exhibit anticoagulant properties. Neutrophil granules contain proteases, primarily elastase, that may exhibit anticoagulant properties by inducing the destruction of blood clots and thrombi.[14] In addition, one study performed by Grau et al. revealed that phagocytosed fibrin pieces were found inside of neutrophils, indicating that they may have some fibrinolytic properties as well.[14]

As stated above, there appears to be many contributing factors to post-resuscitation injuries. These injuries are the primary reason that there is increased mortality in patients who are successfully resuscitated in the hospital following cardiac arrest. The goal of this project was to examine the coagulation of blood and determine if there is a coagulopathy following resuscitation in our laboratory model. If it appears that a coagulopathy does exist, we will characterize it as being hypercoagulable or hypocoagulable and hopefully gain some insight as to why that response may be occurring. In our laboratory, we developed a cardiac arrest-resuscitation model by which we obtained the blood samples used in the research. The method used to analyze the coagulation of the blood was thromboelastography. Thromboelastography, or TEG, is a method of examining the whole blood coagulation and how well the blood coagulates. Rather than pinpointing one individual clotting pathway or studying platelet aggregation alone, TEG takes all of these factors into consideration and measures how well the blood clots as a whole. Using this method, we analyzed blood samples and

attempted to answer the question as to whether a coagulopathy does exist in the early hours following successful resuscitation.

METHODOLOGY

Thromboelastography

The thromboelastograph is a device that measures the coagulability of whole blood. The blood samples, which are collected in a tube coated with sodium citrate to prevent clotting, are placed in a small cuvette and then a small metal pin is inserted into the cup of blood. In order to initiate the clotting cascade, a small amount (20 µL) of calcium chloride is inserted into each cuvette of blood. The pin slowly rotates and eventually the blood starts to coagulate. As coagulation occurs, the blood becomes more viscous and it becomes increasingly difficult for the pin to rotate. Throughout this process, the torque required for the pin to continue spinning at the same rate is recorded and using this information, the computer program calculates other important values. In our study, whole blood samples were taken at PRE, R60, and R120 and analyzed for their respective levels of coagulation.

The important indicators of coagulation that the TEG provides include: R-time, maximum amplitude (MA), and coagulation index (CI). The R-time measures the length of time it takes until a significant level of clot is detected by the machine. The MA measures the overall strength or stiffness of the clot. The CI is an all inclusive value that is comprised of each experimentally determined aspect of the clot and describes how coagulable the clot is. The equation that is used for calculating CI is:

$$CI = -0.2454(R) + 0.0184(K) + 0.1655(MA) - 0.0241(\alpha) - 5.0220$$

An example of a curve generated by the TEG is shown on the next page.

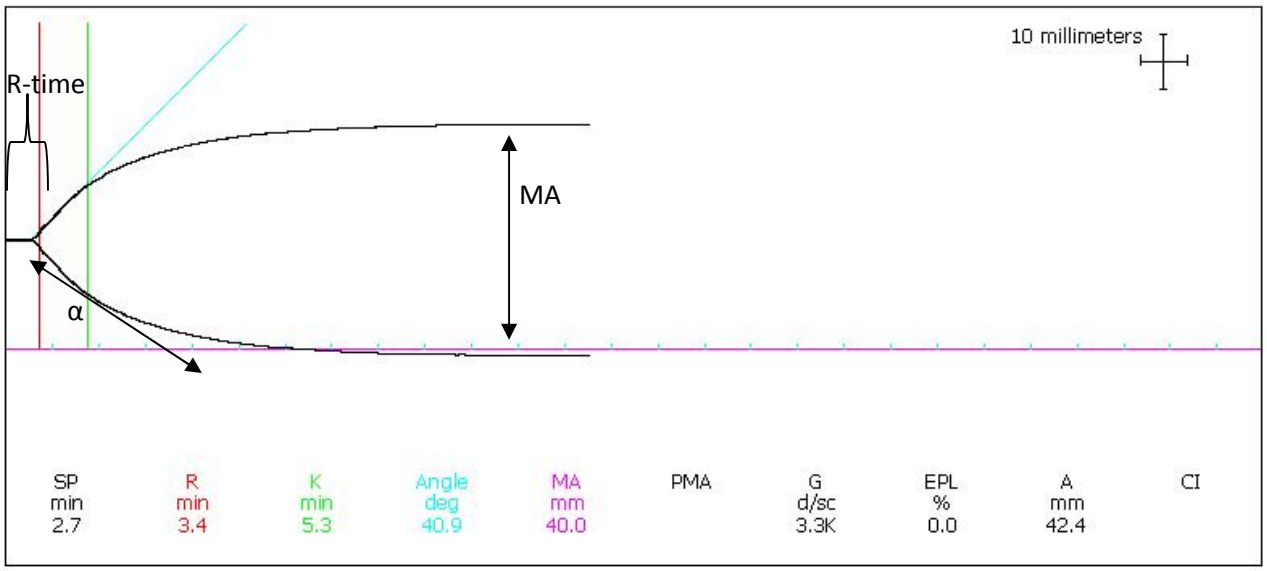

Figure 1: This image is from the thromboelastograph and shows an example of one coagulation curve. Note the graphical representations of R-time, slope (α), and MA.

Arrest-Resuscitation Protocol

Sprague Dawley rats were weighed and deeply anesthetized with 50 mg/kg sodium pentobarbital. A PE-50 catheter was inserted into the femoral artery to obtain blood samples, measure arterial blood pressure, and administer medication throughout the study. Rats were intubated with a PE-200 tube and respiratory function was maintained using a ventilator (Harvard, model #683). A PE-190 catheter was placed in the right jugular vein and advanced into the right ventricle. A J-wire was pushed through the catheter into the base of the right ventricle. Cardiac arrest was achieved by electrical stimulation for a period of three minutes. During the arrest period, the ventilator was turned off. After four minutes of arrest, the ventilator was turned on, epinephrine was administered (20 µg/kg) and cardiac chest compressions initiated (200/min) for two minutes. Defibrillation was then attempted (single shock, Code Master XL, 5 joules for animals < 500g, 7 joules for animals > 500g). If spontaneous cardiac contraction did not occur, another dose of epinephrine was given, compressions were given for two minutes, and defibrillation was attempted again. At least five attempts were made to

resuscitate. Return of spontaneous circulation (ROSC) was achieved when cardiac rhythm was restored and the mean arterial pressure (MAP) reached 50 mmHg. Following ROSC, 1.5 cc of 8.4% sodium bicarbonate was given intra-arterially over a period of three minutes. Blood samples were taken pre-arrest and after 15, 30, 60, and 120 minutes following ROSC.

Throughout the resuscitation process, blood samples were taken to look at different physiological markers. Glucose samples were taken each time blood was extracted from the animal. Hyperglycemia is a common occurrence during any applied stress so it was important to monitor how hyperglycemic these rats were before and after cardiac arrest. Another important biological marker, specifically in our experiment was the blood pH. The pH was important in providing information throughout the resuscitation process about how acidic or alkaline the blood was. The blood pH dictated to us whether changes needed to be made in the respirator or if we had to administer bicarbonate in order to keep the pH as close to 7.4 so the animal would survive the experiment. Blood pressures were especially important during the arrest-resuscitation protocol because the blood pressures were the best indicator as to whether the animal was successfully being resuscitated. In addition, blood pressure had to be constantly checked on throughout the experiment to ensure that the animal's health and heart were not declining.

All of the data taken during the experiments were kept in a notebook. This included all of the printouts, graphs, and notes made during the experiment. The data was then input into a Microsoft Excel spreadsheet on the laboratory computer. All experimental results are presented as Mean±SEM. Statistical analysis was performed using SigmaStat software. $P<0.05$ was considered significant.

RESULTS

A total of twenty-six A/R studies were conducted in this study. Of the twenty-six experiments that were done, five of the animals could not be resuscitated and two of them did not survive the entire two hour recovery period. For the survivors (19/26), most of them exhibited a hypercoagulable response throughout the recovery period (13/19, 68%). Some of the survivors (6/19, 32%) responded during the recovery process with a hypocoagulable response. In addition, a group of eight SHAM control experiments were conducted. During the SHAM experiments, normal experimental procedures were followed except that cardiac arrest and resuscitation were not performed.

ARTERIAL BLOOD pH			
Time Point	**SHAM**	**HYPERCOAGULABLE**	**HYPOCOAGULABLE**
PRE	**7.33 ± 0.02**	**7.32 ± 0.02**	**7.32 ± 0.03**
R15	**7.32 ± 0.03**	**7.12 ± 0.03**	**7.01 ± 0.11**
R30	**7.33 ± 0.03**	**7.15 ± 0.03**	**7.07 ± 0.06**
R60	**7.30 ± 0.04**	**7.23 ± 0.02**	**7.15 ± 0.08**
R120	**7.29 ± 0.03**	**7.28 ± 0.03**	**7.17 ± 0.02**

Table 1: Arterial blood pH values at PRE, R60 and R120 for SHAM, hypercoagulable, and hypocoagulable survivors.

Arterial Blood Gases						
	O_2 (mmHg)			CO_2 (mmHg)		
Time	SHAM	Hyper	Hypo	SHAM	Hyper	Hypo
PRE	405.00 (24.97)	346.92 (38.53)	372.00 (58.40)	44.69 (2.60)	44.92 (2.99)	39.50 (2.88)
R15	352.67 (36.85)	129.08 (9.39)	172.00 (61.62)	48.87 (3.92)	72.50 (7.26)	62.33 (5.04)
R30	385.21 (31.08)	161.75 (16.83)	138.17 (17.54)	44.64 (3.53)	70.67 (5.99)	65.50 (7.76)
R60	380.31 (28.93)	214.83 (27.94)	130.17 (31.06)	44.50 (3.72)	66.58 (6.03)	45.83 (11.48)
R120	415.50 (27.12)	331.31 (29.96)	114.00 (21.70)	45.50 (4.49)	48.85 (4.56)	37.40 (6.54)

Table 2: Arterial blood PO_2 and PCO_2 values at PRE, R15, R30, R60 and R120 for SHAM, hypercoagulable, and hypocoagulable survivors.

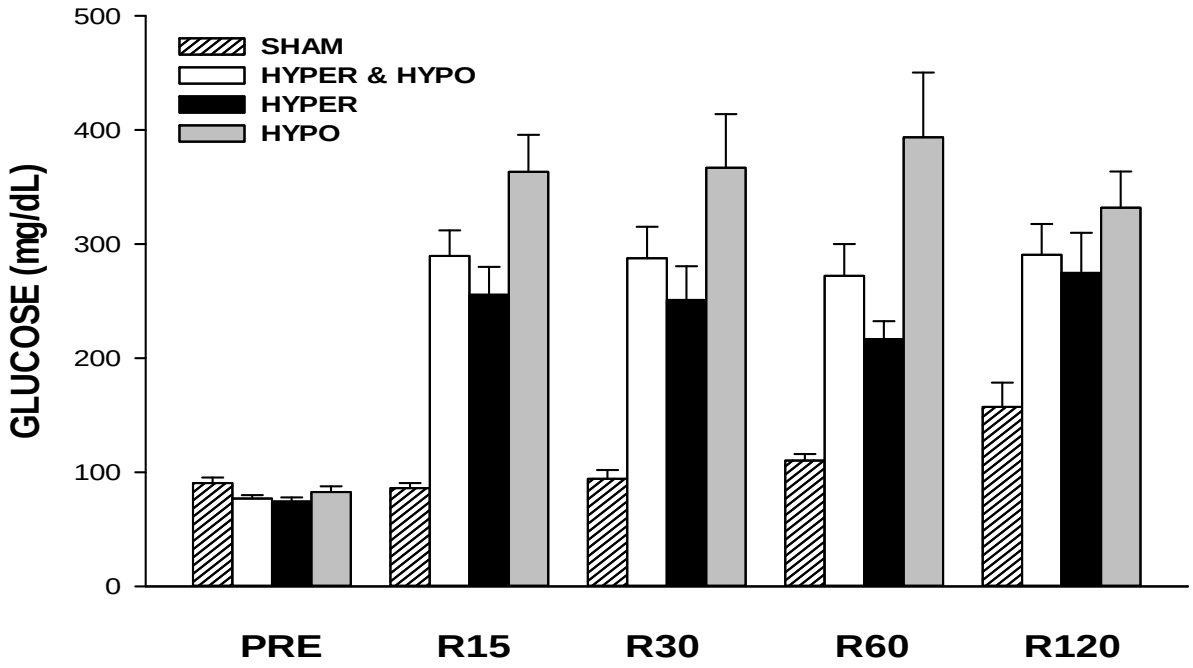

Figure 2: Blood glucose throughout the protocol for SHAM, all A/R survivors, Hypercoagulable and Hypocoagulable survivors.

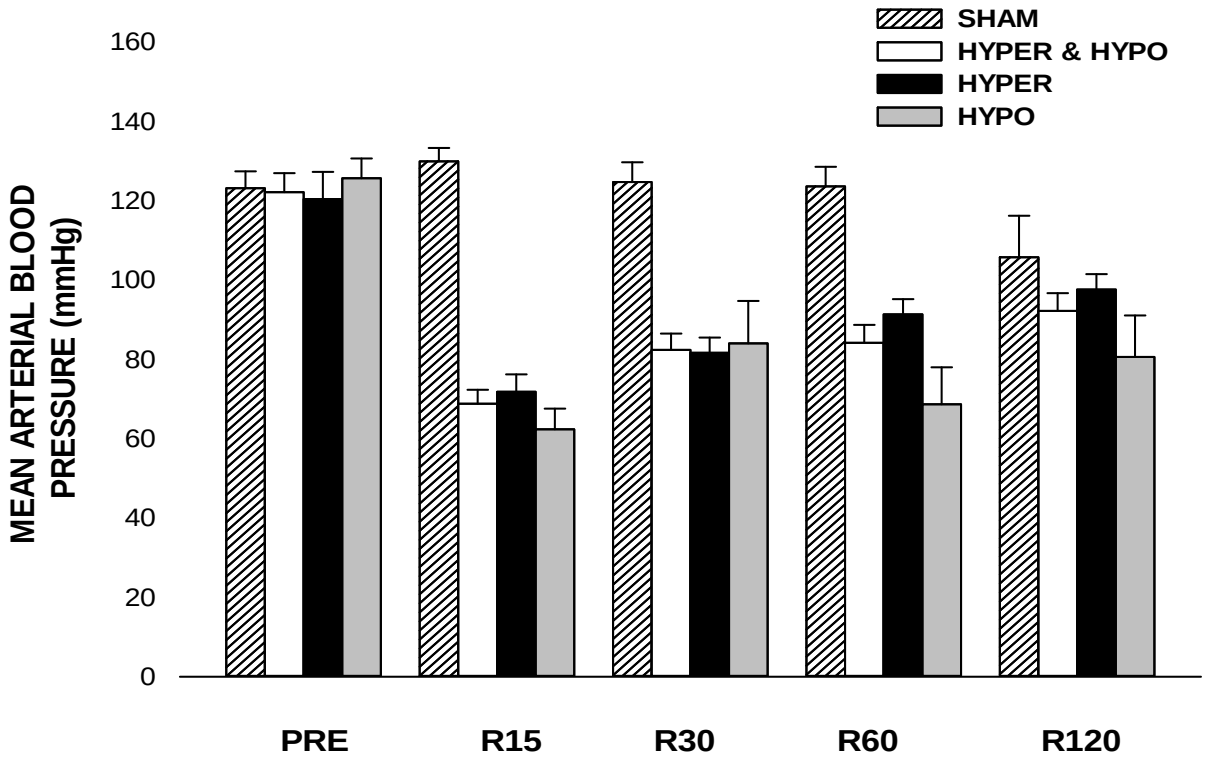

Figure 3: Mean arterial blood pressure (MAP) throughout the experiment for SHAM, all A/R survivors, Hypercoagulable and Hypocoagulable survivors.

THROMBOELASTOGRAPH									
	PRE			R60			R120		
PARAMETERS	Sham (n=6)	Hyper (n=13)	Hypo (n=6)	Sham (n=6)	Hyper (n=13)	Hypo (n=6)	Sham (n=6)	Hyper (n=13)	Hypo (n=6)
R (min)	8.90 (1.18)	10.43 (1.12)	8.47 (2.78)	8.02 (130)	6.09 (0.67)	13.76 (4.00)	4.73 (0.68)	5.12 (0.57)	36.87 (15.11)
K (min)	4.58 (0.84)	6.07 (0.95)	7.97 (3.26)	5.48 (1.20)	2.12 (0.30)	7.15 (2.74)	2.55 (0.72)	1.50 (0.23)	16.70 (7.76)
MA (mm)	66.73 (2.27)	64.17 (1.12)	68.25 (1.98)	60.18 (5.65)	69.35 (1.24)	36.80 (11.17)	70.78 (1.97)	63.60 (3.97)	36.73 (11.94)
ANGLE (α)	41.42 (4.68)	38.85 (3.75)	38.20 (7.98)	38.02 (6.28)	59.46 (3.69)	28.02 (11.76)	59.20 (5.81)	60.32 (3.95)	24.93 (16.30)

Table 3: TEG parameter results at PRE, R60 and R120 for SHAM, hypercoagulable and hypocoagulable survivors.

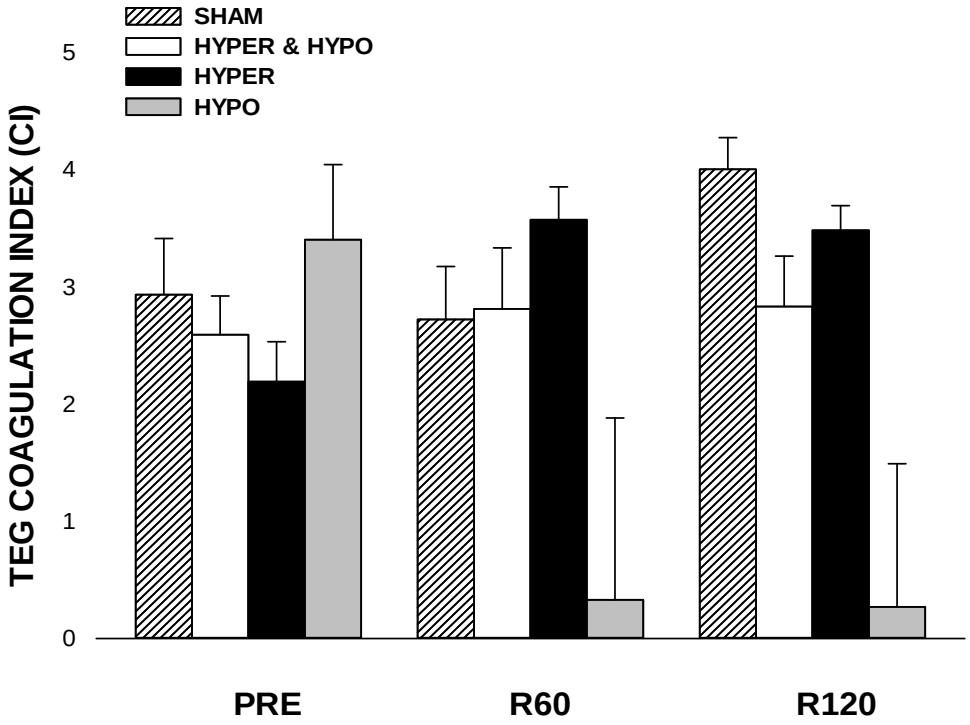

Figure 4: This figure demonstrates the change in the calculated coagulation index (CI) from pre-arrest to R120. Lined bar represents SHAM, Open bar represents ALL A/R animals, Black bar represents those animals who demonstrated a hypercoagulable response and the Grey bar represents the hypocoagulable responders.

Hematology												
	WBC (cells/µL)			RBC (10^3/ µL)			HCT (%)			PLT (10^3/ µL)		
Time	SHAM	Hyper	Hypo	SHAM	Hyper	Hypo	SHAM	Hyper	Hypo	SHAM	Hyper	Hypo
PRE	4441.67 (377.58)	4107.69 (261.70)	4675.00 (307.34)	7.94 (0.17)	8.17 (0.10)	8.28 (0.32)	43.71 (1.16)	44.87 (0.57)	45.73 (1.10)	400.00 (20.97)	407.38 (11.08)	448.92 (18.82)
R15	3516.67 (488.99)	5223.08 (631.81)	2725.00 (723.39)	7.40 (0.42)	7.18 (0.25)	5.87 (0.62)	40.63 (2.46)	40.22 (1.27)	32.39 (3.55)	374.58 (19.49)	359.85 (32.61)	317.58 (34.04)
R30	3658.33 (517.75)	4407.69 (399.55)	4208.33 (747.60)	7.17 (0.31)	7.38 (0.17)	6.76 (0.50)	39.25 (1.78)	40.97 (0.93)	37.42 (2.88)	369.42 (1.78)	384.48 (31.58)	402.17 (39.90)
R60	4575.00 (1132.02)	3561.54 (455.42)	2925.00 (493.58)	6.65 (0.30)	7.27 (0.15)	6.71 (0.67)	36.39 (1.71)	40.15 (0.92)	37.35 (3.64)	352.58 (22.18)	430.54 (14.49)	416.50 (28.13)
R120	7741.67 (1468.70)	3646.15 (524.33)	2708.33 (639.98)	6.35 (0.29)	6.41 (0.14)	5.59 (0.62)	34.35 (1.57)	35.23 (0.80)	30.98 (3.70)	351.42 (23.19)	395.08 (14.09)	370.83 (39.95)

Table 4: Blood hematology values at PRE, R15, R30, R60 and R120 for SHAM, hypercoagulable, and hypocoagulable survivors.

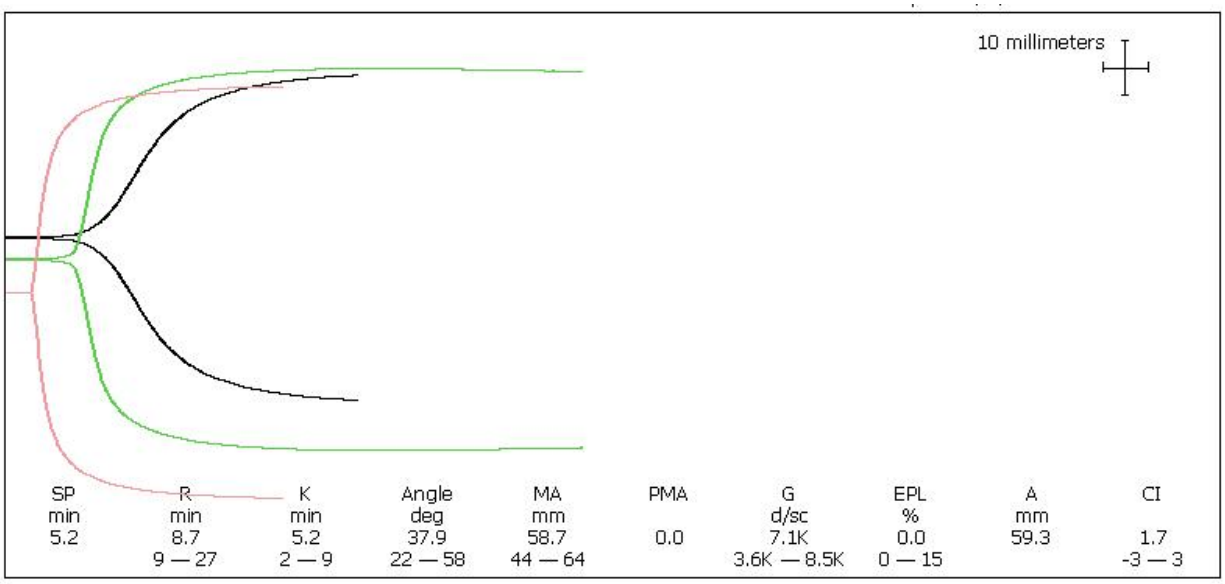

Figure 5: This image is from the thromboelastograph and depicts a state of hypercoagulable blood during recovery. PRE (BLACK), R60 (GREEN) and R120 (PINK). Note the decrease in R and the increases in slope and MA.

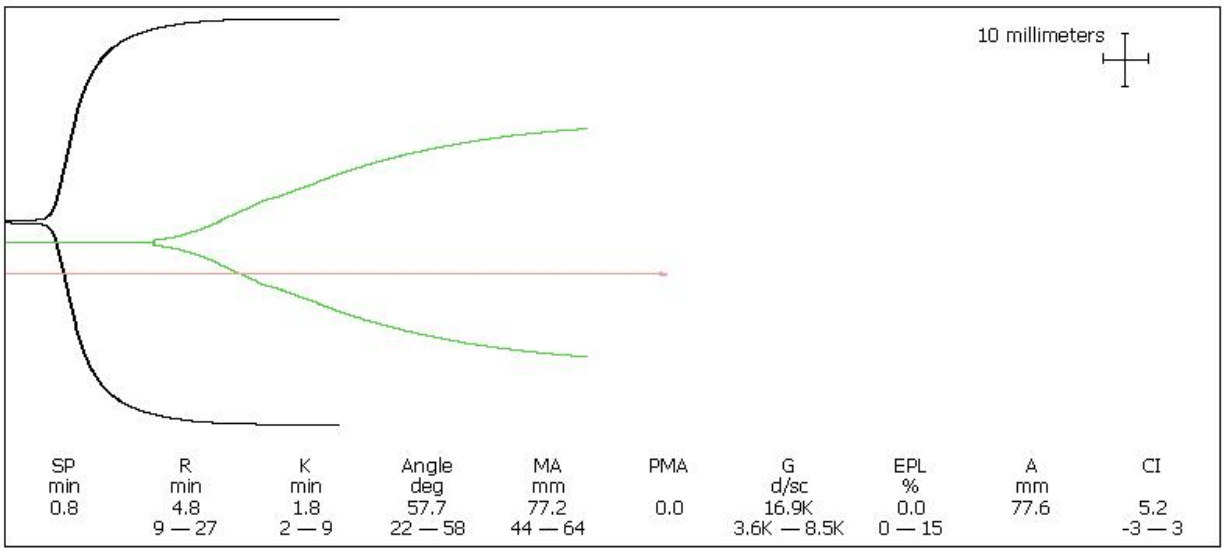

Figure 6: This image is from the thromboelastograph and depicts a state of hypocoagulable blood during recovery. PRE (BLACK), R60 (GREEN) and R120 (PINK). Note the increase in R and the decreases in slope and MA.

DISCUSSION

The purpose of this research study was to (1) establish a small animal model of cardiac arrest, resuscitation, and recovery and (2) determine if a coagulopathy occurs in the first two hours of recovery following successful resuscitation. We observed an increase in whole blood coagulability during recovery for most (13/19) of the animals that were successfully resuscitated. It was surprising however to find that 32% (6/19) of the successful resuscitations were accompanied by a hypocoagulable state during recovery. At necropsy, the hypocoagulable animals had more severe intestinal hemorrhage and abdominal fluid accumulation than the hypercoagulable animals. Some possible explanations for the hypocoagulopathy may be marked hemodilution and an effective loss of coagulation factors.

It should also be noted that the six animals that were hypocoagulable at R60 and R120 required much more epinephrine and bicarbonate solutions during recovery to maintain MAP and acceptable blood pH values. On average, we administered 3.1 mL of epinephrine and 1.5 mL of bicarbonate throughout the two hour resuscitation period to the hypercoagulable animals. In comparison, the hypocoagulable animals received 7.3 mL of epinephrine and 3.3 mL of bicarbonate on average. Tables 1 and 2 and Figure 3 demonstrate that the hypocoagulable animals had a more difficult time keeping their pH, blood gases and arterial blood pressures near normal as compared to the hypercoagulable sub-group.

The hypocoagulable animals' glucose concentrations were significantly increased during recovery compared to the hypercoagulable sub-group, as shown in Figure 2. Because glucose is released in stressful situations in order to prepare the body to take action, pronounced hyperglycemia suggests that the HYPO sub-group was relatively more stressed than the HYPER sub-group during

recovery. In addition, the six animals in the HYPO sub-group were initially more coagulable than the HYPER sub-group (see PRE values in CI Figure). Perhaps this initial difference and early increased coagulation that is observed led to many of the coagulation factors being utilized before resuscitation occurred. During recovery, around R60 and R120, it is possible that the coagulation factors would then be significantly depleted due to them being used up earlier in the study; thus contributing to a hypocoagulable state.

During the experiment, hematology data were recorded and can be found in Table 4. These findings demonstrate that for the first hour of the study, the white blood cell count for the SHAM sub-group remains constant. After the first hour of the study, the white blood cell count increases by nearly 70%. This could provide evidence that during prolonged time of anesthesia and artificial ventilation, the body begins to show an inflammatory response even without undergoing cardiac arrest and resuscitation. It is also important to note that for the HYPO sub-group, both the hematocrit and red blood cell values are consistently lower than the SHAM and HYPER groups. This observation supports the idea that hemodilution may have been a contributing factor for the hypocoagulable state that was seen in some of the animals.

Although the presence of a hypocoagulable response was surprising in our study, the majority of the animals exhibited a hypercoagulable state following resuscitation. These animals had decreases in R-time and increases in both MA and α between the times PRE and R60. Thus, the increase in coagulation was most evident in the first hour. The blood remained hypercoagulable by the time that R120 reached but was really no different than it was at R60. This result suggests that the body's response to cardiac arrest occurs rapidly and may remain effective for several hours. When examining the results for the SHAM experiments, it is important to draw attention to Table 1 and Figures 2 and 3. These graphs clearly indicate that the animals that do not undergo the cardiac arrest and resuscitation

protocol did not exhibit large changes in blood pH, glucose concentrations, and blood pressures for the first hour of the study. Although the SHAMs do not undergo the full A/R protocol, their glucose concentrations increased somewhat, pH values decreased, and MAP decreased between the first and second hour. It was also surprising to observe that the SHAMs demonstrated increases in coagulation during that same period of time as shown on Figure 4. These results suggest the possibility that after three total hours of anesthesia and artificial ventilation, the body begins to lose its ability to maintain proper MAP, pH, glucose concentrations, and coagulation. Thus it raises the question as to whether the model we established needs to be altered, specifically shortened. It may be beneficial to run the tests for 90 minutes rather than 120 if we continue to see the decline in health in the SHAMs. We would need to perform more SHAM experiments in order to conclude that the model needs to be shortened or whether the stress put on the animals is causing the decline in health.

Although there is not a large number of studies investigating cardiac arrest and the post-resuscitation period, there have been some other cardiac arrest and resuscitation coagulation studies performed. Among these studies, there appears to be a large variability in the length of time for the post-resuscitation period. One study by Lopez-Herce et al., in which pediatric piglets experienced cardiac arrest and resuscitation to test correlations between hemodynamic parameters, the animals were only followed for thirty minutes following ROSC.[15] Another study, performed by Ichinose et al. administered heparin before cardiac arrest and observed the animals following resuscitation for 120 hours.[16] This study found that for the animals that were given a pre-arrest dosage of heparin, survival rate increased and no coagulation complications were observed.[16] Aside from these two studies, the others all that utilized an animal model for cardiac arrest and resuscitation used a timeline somewhere in between thirty minutes and 120 hours.[12, 17] The large inconsistency in post-resuscitation times between research studies indicates that there is still a lot that needs to be learned about the post-resuscitation period. Although most of the studies used a timeline between two and four hours, there is

the possibility that physiological alterations may occur outside of this timeline. Based on our model and the data we received, it appears that between one and two hours there is a large decline in the bodies' ability to maintain homeostasis. For this reason, we may want to begin including R90 in the study to focus more clearly on when these changes actually occur.

In conclusion, the majority of the subjects exhibited an increase in coagulation following cardiac arrest and resuscitation while the other animals displayed signs of hypocoagulation. Based on this, we can conclude that there does appear to be a coagulopathy present following cardiac arrest and resuscitation. Although it is not clear, at this point, exactly why either a hypercoagulable or hypocoagulable response occurred, both responses may compromise recovery following successful resuscitation from cardiac arrest.

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