From THE DEPARTMENT OF CLINICAL SCIENCE AND EDUCATION
Karolinska Institutet, Stockholm, Sweden

TRANSLATIONAL STUDIES ON MECHANICAL HEMOSTASIS AND COAGULOPATHY IN TRAUMA

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M.D.

Karolinska Institutet

Stockholm 2021

Published by Karolinska Institutet.
Printed by Universitetsservice US-AB, 2021

ISBN 978-91-8016-139-8
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TRANSLATIONAL STUDIES ON MECHANICAL HEMOSTASIS AND COAGULOPATHY IN TRAUMA
THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

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The thesis will be defended in public at Södersjukhuset - Aulan, Sjukhusbacken 10, 118 83, Stockholm Friday May 28, 2021 at 09.00

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Dedicated to:

Jesper Lindblom and Tomas Bergqvist.

K.I.A. Afghanistan, 2005.

"The most intelligent men, like the strongest, find their happiness where others would find only disaster: in the labyrinth, in being hard with themselves and with others, in effort; their delight is in self-mastery; in them asceticism becomes second nature, a necessity, an instinct. They regard a difficult task as a privilege; it is to them a recreation to play with burdens that would crush all others".

- Nietzsche

If I have seen further, it is by standing on the shoulders of Giants.

- Isaac Newton, 1675.

POPULAR SCIENCE SUMMARY OF THE THESIS

Physical injuries after accidents or violence are a major cause of death worldwide in both civilian societies and armed conflicts, especially among children and young adults. Massive bleeding is an important cause of possibly preventable deaths after physical injuries and needs to be managed in the acute phase of patient care. External bleeding can often be controlled by compression of the wound or by using adjuncts for decreasing the blood flow to the site of injury. Such devices are called tourniquets, derived from the French word *tourner* – "to turn". However, these methods for control of bleedings are principally limited to extremity wounds. Internal bleedings in the abdomen and pelvis are often associated with significant blood loss and cannot be compressed by manual force or standard tourniquets. Furthermore, severe tissue injury has been shown to cause systemic reactions on a molecular level that can further aggravate blood loss due to dysfunctions in the ability to form stable blood clots – this involves both the process of coagulation, and fibrinolysis which is the resolve of a clot. This is referred to as *Trauma Induced Coagulopathy* (TIC). Coagulation dysfunction is an individual and important factor for mortality after physical injuries. The specific causes for the development of TIC remains unknown but involves many different sources that needs to be discovered and preclinical research, using animal models, are needed for further understanding of this process. Methods for early, specific, assessment of the bloods ability to form stable blood clots can provide information needed to develop treatments to increase survival after injury and surgery (which is often needed to obtain definitive bleeding control). Therefore, early interventions for prevention of massive blood loss and detection methods for specific dysfunctions in the coagulation system may have a significant impact on mortality and functional outcome after severe injuries.

Novel methods have been developed for the possibility to mechanically control or limit hemorrhage from non-compressible sources. These include a device for external compression of the abdominal aorta (Abdominal aortic and Junctional Tourniquet, AAJT) and a method for occluding aortic blood flow using an inflatable balloon inside the vessel (Resuscitative Endovascular Balloon Occlusion of the Aorta, REBOA). However, these interventions can be dangerous because of their dramatic circulatory effects and the decreased exchange of oxygen and cellular byproducts from the tissues distal to the occluded blood flow - so called *ischemic* injuries. Animal studies targeting the effectivity and specific harmful effects of the AAJT and REBOA can provide important knowledge regarding safe application-time limits and specific management strategies, including the removal of the devices. As for TIC, a robust animal

model with methods for induction of TIC and detection of the specific components of coagulation dysfunction after trauma is needed for future studies on treatment options.

The overall aim of this thesis was to provide physiological animal data on the above mentioned novel mechanical hemostatic adjuncts to facilitate their implementation into clinical practice (translational research) and to contribute to the important research area of traumatic coagulopathy. All studies in the present thesis was conducted on pigs averaging 60 kg because of the similarities in anatomical dimensions as well as physiological likeness in cardiovascular function, response to hemorrhage shock and pharmacological therapies.

In study I, we investigated the physiological consequences of an abdominal aortic tourniquet in relation to a 60- versus 240-minute application time and compared the resuscitative effects to standard fluid resuscitation. We found that the device effectively stopped blood flow at the level of the abdominal aorta with sustained circulation to the kidneys and thus show potential as a hemostatic device for control of bleeding from distal injuries. However, the mechanical compression caused significant injuries to the intestine and liver after 240 minutes but not after 60 minutes. The abdominal tourniquet also produced an increased blood pressure and therefore, less un-physiologic fluids were needed to restore and maintain blood circulation of the heart, lungs and brain in the animals.

In *study II*, we compared the circulatory resuscitative effects, and the total need for clear fluids (Ringer´s acetate), i.e. non-blood products, between the abdominal aortic tourniquet device and REBOA with the balloon placed in the abdominal aorta between the kidney branches and the division of the aorta into the inguinal arteries (zone III). We found a significantly higher need for resuscitation fluids to restore and maintain circulation of the organs above the aortic occlusion with REBOA compared to the AAJT. These findings indicate that the AAJT, which is also easier to use by non-medical professionals, may be more suitable in the first phase of care in the field. This study also implicated a lesser physiological penalty with zone III REBOA compared to the AAJT.

In *study* III, we investigated the possibility to replace the AAJT with zone III REBOA because of the possibility to operate a patient during REBOA which cannot be done with the AAJT in place on the abdomen. The findings in study II, of a possibly longer physiological tolerance of zone III REBOA also implicated this study. We found that a transition between these interventions can be performed, but may be technically challenging due to the decreased diameter of the femoral artery (which is used for insertion of the REBOA balloon catheter) after blood loss and AAJT application. Furthermore, in line with the findings in study I, a

decrease in blood pressure occurred after the transition procedure which required blood volume replacement and/or drugs for constriction of the blood vessels to maintain a sufficient blood pressure.

In *study IV*, we investigated a strategy of a zone I (thoracic) REBOA balloon inflation for 10 min followed by complete deflation for 3 min during 60 minutes' total intervention time. Placement of the balloon in the thoracic aorta may be needed to control bleedings in the abdomen and to restore circulation in patients with extremely low blood pressure. Previous studies have found that thoracic aortic occlusion (close to the heart) may risk cardiac injury because of the high mechanical resistance towards the cardiac output. Study IV was designed to evaluate the possibility to mitigate the ischemic injury associated with REBOA, by permit intermittent circulation (hence the term intermittent REBOA) and partial oxygenation of the body regions that becomes uncirculated during REBOA. Furthermore, we studied the relationship between blood flow in the aorta (as restored by the balloon deflation) and the major kidney arteries and found that these did not correlate directly. The findings in study IV implicated that intermittent release of the high resistance close to the heart may decrease the risk of cardiac injury during 60 min intervention. We also showed that the low-grade return of circulation of the kidneys, during the balloon deflation intervals, resulted in superior urine production which may be viewed as a predictor of decreased risk for kidney injury after REBOA. The intermittent REBOA protocol was, however, associated with large fluctuations in blood pressure and the 3 minute deflations had to be aborted increasingly often due to dangerously low blood pressure.

In *study V*, we addressed the development of an animal model that can be used for further studies in the process of acute traumatic coagulopathy. We designed a research model which included two different physical injury modalities, massive bleeding, low body temperature and dilution of the blood with clear fluids which is also associated with coagulation dysfunction. We used a test called rotational thromboelastography, often used in human patients, which can discover a variety of coagulation abnormalities. With this method, a summarization of all components in the bloods function of coagulation and fibrinolysis is visually presented. We further added a mathematical method, called principal component analysis, which allowed for multiple comparisons between individual animals and the different trauma modalities at specific time points. We were able to induce TIC and further to detect specific differences between the study groups and therefore this study showed promising results for future studies in this area.

In general, prospectively collected data from human patients are needed to advance the possibility to use these novel mechanical hemostatic adjuncts in clinical scenarios and further animal studies are needed to study the process of TIC.

ABSTRACT

Trauma, in terms of physical injury, is the leading cause of death and disability among children and young adults worldwide. Hemorrhage and coagulation dysfunction are two important causes of preventable deaths after traumatic injuries. Novel methods for management of non-compressible hemorrhage and treatment of trauma induced coagulopathy (TIC) may reduce mortality. In this thesis we investigate two fundamentally different methods for mechanical hemorrhage control after potentially lethal truncal hemorrhage: intravascular occlusion- and external compression of the aorta. The Abdominal Aortic and Junctional Tourniquet® (AAJT) and Resuscitative Endovascular Balloon Occlusion of the Aorta (REBOA) were initially introduced in military medicine as possible interventions to avoid exsanguination from large vessel injuries. We assessed these interventions during 60 to 240 minutes with the aim to identify specific physiological effects and suggest tolerable application times. The feasibility of a transition between the methods was studied. We investigated the hemodynamic effects of crystalloid- and whole blood transfusion in conjunction with the interventions. Intermittent reperfusion during REBOA, with the aim to decrease organ damage was investigated. We introduced a method for advanced analyzation of viscoelastic tests by principal component analysis to detect TIC. The AAJT and REBOA were both effective to stop hemorrhage distal to the aortic bifurcation and restored critical circulation proximal to the aortic occlusion. Infra-renal REBOA required more crystalloid fluids to restore the circulation than AAJT. Both methods caused ischemic injuries which became significant after 1 hour of infra-renal aortic occlusion. The ischemic injuries were alleviated by intermittent reperfusion during thoracic REBOA application. TIC was detected in pigs by principal component analysis of rotational thromboelastometry, which comprised a new method for possible identification of TIC phenotypes. In conclusion, mechanical hemostasis was effective by both abdominal tourniquet- and intravascular aortic occlusion. We also identified potentially severe complications due to hemodynamic- and metabolic consequences, particularly when application times exceed one hour.

LIST OF SCIENTIFIC PAPERS

This thesis is based on the following papers, which will be referred to by their Roman numerals as indicated below:

I. **Abdominal Aortic and Junctional Tourniquet release after 240 minutes is survivable and associated with small intestine and liver ischemia after porcine class II hemorrhage.**
Brännström A, Rocksén D, Hartman J, Nyman N, Gustavsson J, Arborelius UP, Günther M.
J Trauma Acute Care Surg. 2018 Oct;85(4):717-724.

II. **Increased crystalloid fluid requirements during zone 3 Resuscitative Endovascular Balloon Occlusion of the Aorta (REBOA) versus Abdominal Aortic and Junctional Tourniquet (AAJT) after class II hemorrhage in swine.**
Brännström A, Dahlquist A, Gustavsson J, Arborelius UP, Günther M.
Eur J Trauma Emerg Surg. 2021 Jan 30:1-10. Online ahead of print.

III. **Transition from abdominal aortic and junctional tourniquet to zone 3 resuscitative endovascular balloon occlusion of the aorta is feasible with hemodynamic support after porcine class IV hemorrhage.**
Brännström A, Dahlquist A, Gustavsson J, Arborelius UP, Günther M.
J Trauma Acute Care Surg. 2019 Oct;87(4):849-855.

IV. **Improved Renal Blood Flow and Indices of Organ Function after 60 minutes Intermittent versus Continuous Zone 1 REBOA in an Animal Model of Lethal Hemorrhage.**
Brännström A, Hultström M, Gustavsson J, Aurfan Z, Günther M.
Manuscript

V. **The Swine as a vehicle for research in trauma-induced coagulopathy: Introducing principal component analysis for viscoelastic tests.**
Brännström A, von Oelreich E, Degerstedt LE, Dahlquist A, Hånell A, Gustavsson J, Günther M.
J Trauma Acute Care Surg. 2021 Feb 1;90(2):360-368.

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LIST OF ABBREVIATIONS

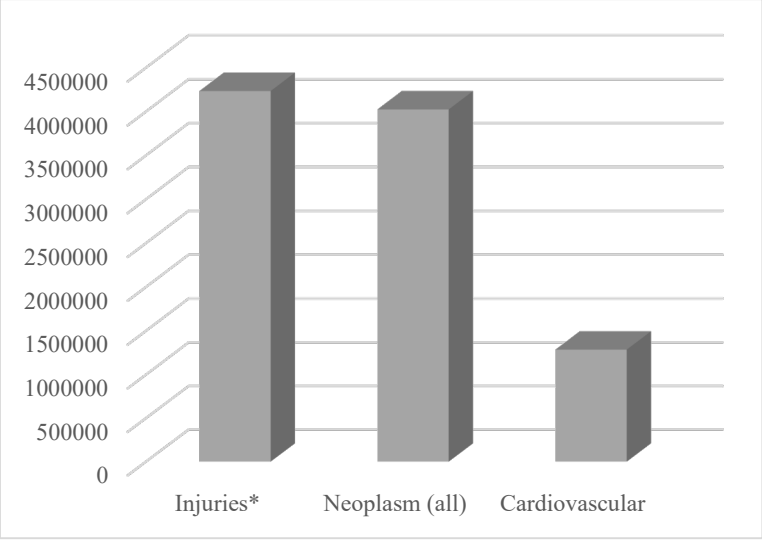
AAJT	Abdominal Aortic and Junctional Tourniquet
aPTT	Activated partial thromboplastin time
aPC	Activated protein-C
AKI	Acute kidney injury
ATC	Acute traumatic coagulopathy
ATLS	Advanced Trauma Life Support
ANOVA	Analysis of variance
AO	Aortic occlusion
BP	Blood pressure
CO	Cardiac output
CVP	Central venous pressure
cREBOA	Continuous REBOA
DCR	Damage control resuscitation
Hct	Hematocrit
ISS	Injury severity score
iREBOA	Intermittent REBOA
INR	International normalized ratio
IRI	Ischemia reperfusion injury
LPS	Lipo-polysaccharide
MAP	Mean arterial pressure
NCH	Non-compressible hemorrhage
NS	Normal saline
VO_2	Oxygen consumption

DO_2	Oxygen delivery
RBCs	Packed red blood cells
pREBOA	Partial REBOA
PPH	Post-partum hemorrhage
K^+	Potassium
PCA	Principal component analysis
PT	Prothrombin time
RBF	Renal blood flow
REBOA	Resuscitative Endovascular Balloon Occlusion of the Aorta
RT	Resuscitative thoracotomy
RA	Ringer´s acetate
ROTEM	Rotational thromboelastometry
SVR	Systemic vascular resistance
SBP	Systolic blood pressure
TCCC	Tactical Combat Casualty Care
TV	Tidal volume
TBV	Total blood volume
TIC	Trauma induced coagulopathy

1 INTRODUCTION

Traumatic injuries are the leading cause of death among children and middle-aged adults worldwide and constitutes approximately 10% of total mortality in all ages (1). More than 12 000 people die each day from injuries as a result of accidents or violence (1). In perspective, the number of deaths caused by trauma (in this thesis defined as *physical* injury) is around 1.7 times higher than the overall deaths of the infectious diseases AIDS, malaria and tuberculosis combined (2). The global mortality as a result of conflicts and terrorism is approximately 130 000 each year, or approximately 15 people every hour (1). Survival, clinical- and functional outcomes after severe injuries is highly dependent on the quality of, and timely access to adequate care. The Birmingham Accident Hospital, founded in the UK 1941, is considered as the first medical facility dedicated to trauma-victims and the start of a medical evolution leading to today's modern trauma-centres (3). Reflecting upon the time for the foundation of this first trauma-hospital, it must be noted that, sadly, a substantial amount of the knowledge foundation for the care of trauma-victims have been congregated during times of war. Recognizing the impact of early, life-saving interventions and transferral to definitive care - in 1966, the National Academy of Sciences in the US, published "Accidental Death and Disability: The neglected disease of modern society" (4). That report influenced the development of the first prehospital emergency medical services, as an integrated part of a regional trauma system.

Figure 1. Major causes of global deaths (2017) in ages 1-49 years.



**Including self-harm and violence*

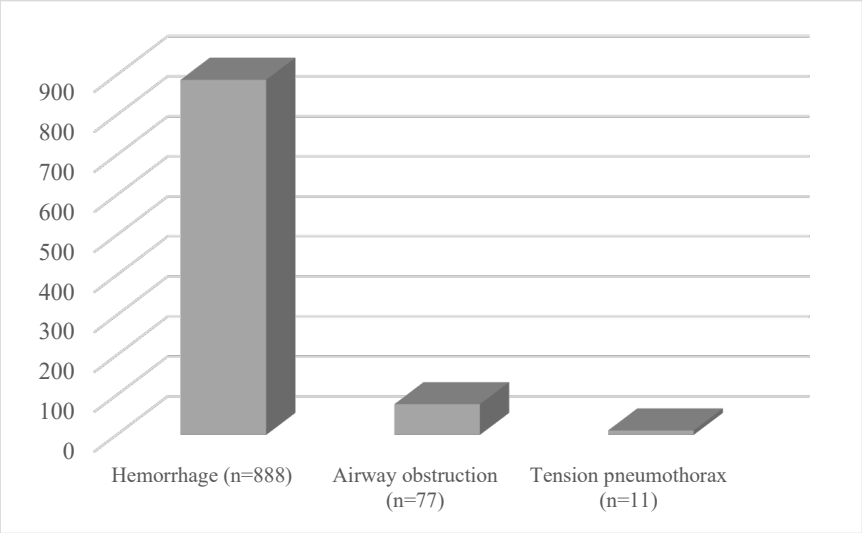
Hemorrhage is the most important cause of deaths that possibly could have been prevented after trauma (5-7). It accounts for approximately 30-40% of deaths following injuries every year, of which 50% occurs outside hospitals. In a Swedish report investigating injury-related mortality, the vast majority (90%) died before hospital admission. Furthermore, the injury characteristics differed significantly from the hospital deaths including firearm injuries where 67% of deaths occurred prehospital (8). Analyses of prehospital deaths in military (7) and civilian trauma (8) show similarities with a majority of deaths regarded as nonsurvivable. However, of the potentially survivable deaths on the battlefield up to 91% are related to hemorrhage (9-11). This thesis is largely based on studies conducted in military populations. Considerable research- and clinical efforts the past two decades have resulted in unprecedented improvements in mortality rates of combat casualties (12). These determinations are paving the way for the management of severely injured patients in civilian trauma as well, especially regarding traumatic hemorrhage and shock.

2 LITERATURE REVIEW

2.1 MILITARY INJURY PATTERNS IN RELATION TO CIVILIAN TRAUMA

Understanding the causes of death after traumatic injuries is fundamental for research and development of new treatments as well as the organization of trauma-systems. In 1996, Tactical Combat Casualty Care (TCCC) (13) was introduced in the US military as a result of a 2-year study identifying casualty care techniques with more appropriateness than the previously used principles of Advanced Trauma Life Support (ATLS) (14), adopted from the civilian care. The need for these modifications were based on the combined complexity of severe injuries from high energy- and blast trauma, limited medical equipment, hostile environments, inconstant evacuation times and a high proportion of penetrating trauma. The TCCC concept has since been continuously revised and updated in accordance with evidence based, proposed changes. In 2010, after ten years of war in Iraq and Afghanistan, the outcomes after severe combat injuries where equal to, or better than those in the US civilian trauma systems (15). Still, a 24% of battlefield mortalities has been deemed as potentially survivable (7).

Figure 2. Aetiology of potentially salvageable combat fatalities 2001-2011[7].



The most common causes of deaths, that were judged as non-survivable and occurred instantaneously or in the acute phase after injury were: brain injury (43%), heart/thoracic injury (21%), dismemberment (16%), other (13%), open pelvic injury (3%) and high spinal cord injury (4%).

In the subgroup of patients who die from hemorrhage but have been regarded as potentially survivable (80%) (16), the term “exsanguination shock” has been introduced to further characterize the physiological causes of death due to massive hemorrhage before the onset of

multiple organ failure in later stages of hemorrhage shock (17). These patients typically present severe hypotension or even arrest due to massive blood loss, but respond to resuscitation and surgical hemostasis before passing away. The underlying pathophysiology behind death in these patients is unknown but likely involves complex molecular dysregulation after profound ischemia, and as a result of inflammation, oxygen debt, coagulation dysfunction, CNS dysfunction and immune-related mechanism with capillary occlusion (17). Furthermore, respiratory arrest rather than circulation failure is often the first sign of deterioration.

Penetrating injuries from explosive fragments or gunshot wounds can be expected in up to 75% of casualties in combat (7, 18). These injuries often involve vascular- or solid organ disruption (19) which can lead to rapid external or intra-cavital, fatal loss of circulating blood volume (exsanguination) following the rationale of Poiseuille´s law – the larger the vessel, the greater the blood flow (20, 21). Massive hemorrhage from intra-abdominal or thoracic sources are historically only amendable for surgical control due to the severity to manually exert counter pressure against the injury site. Hence, the term non-compressible hemorrhage (NCH).

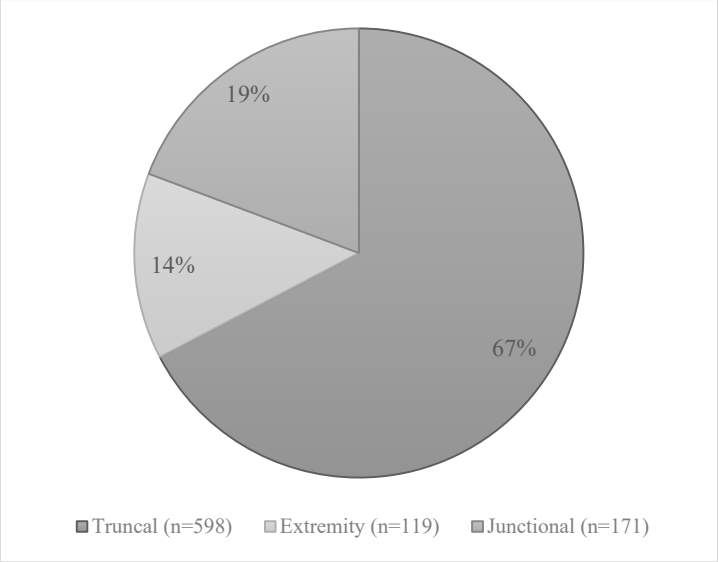
Figure 3. A "pressure-plate" improvised explosive device found buried in a road in Afghanistan before it could do harm.

Injuries caused by explosive ordnance has increased significantly in recent wars in Afghanistan and Iraq and is the most common (72%) mechanism of injury [(18)]. Photo: Personal stock.

To date, no formal definition of NCH exists allowing for comparisons of epidemiology and treatments. However, the concept includes vascular disruption with hemorrhage from ruptured vessels or solid organs in the abdomen and thorax including the pelvis and junctional regions

between the proximal extremities and the torso (22, 23). In particular, bleeding from the lower trunk and pelvic regions poses a great opportunity for reduction of mortality rates in rural civilian and military environments (7, 24). Analyses of data between 2002-2010 of the frequency and mortality of NCH (further categorized to anatomical subgroups of thoracic, solid-organ, axial vessels and pelvic fractures) in Iraq and Afghanistan summarized that 13.7% of all injuries were classified as torso injuries with a mortality rate of 19% (16). In the civilian environment, similar figures of frequency and mortality exists but with a majority of blunt rather than penetrating mechanisms of trauma (25). The injury patterns in the military population with up to 90% penetrating trauma and a significant need for massive transfusions (>10 units of packed red blood cells (RBCs) (26), encouraged the development of a resuscitation strategy more specifically targeting the physiological consequences of severe blood-loss and profound hypoperfusion.

Figure 4. Anatomic focus of potentially survivable hemorrhage-related deaths on the battlefield 2001-2010[11].



The development of body armour effectively protecting thoracic organs have increased the ratio of potentially preventable deaths from lower abdominal, pelvic and inguinal combat related injuries.

2.2 HEMORRHAGE SHOCK AND TRAUMA INDUCED COAGULOPATHY (TIC)

The relationship between blood loss and hemorrhage shock was first described in animal experiments performed by Blalock in 1930 (27). Shock occurs during inadequate oxygen delivery or utilization in peripheral tissues and develops secondary to a circulatory failure (28). Blood loss will lead to hypovolemic shock when the venous return decreases below the threshold for a sufficient cardiac output (reduced preload) and a mean arterial pressure (MAP)

of approximately 65 mm Hg (29). The pathophysiology of shock can be understood as insufficient aerobic energy production and a developing oxygen debt (30). The standard metabolic variable for detection and quantification of anaerobic glycolysis from imbalance between oxygen delivery (DO_2) and demand (VO_2) is lactate (31, 32). Hyperlactatemia is generally defined as blood lactate levels > 1.5-2 mmol/L and has been shown as a reliable predictor of outcome in practically all types of circulatory shock (33, 34). However, important exceptions exists, for example a prompt elevation of lactate levels may reflect as a part of reperfusion of hypoxic tissue and thus a step towards clinical improvement (35). To avoid extensive cell death and organ damage, the perfusion must be restored and the oxygen debt compensated (36). The degree of blood loss may not reflect the susceptibility to shock due to physiological differences (such as cardiac performance, capillary density and mitochondrial concentration- and function) in DO_2 or VO_2, (30, 36, 37) and therefore hypotension is *not* an obligate criteria in the diagnosis or definition of clinical shock (38). Signs of shock is characteristically often presented as tissue hypo-perfusion which can be revealed through the three "windows of the body" by cold, damp and discoloured peripheral skin, low or absent urine production and affected mental status (39). Blood loss is offset by sympathetic-mediated, and hormonal compensatory mechanisms, such as cardiovascular adaptations and hyperglycaemia (which osmotically recruits extravascular body-water into the circulation) to uphold critical organ perfusion. Therefore, blood pressure changes after hemorrhage shows a non-linear relationship to blood-loss up to a point where these mechanisms are depleted. As a consequence, hemorrhage *with* manifest hypotension, may reflect a severe grade of blood loss and ongoing development of profound hemorrhage shock, especially in young and otherwise healthy individuals. In trauma populations with clinical signs of hypoperfusion, hemorrhage should therefore *always* be suspected until proven otherwise.

Table 1. Hemorrhage shock classes and clinical manifestations.

Class	***I***	***II***	***III***	***IV***
Hemorrhage (%)	< 15	15-30	30-40	> 40
Heart-rate	Unchanged	> 100	> 120	> 140
Arterial pressure	Unchanged	Unchanged	Decreased	Very low
Capillary refill	Unchanged	Delayed	Slow	None
Breathing rate	Unchanged	High normal	Tachypnea	Distinct Tachypnea
Mental status	Unchanged	Uneasy	Disoriented	Lethargic
Urine output	Unchanged	15-30 mL/h	< 20 mL/h	None

Adopted from the ATLS student course manual, tenth edition [(40)].

Severe traumatic injury is now known to cause massive endogenous reactions on a molecular level. Changes in expression of up to 75% of the genome has been reported as a response to severe injury (a "genomic storm") with crucial impact on immunologic and inflammatory pathways (41). Coagulation dysfunction is present in at least 25% of severely injured patients (depending on the definition used) upon hospital admission (42) and constitutes a major, independent cause of mortality (43, 44). The understanding of coagulopathy associated with trauma have recently evolved from an iatrogenic condition sprung from deranged homeostasis (acidosis and hypothermia) and loss of effective coagulation factors due to hemodilution (45). Recent knowledge suggests that the process of acute traumatic coagulopathy (ATC) more importantly arises as an endogenous condition in the presence of tissue injury and hemorrhage shock and that this onsets as early as minutes after injury (42, 46-48). The concept of ATC involves both disrupted plasma coagulation (the crucial part of fibrin mediated stabilization of blood-clots secondary to primary hemostasis by vasoconstriction and formation of platelet plugs) and fibrinolysis (49). The pathogenesis of ATC is multifactorial, however, to a large degree not understood. It is generally believed that the activated protein C (aPC) pathway is a central part of the pathologic condition of ATC (50). Translational research plays a fundamental role in the search for the mechanisms behind the pathogenesis of ATC and potential treatment options. The traditional assays for detecting ATC are prothrombin time (PT), activated partial thromboplastin time (aPTT) and international normalized ratio (INR), using a 50% increase in these parameters as a diagnostic criteria (51). These tests are limited by the low concentration of, and only measuring the initial function (30-60 s) of, the platelets and the laboratory delay in the situation of acute and often time-sensitive management of

trauma patients. Viscoelastic tests such as thromboelastography and rotational thromboelastometry (ROTEM) reflecting a more global coagulation performance of the blood at the bedside has therefore been introduced but no general diagnostic criteria of ATC using viscoelastic tests are currently adopted.

2.3 ASPECTS OF FLUID RESUSCITATION AND USE OF BLOOD IN TRAUMATIC HEMORRHAGE

It is well known that resuscitation using fluids and transfusion of blood products may be necessary to resuscitate a hypovolemic patient (52). The golden questions in resuscitation are when, what type of and to what degree administration of fluids should be performed (53, 54). Furthermore, the preferential use of isotonic crystalloid- versus hypertonic colloid fluids has been debated for many years (55). Sodium, constituting the principal osmotic factor in crystalloid solutions, will distribute along the concentration gradient across capillary membranes and thus, the main part of administered fluids (75-80%) will follow to the extravascular space within approximately one hour, which in turn leads to loss of circulating volume and (with excessive administration) formation of oedema (56, 57). Colloids on the other hand, contains osmotic molecules designed to remain intravascular and expand the circulating volume for up to 8 hours (58). In prehospital environments there is a consensus that fluids (any) should be administered in bleeding patients with signs of deteriorated mental status, reflecting an insufficient perfusion of the brain, and thus, end-state hypovolemia (52).

In the early -90s, the ATLS protocol advocated liberal use of crystalloid fluids (2000 mL as soon as possible) to normalize blood pressure (59). That strategy has subsequently been proven to be harmful in patients where hemorrhage control is not obtained, due to risk of worsened bleeding and coagulation dysfunction, which in turn is due to hypothermia and dilution of coagulation factors and oxygen carrying haemoglobin (60-62). Animal data has shown survival benefits with permissive hypotensive resuscitation to 20 mm Hg below baseline MAP over resuscitation to baseline values and restraining of fluids until after surgical hemorrhage control (63). The damage control resuscitation (DCR) strategy was introduced in military medicine in 2007 as a response to the increased understanding of injury patterns and causes of mortality of the casualties. In this concept the targeted systolic blood pressure (SBP) during initial fluid resuscitation is around 80-90 mm Hg, using plasma and RBCs in a ratio of 1:1 or as close relation as possible (64, 65). This strategy is now also advocated by prestigious civilian hospitals like the Mayo Clinic in the US (66). During the following years, the sentience of

appropriateness of replacing lost blood with whole blood was growing and several studies provided evidence of this being correct (67-70). The use of fresh whole blood has further been shown to improve outcome in comparison with component therapy, which partly may be attributed to the mechanic properties of the red blood cells and their capillary interaction (69). Of course, several logistical and potential medical risks limit the use of fresh whole blood both in the prehospital and hospital settings. The possibility to perform comparability tests to avoid allergic transfusion reactions, storage (more than a few days) and screening for transmittable diseases are the major limitation factors for the use of fresh whole blood in emergency situations outside hospitals. Since administration of whole blood or blood-products (fresh plasma, RBCs and platelets) is foremost a logistical problem in the military prehospital environment – the secondary resuscitation fluid of choise is considered to be dried plasma (71).

Figure 5. A medic during World War II infusing plasma to a wounded soldier.

Resuscitation with blood products was used before the era of synthetic fluids. During the Vietnam-war, crystalloids was the principally used resuscitation fluid, often excessively, which led to the encounter of their potentially harmful effects. Hence the word "shock-lung" or "DaNang Lung" which refers to the formation of pulmonary oedema and respiratory failure after successful hemodynamic resuscitation with voluminous administration of crystalloids. Photo from U.S. Army Medical Department Office of Medical History Website. Reproduced with permission.

2.4 HEMORRHAGE CONTROL AFTER SEVERE INJURY

In modern history, treatment of severe traumatic hemorrhage have improved despite the lack of novel revolutionary means to achieve early control of bleeding. Extremity bleedings can be controlled with manual wound pressure, proximal control using tourniquets, and hemostatic agents (72-74). These interventions are principally limited to extremity injuries. Immobilisation of long bone fractures and the pelvis reduces blood loss from venous and bone-fracture-surface bleedings by counteracting mechanical disturbance of clot-formation. Pharmacological treatment including early administration of anti-fibrinolytic agents

(administered within 3 h of injury) (75, 76), coagulation proteases (77), reversal of oral anticoagulants (78) and antibiotics are also a part of the clinical management of patients with massive traumatic hemorrhage.

Rapid hospital transfer is the most important factor to improve survival after massive NCH. Traditionally, the in-hospital treatment of intra-abdominal hemorrhage, in the presence of profound hemorrhage shock, is thoracic aortic occlusion by means of thoracotomy (79). This procedure can provide temporary control of bleeding and increase cerebral and coronary perfusion. However, the outcomes associated with this intervention is poor (80). The procedure itself also causes additional trauma in a patient with depleted physiologic reserves and it requires that the patient can survive transportation to a facility with the appropriate capability.

2.5 MECHANICAL HEMOSTATIC ADJUNCTS

2.5.1 Extremity tourniquets

The most important cause of reduced mortality after extremity hemorrhage in military medicine, is the established use of tourniquets. Controversies exists regarding the use of this ancient device to control extremity hemorrhage. However, the emphasis of its use in the battlefield have increased the survival of extremity injuries to over 90%. Possible complications include unnecessary amputations resulting from limb ischemia, organ damage including renal failure, clots, myonecrosis and nerve palsies. In summary, Kragh et al. have, in a series of reports, based on extensive military data, shown evidence supporting the efficacy and safety of appropriately used tourniquets to control lethal hemorrhage from the extremities (81-83). Importantly, survival was strongly associated with application before the onset of shock (83).

Figure 6. *Ancient tourniquet device. From Alamy stock photo. Reproduced with permission.*

Generalization to civilian care is not uncomplicated. Differences in patient populations, including higher proportions of elderly and paediatric patients, and significantly higher ratios of co-morbidities exists. Data regarding use of tourniquets in the civilian care is limited and recommendations for use are vaguer than in the military (84). Furthermore, military indicated use of a tourniquet may result from tactical reasons not applicable to civilian prehospital care.

However, fear of complications must be rational and with the judgemental and proper use it may be an increasingly utilized intervention also in the civilian environment (85-87).

2.5.2 Truncal tourniquets

The widespread use of extremity tourniquets and their effectiveness in military operations resulted in a shift of focus in military medical research, pragmatically addressing the abdomen, pelvic- and junctional regions as the now most common sources of preventable death from exsanguination. New devices that could close off the arterial inflow to these regions were proposed for evaluation.

Compression of the abdominal aorta to decrease blood flow to distal bleeding sites is a medical procedure that has been used in both trauma and post-partum (PPH) hemorrhage for decades. Manual pressure over the distal abdominal aorta or proximal inguinal artery can be effective to stop distal bleeding. However (beside the case of a recently pregnant woman with diastasis of the abdominal rectus muscles), the caregiver may need to apply around 588.4 N of pressure, comparable to a body weight of 92 kg during short-term periods or 115 kg to maintain occlusion during longer transports (24, 88).

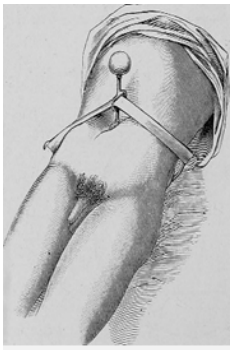


Figure 7. *Esmarchs abdominal aortic tourniquet from around 1870 $_{AD}$. From Alamy stock photo. Reproduced with permission.*

The first published report of a truncal aortic occlusive device, an abdominal tourniquet, was published in 1964 by Edwards et al (89) describing the idea of regional perfusion of the lower extremities with therapeutic agents (chemotherapy and antibiotics) in doses that would have been deleterious if administered systemically. The authors summarize the procedure as "*...The major problems encountered were technical difficulties in an epidural catheter* [which was used for injection of a saline bolus to compress the epidural venous plexus that risked shunting of blood between the regionally perfused body parts]*, circulatory disruption when the tourniquet was applied and (especially) removed, blood loss and a critically ill patient in the postoperative period*". Other external devices designed to mechanically occlude large arteries go back in history to at least around 1830. One example is the Esmarch´s aortic tourniquet that, in the hands of a surgeon with comprehensive anatomical knowledge, was successfully used to control hemorrhage during surgery (90). In modern history (2004) another custom made abdominal aortic tourniquet was assessed as a first aid for PPH (91). The authors conclude the intervention as a "*cost effective and easily applied manoeuvre that allows satisfactory management of PPH without maternal mortality or*

morbidity. It is of value in developing countries". Given the historical background, and the physiologic prudence of hemorrhage control by external compression of truncal arteries, a modern, well-designed truncal tourniquet was re-introduced in combat casualty care around 2012.

The Abdominal Aortic and Junctional Tourniquet (AAJT; Compression Works, Alabama, US) is a device designed to occlude blood flow through the infra renal aorta by pneumatic compression of the abdomen. It has been shown to effectively reduce bilateral iliac blood flow in human volunteers and to be hemodynamically tolerable for two hours in porcine models (92-94). A major advantage of the AAJT is that it is easy to use, and thus may be managed by non-medical professionals. In addition to hemorrhage control, the increased systemic vascular resistance (SVR) from aortic occlusion can provide a resuscitative effect and lower the need for resuscitation fluids (95). A single, anonymous case report from 2013 described the first use of the AAJT in a successful resuscitation of a battlefield casualty (96). In addition, two case reports of clinical use with focal application to injuries in the axilla and groin has been published (97, 98).

Figure 8. The Abdominal Aortic and Junctional Tourniquet®

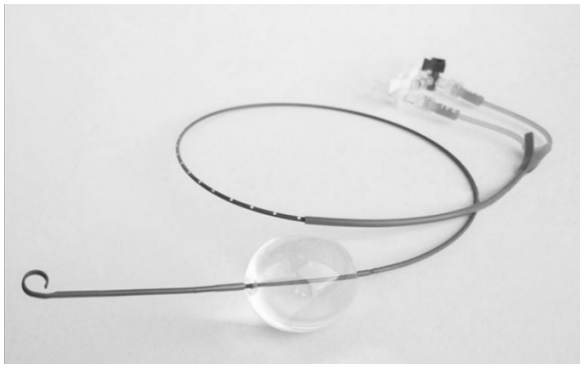
The AAJT is applied over the umbilicus and secured with a waist-strap before inflation of the wedge shaped, pneumatic bladder which compresses the abdominal aorta between underlying tissues and the vertebral column. Photo: Personal stock

A limitation for establishing the AAJT into clinical practice is insufficient physiological data regarding the adverse effects, particularly in case of prolonged application and the evidence of any specific organ damage related to the AAJT had not been addressed prior to study I in this thesis. Assumed complications of the device include severe ischemic injuries to abdominal organs and lower extremities and life-threatening metabolic derangement after removal (93, 99). The current abdominal application time-limit of 60 minutes have not been associated with

any long-term detrimental effects in animal studies, however, removal after two hours may cause reversible metabolic and respiratory complications in pigs (93). Further knowledge of complications with AAJT application and subsequent removal can increase patient safety and use of the device. These aspects were addressed in study I in this thesis.

2.5.3 Resuscitative Endovascular Balloon Occlusion of the Aorta (REBOA)

Figure 9. The ER-REBOA™ catheter.

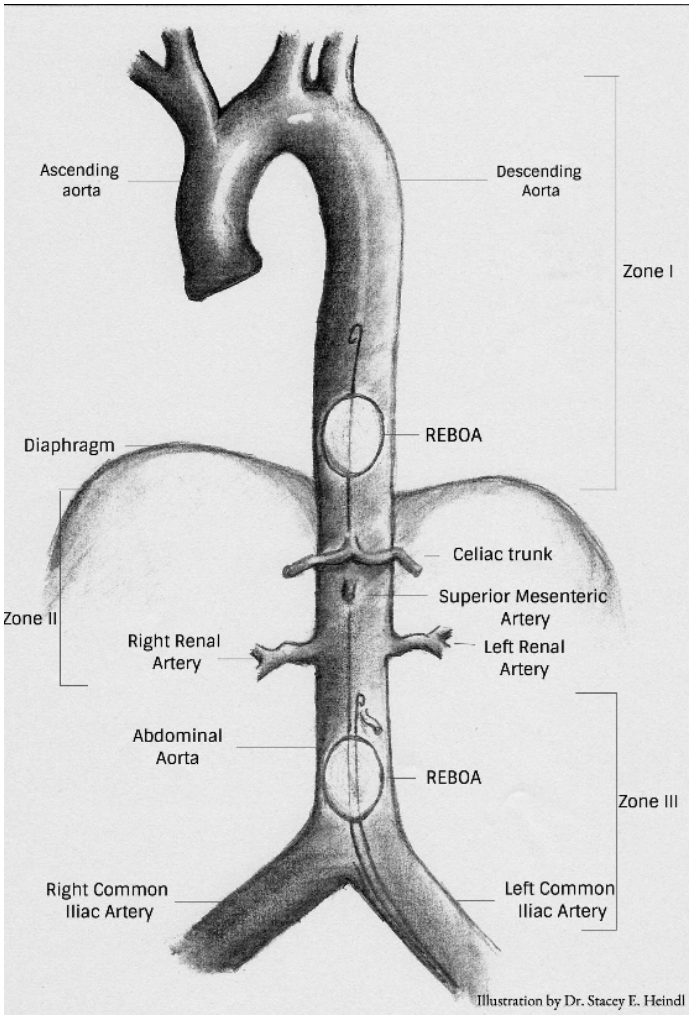


A commercially available device with the desired traits of a REBOA catheter. It contains a curved tip for atraumatic insertion through the vessels. The compliant balloon is distally- and proximally demarked by radiopaque bands. A double-lumen catheter (one communicating with the balloon and one for proximal arterial pressure monitoring) with length markers. From Alamy stock photo. Reproduced with permission.

History and objectives with REBOA

Resuscitative endovascular balloon occlusion of the aorta (REBOA) was first described 1954 during the Korean war (100) and the first report of an animal model (canine) for studies on the feasibility of intra-aortic balloon occlusion in massive traumatic hemorrhage was published in 1953 (101). In this technique, a catheter based inflatable balloon is inserted via an introducer (usually 7F size) in the common femoral artery and positioned in the infra-renal abdominal (zone III) or thoracic aorta (zone I) (102).

Figure 9. Aortic zones for REBOA placement.

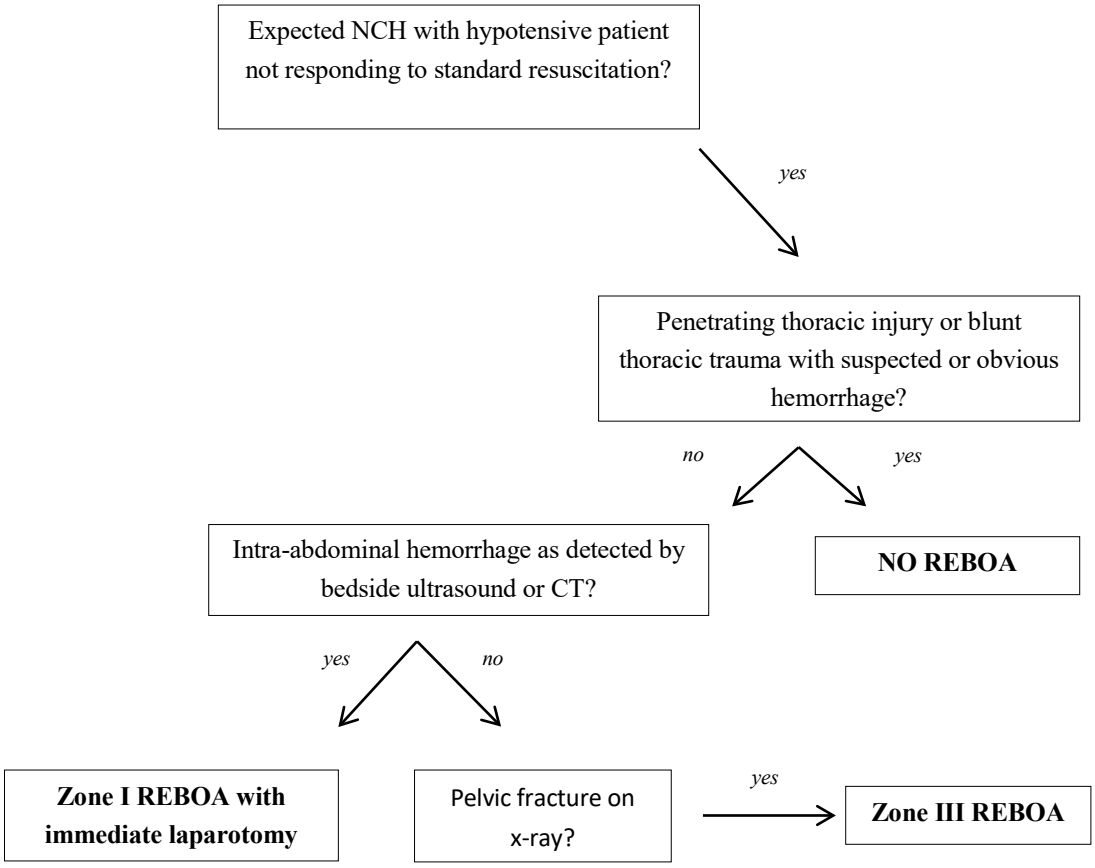


Zone I (thoracic): The left subclavian artery to the celiac trunk (aorta descendens); Zone III (infra-renal): Below the most distal renal artery to the bifurcation. The para-visceral aorta in zone II is considered as a "no-go" zone for REBOA due to decreased possibility to control hemorrhage and risk of abdominal organ injuries. From Heindl S, Et al. *Partial Versus Complete Resuscitative Endovascular Balloon Occlusion of the Aorta in Exsanguinating Trauma Patients With Non-Compressible Torso Hemorrhage. Cureus. 2020;12(7): e8999.* [(103)]. *Reproduced with permission.*

When the balloon is inflated, the distal aortic blood flow is occluded. The objective with REBOA is similar to the resuscitative thoracotomy (RT) manoeuvre with temporal decrease in arterial inflow to the injury site and augmentation of cerebral and coronary perfusion by increased cardiac afterload (104). After the first report by Hughes in 1954, publications regarding REBOA were sparse for decades. In recent years, with developments in endovascular medical procedures and technical advancements in catheter-based interventions, REBOA have been reintroduced as an adjunct for hemorrhage control (102). In response to the increase of vascular injuries, during the conflicts in Iraq and Afghanistan, the US military reconsidered

REBOA as an alternative for casualties with potentially lethal NCH (105). Indications and recommendations for clinical use in civilian trauma has later been published in the US (106).

Figure 10. A sample emergency-room REBOA algorithm.



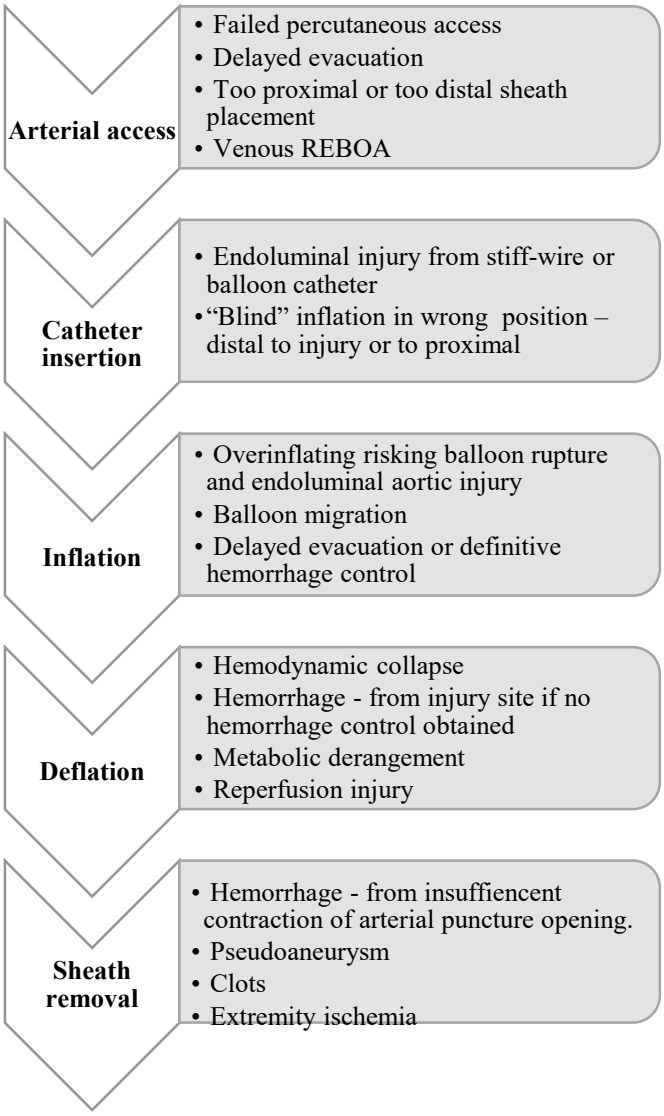
Example of an algorithm for consideration of REBOA in trauma patients. Modified from Stannard et al[102].

The implementation of REBOA in civilian trauma care has not yet been shown to improve long term mortality rates, however, superior outcomes compared to RT have been reported (107, 108). Translational research also provided evidence of less metabolic insults with REBOA over RT (80). The principal expected physiological response after inflation of the balloon is a prompt increase in proximal SBP of around 50-70 mm Hg (109-111). The result of the increased cardiac afterload from aortic occlusion is restoration of organ perfusion proximal to the balloon (110). However, the high resistance to cardiac outflow is also associated with an increased myocardial workload, and thus, VO_2 (112). The profound cardiac burden associated with zone I REBOA has further been shown to risk a type-2 myocardial injury after prolonged intervention times (> 2 h) (113). Similar to the AAJT, distal ischemia and impending reperfusion injury with severe systemic changes (hypotension, acidosis, hyperkalemia and hyperlactatemia) poses the great challenges with REBOA (114, 115). Current recommendations state a 30 minute (for zone I) and 60 minute (for zone III) maximum occlusion time based on both human and translational research (115). Acute kidney injury

(AKI) has also been shown as a common and potentially deleterious complication associated with REBOA (111).

The demonstrated effectiveness of REBOA, to achieve temporary hemorrhage control, has also led to propagations for its use prehospital (116) and a few reports have been published showing its potential (117, 118). The use of REBOA in prehospital locations raises new questions regarding its appropriate use. The category of possible providers may differ significantly from civilian to military trauma systems. Furthermore, the anaesthetic management of the patient without standard intra-operative (112) monitoring possibilities is challenging (119). Technical improvements and training in REBOA is likely to increase safety. However, several critical moments, which are summarized in Figure 11, need to be recognised by the care-giver performing REBOA (38).

Figure 11. Steps for REBOA placement with associated potential complications.



Aspects of aortic occlusion zones and resuscitation

Increasing evidence suggests important differences in hemodynamic effects between thoracic and infrarenal REBOA. As the primary objectives of REBOA are hemorrhage control *and* proximal hemodynamic support this may have significant implications for the further management of patients eligible for REBOA. It seems likely that a zone III aortic occlusion (AO) provides lesser hemodynamic support than a zone I occlusion. Examination of human patients with REBOA in zone I or III has shown that the effects on blood pressure and heart rate differ. The mean increase in SBP after zone I REBOA were 94% (64 to 124 mm Hg) versus 31% (75 to 98 mm Hg) after zone III REBOA (120). The mean decrease in SBP after deflation however, did not differ (14 mm Hg, zone I versus 12 mm Hg in zone III respectively). Another study confirmed these findings after an analysis of patients included in the prospective observational AORTA registry between 2013-2017 (121). In that study the mean increase in SBP was significantly associated with the aortic zone of occlusion with lesser improvements in hemodynamics after infra renal REBOA. Animal studies have further questioned the use of zone III REBOA in hemodynamically unstable patients (122).

The similar indications for the AAJT and zone III REBOA prompted an investigation of physiological differences after resuscitation with the devices (123). In this animal study both devices were found to obtain 100% hemostasis and no difference in mortality was observed. The AAJT animals had higher MAP but also increased lactate levels after 1h intervention time. This indicates that the AAJT may be a more suitable option for hemorrhage control and hemodynamic resuscitation in the early phases of care. We addressed this question in study II were the physiological and metabolic effects were further compared in relation to fluid requirements and application time. Furthermore, as the logical step for patients resuscitated with the AAJT, we examined the feasibility of transition between the interventions in study III. This manoeuvre is likely necessary to obtain hemorrhage control, avoid hemodynamic collapse and provide ability to perform surgery on the patient.

Aspects of prehospital REBOA

In a retrospective analysis of combat casualties (n=244), 19% were found to may have benefited from prehospital REBOA intervention (124). These patients had remaining circulation during evacuation but died en route or after hospital admission with a median time of 75 min from injury to death. The distribution of deaths was 46% at the scene, 37% during transport and 17% in-hospital. The median prehospital time for the patients transferred to a hospital was 61 minutes. A panel of international civilian and military experts on the field of NCH reached consensus on the military prehospital environment as a potential location for REBOA intervention, but not in civilian prehospital care (125). However, successful REBOA

has been performed (as described in a case report) in the civilian prehospital emergency care by the London Air Ambulance (116). In the military operations, the first use of "prehospital" REBOA on 4 patients was reported by Manley et al. in 2017 (126). These patients were treated by a forward surgical team within 10 minutes' transportation from the point of injury but 2 hours from the next level of care. None of the provider physicians had previous specialty training in endovascular techniques, but all had undergone a standardized training course in REBOA. Furthermore, these care-givers were originally assigned to a level I trauma centre and thus highly experienced in management of trauma patients. This case series also underlined the usefulness of REBOA to facilitate induction of anaesthesia in hemodynamically unstable patients. All patients survived to be transported with stable physiology to the next level of care and no procedural complications were reported.

The lack of diagnostic and monitoring equipment in austere environments, for example x-ray, warrants different approaches to the question of when to perform REBOA in these environments. Another case-series report provided one example of a pragmatic algorithm for REBOA indications during these circumstances. Here, the mechanism of injury (high energy trauma), injury type (sub-diaphragmatic bleeding as confirmed by ultrasound), and vital signs (SBP < 90 mm Hg) were used to guide the initiation of zone I REBOA (127). The crucial aspect of balloon positioning without imaging technology has been specifically addressed by correlation of skeletal landmarks and vascular anatomy as investigated by computer-tomography (128). For average height humans, the investigators suggest a fixed length of 48 cm for zone I insertion and 28 cm for zone III. Importantly, and in parallel with the length of the aortic zones, I: 21.6 cm; III: 8.7 cm (Fig. 9), the error of these fixed insertion lengths was 0.4% and 33.3% in blind zone I versus zone III placement.

Finally, deflation of the balloon may be the most challenging step in REBOA provided that the patient has survived definitive hemorrhage control surgery. Hemodynamic instability from the reduction in afterload and ischemia induced vasoplegia and capillary leakage often requires vaso-active pharmacological treatment. The profound metabolic derangements associated with ischemia reperfusion has been shown to develop for up to 90 minutes (animal data from 45-, 60 and 90 minute zone I occlusion times) after balloon deflation (114). Severe electrolyte derangements (hyperkalemia and hypocalcaemia), hypoglycaemia and acidosis must be expected and treated to prevent lethal arrhythmias and organ failure. Therefore, balloon deflation should only be performed under vigilant monitoring with capability to treat these expected physiologic abnormalities.

Aortic occlusion time and ischemic injury

Much research interest has been put into mitigation of the adverse effects of REBOA in recent years. Ischemia reperfusion injury (IRI) is the complicated process causing further damage to ischemic tissues after return of blood circulation and oxygenation. Fundamentally, the grade of severity of IRI depends on the organ ischemic insult and thus, with REBOA, total occlusion time (129). Tissues are differently sensitive to hypoxia. For example, skeletal muscle can tolerate much longer ischemic time than the brain or the kidneys. Translational research has shown an undisputed correlation between occlusion times and degree of acidosis and pro-inflammatory cytokine levels (115, 130). In other clinical backgrounds such as transplantation of organs and coronary interventions, specific pharmacological treatment of IRI exists. Mitigation of inflammatory pathways, formation of free oxygen radicals and nitric oxide constitutes important targets for these drugs (131-133).

The degree of ischemic injury developing during REBOA may also be altered with modified management strategies of complete aortic occlusion. Two possible strategies, that both includes permissive distal aortic flow, have been proposed. In partial REBOA (pREBOA) incomplete AO is used balancing proximal hemodynamics and risk for further blood loss with the aim of prolonging the tolerable ischemic time. Several reports have shown promising results with this technique and with future development of more advanced catheters, variable and individually titrated AO is thought to form a new standard for REBOA management (134-137). Another technique used for reducing the total ischemic time to distal organs is intermittent REBOA (iREBOA), where restoration of aortic blood flow in a time- or blood pressure controlled manner is allowed. No human data exists supporting the use of iREBOA. However, a few animal studies has shown that after an initial inflation time of 15 minutes, continuing with iREBOA was associated with decreased levels of lactate and whole blood requirements to maintain MAP and decreased acidosis compared to continuous and complete AO (138, 139). These studies also suggested a more favourable metabolic and hemodynamic profile, using blood pressure limits to guide re-inflation of the balloon over a strict time-based protocol.

In austere environments, the use of REBOA with permissive distal flow strategies may be challenging. Titration of pREBOA is limited by the monitoring capabilities in these environments. Deflation of the balloon (total balloon volume) has also been shown to not correlate with the return of blood flow in the distal aorta. Rather, a “steep inflection point” occurs at an unpredictable degree of deflation that results in an abrupt decrease in proximal arterial pressure (140). This is in part understood as the combined effects of intravascular volume, aortic vessel-wall compliance and mechanistic properties of the balloon. Blood-flow

dynamics during REBOA is complex and poorly understood. The postulation that organ blood flow is principally following the aortic flow rate has been shown to be wrong. Instead, REBOA was shown to not only affect aortic branch vessel flow during intervention, but also up to 45 minutes after balloon deflation (141). In that study, the worst recovery of blood flow was observed in the renal arteries. This phenomenon is at least in part thought to result from IRI. Lastly, AO does not *completely* stop distal blood flow due to collateral circulation to a differing extent. Remaining blood flow both at the site of vascular injury and distal to the introducer sheath have been shown in human patients undergoing REBOA (142). In study IV, we therefore aimed to study the effects of an iREBOA protocol on IRI and organ injury of the heart and kidneys in correlation to overall hemodynamic variables and renal artery blood flow (RBF).

2.6 EXPERIMENTAL ANIMAL MODELS IN TRAUMA RESEARCH

Preclinical animal research, in the setting of trauma and hemorrhage shock, constitutes an irreplaceable source of evidence for development of new interventions and studies on complex physiological courses such as coagulation and shock (143). However, a general lack of uniformity regarding laboratory animals (144) and experimental designs hampers the translation of experimental results into clinical praxis (145). A useful animal model for trauma research should include the combination of early mechanical and subsequent innate physiologic reactions associated with tissue trauma, hypovolemia, regional or systemic hypoxia, inflammation and immune reactions to organ damage and shock (146). Swine is the most commonly used animal in preclinical, traumatic hemorrhage research (146). In terms of a, non-primate, *large animal model* the pig is reasonably similar to humans in vascular and visceral dimensions (147), physiological responses to tissue damage (148), cardiovascular dynamics and pharmacological therapy (149). Furthermore, pigs are cost-effective. Induction of hemorrhage shock is principally performed by removing blood from the animal through a controlled or uncontrolled extravasation (150). Furthermore, the simulated hemodynamic effects and depth of shock can be achieved by either a fixed targeted blood pressure, *pressure controlled*, (149, 151) or a fixed hemorrhage volume, *volume controlled mode*, (152-154). The infliction of tissue trauma to a hemorrhage research model can be performed by a single- or multiple insults to the organ of interest based on the aims in the specific study. In conjunction with human trauma populations, the large heterogeneity of clinical scenarios should be addressed specifically. Commonly used trauma models include blunt or penetrating injuries to

the head, chest, abdomen or extremity bone-fractures in combination with soft tissue damage (143). A systemic inflammatory response, mimicking a septic-reaction, can further be induced by injection of endotoxins (155, 156).

Coagulation effectivity and levels of parameters used for its evaluation differ between species (157, 158). Swine are known to be hypercoagulable compared to humans in response to traumatic hemorrhage (159). Nonetheless, porcine models are the most commonly used in TIC research due to the otherwise similar organ and hemodynamic physiology. Numerous studies have been performed trying to create a robust porcine model for TIC research. However, the large majority are disadvantaged by having only established coagulopathy by means of the traditional homeostatic derangement, induced by hemodilution and hypothermia in combination with hemorrhage shock. For ATC to ensue after trauma in humans, severe injury (injury severity score; ISS of 25 or greater) along with hemorrhage shock corresponding to a base deficit of 6 mmol/L is required (43). We therefore, in study V, aimed to establish a reproducible porcine model for future TIC research fulfilling the requirements of mechanistic and metabolic preconditions for TIC in humans, along with investigating a ROTEM profile for its detection in pigs.

In summary, no single generic animal model can address the large variety of pathophysiological courses arising from trauma and the weaknesses and strengths of the particular model must be assessed according to the problem addressed.

3 RESEARCH AIMS

The overall aim of this thesis was to provide physiological data on novel, mechanical hemostatic adjuncts to facilitate their implementation into clinical practice and to contribute to the important research area of trauma-induced coagulopathy.

The specific aims of the contributing studies in this thesis were as follows:

I. To investigate the physiologic consequences of an abdominal aortic tourniquet, in relation to application times.
II. To compare crystalloid fluid requirements during resuscitation with an abdominal aortic tourniquet versus infrarenal Resuscitative Endovascular Balloon Occlusion of the Aorta.
III. To investigate the feasibility and physiologic effects of transition from an abdominal aortic tourniquet to infra-renal Resuscitative Endovascular Balloon Occlusion of the Aorta.
IV. To study the hemodynamic effects and organ function of the heart and kidney of recurrent reperfusion intervals during 60-minute thoracic Resuscitative Endovascular Balloon Occlusion of the Aorta.
V. To investigate a porcine research model for studies in acute traumatic coagulopathy in relation to trauma modalities and detection by ROTEM.

4 MATERIALS AND METHODS

4.1 ETHICAL PERMITS

The studies in the present thesis was approved and conducted in accordance with the Swedish regional ethics approval board for animal research in Stockholm. Study I: ID S3-15, Study II: ID S3-15, Study III: ID S3-15, Study IV: ID S3-15 and 1470, Study V: ID S3-15 and 1470.

4.2 LABORATORY ANIMALS

Sexually immature female (n=3) and castrated male (n=57), Yorkshire-Landrace cross pigs (Sus Scrofa) of 6 months old were utilized in the experiments. Pigs were obtained from an accredited breeder which ensured healthy and physiologically uniform animals. Mean body weight of the animals were in study I: 60 kg, study II: 60 kg, study III: 57 kg, study IV: 60 kg and study V: 63 kg. These animals were utilized based on their human similarity in torso and abdominal organ dimensions. The animals were housed at least three days prior to the experiments in an accredited laboratory animal facility with an ambient room temperature at 21-22°C and 12-hour day/night cycles. Prior to the experiments animals were fasted for 12 hours with free access to tap water. No animal was excluded due to any abnormal behavior, obvious signs of sickness or injury or outlying base-line data. Randomization to study groups was conducted in all studies after completion of surgical preparation by the sealed envelope method.

4.3 ANESTHESIA AND EUTHANASIA

Animals were sedated with an intramuscular injection of 150 mg tiletamine/zolazepam (Zoletil 100 Vet) and 6 mg medetomidine (Domitor) and transported sleeping to the operating room. After recording of weight the animals were placed supine on a standard operating table and pre-oxygenated for three min with 100% O_2. Non-invasive blood pressure was measured in the hind leg and a tail probe was used for pulse-oximetry monitoring. General anaesthesia was induced through an auricular vein with pentobarbital, 6 mg/kg (Pentobarbitalnatrium vet., 60 mg/mL); atropine, 0.02 mg/kg (Atropin Mylan, 0.5 mg/mL); and fentanyl, 2.5 µg/kg (Fentanyl B. Braun, 50 µg/mL) and perioperatively maintained with a continuous i.v. infusion of

ketamine 25 mg/kg (Ketaminol vet. 100 mg/mL); midazolam 0.0485 mg/kg (Midazolam Hameln, 1 mg/mL) and fentanyl 3.5 µg/kg (Fentanyl B. Braun, 50 µg/mL) per hour. A standard size 8, cuffed endotracheal tube was inserted through a midline neck incision (study I-II) or orally, using a custom-made Miller-type, laryngoscope (study III-V). The animals were mechanically ventilated using a Hamilton C2 ventilator (Hamilton Medical, Geneva, Switzerland) to maintain a $PaCO_2$ between 4.7 -5.5 kPa. Standard ventilator settings were: inspiratory oxygen fraction (FiO_2), 21%; peak inspiratory pressure, 16-24 cm H_2O; peak expiratory end pressure, 4-10 cm H_2O and respiratory rate, 16-24 per minute. During preparation an infusion of 500 mL Ringer´s Acetate (RA) was administered to adjust for base-line variances in fluid balance. To compensate for insensible losses during experiments, an infusion of 3 mL/kg per hour of Ringer´s Acetate was administered after induction of hemorrhage shock. At completion of the experiments, animals were euthanized with 35-70 ml pentobarbital sodium (Alfatal vet. 100 mg/ml). Criteria of death was $etCO_2 < 2$ kPa, MAP < 20 mm Hg and asystole on EKG.

4.4 SURGICAL PREPARATION AND INSTRUMENTATION

A 7.5F Swan-Ganz pulmonary artery catheter (Edward Lifescience, Irvine, CA) was introduced through the surgically exposed right external jugular vein and used for core temperature, central venous pressure (CVP), cardiac output (CO) and mixed venous oxygen saturation (SvO_2). In study I-II, the left carotid artery was exposed via the midline neck incision and used for blood pressure (BP) monitoring, blood sampling and blood withdrawal. In study III-V, a standard arterial catheter was placed in either brachial artery under ultrasound guidance (SonoTouch 20, Chison Medical Imaging CO., China) and used for BP monitoring and blood sampling. In study II-V, either femoral artery was percutaneously cannulated with a (II-IV) 7F introducer sheath (Merit Medical Systems Inc., UT) or a (V) 13.5F, 15 cm silicone dialysis catheter (Medical Components Inc., Harleysville, PA) and used for blood withdrawal and REBOA insertion. A suprapubic urine catheter and three intracutaneous EKG electrodes were placed in all animals. In study III-IV, the contralateral femoral artery was surgically exposed for later transection. In study IV the left renal artery and vein was surgically exposed via laparotomy. A perivascular ultrasound flow probe (Transonic Systems Inc., NY, USA) was placed on the renal artery and a standard 1.5 mm i.v. catheter was inserted in the renal vein. The abdomen was temporarily closed with staples during the experiments. In study I-II, the AAJT was positioned and left unbuckled.

4.5 INDUCTION OF HEMORRHAGE SHOCK

Total blood volume (TBV) was calculated by (I-IV) 67 mL/kg and (V) 65 mL/kg. Hemorrhage was completed (study I-II, V) or initiated (study IV) by blood withdrawal through the carotid (I-II) or femoral (IV-V) artery catheter using a peristaltic pump (Masterflex L/S, Cole Parmer) until the target blood loss or collection to citrated bags for later autologous transfusion was achieved. In study III and IV a free bleeding of the total or remaining blood loss was used via transection of either femoral artery. Hemorrhage volume was measured by weight of the collection container, citrate bags and gauzes. Citrate bags used for autologous transfusion were stored at 38°C. No splenectomy was performed in the experiments.

Study I-II: A two-phased, pressure controlled bleeding was used to induce a class II hemorrhage shock. Initially, 12.5% of TBV was drawn for 7 minutes. After a stabilization period of 10-15 min the bleeding was continued at halved rate to a MAP < 40 mm Hg.

Study III: A free bleeding of 40% of estimated TBV was used via transection of the femoral artery. Extravasated blood was collected by suction to a container and collected in gauzes. The bleeding was periodically paused by manual pressure while total blood loss was calculated. After completion of hemorrhage the bleeding was paused and the animals left for 15 min to induce hemorrhage shock.

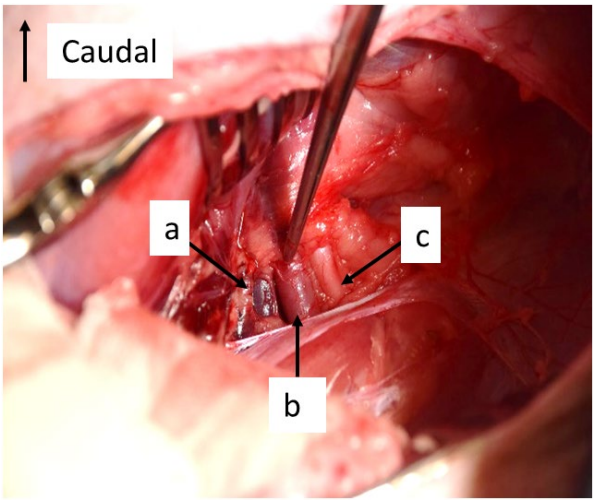


Figure 12. *A detail picture of the left femoral sheath with a: vein; b: artery and c: nerve. Notably, this exposure of the injury caused an uncontrolled external hemorrhage. The rate of blood-loss may vary considerable with remaining overlying tissue and formation of a hematoma exerting counter-pressure to the vessel.*

Study IV: A hybrid hemorrhage protocol of a 40 % estimated TBV was utilized to produce a class IV hemorrhage with severe hypotension. Hemorrhage was initiated by blood withdrawal to citrate bags (2 x 450 mL at 100 mL/min) followed by transection of the femoral artery. The

free bleeding was periodically paused by manual pressure of the femoral artery while total blood loss was calculated. When the targeted hemorrhage volume was achieved, the femoral artery was temporarily clamped during a period of 15 min to induce shock. The femoral artery was left unclamped during REBOA in order to investigate occurrence of rebleeding during the balloon deflation intervals. The hemorrhage protocol was found to be 100 % lethal without resuscitation in pilot studies. At 60 min, the transected femoral artery was clamped using a standard hemostatic forceps.

Study V: Animals were subjected to a controlled hemorrhage; in *positive controls* of a 60% of TBV and in the groups *pulmonary contusion* and combined *hemorrhage*/*polytrauma* of a 25% of TBV respectively.

4.6 TRAUMA MODALITIES (V)

4.6.1 Pulmonary contusion

The animals were subjected to pulmonary contusion by blunt trauma using a 59 g, 65 (diameter) x 55 mm polyethylene projectile with a mean velocity of 82.3 m/s from a firing distance of 3.3 m. The projectile was fired using a custom made, compressed air gun, producing a 300-joule energy burst to the thorax at a fixed point (5 cm caudally and 2 cm ventral to the tip of the right scapula, 22-24 cm dorsally to the xiphoid process with the right front leg maximally abducted). Pulmonary contusion was confirmed by B lines and C lines by chest ultrasound and haemoptysis in the endotracheal tube. Additionally, post mortem calculations of lung injury volume and skin lesion was conducted for comparison of consistency.

4.6.2 Blast polytrauma

The animals were subjected to separate blast injuries at two sites. First, a blast injury to the groin was performed were the point of impact was above the midlevel of the femur. Second, a blast injury to the abdomen was performed with the point of impact one cm medially of the rib cage at level I6-I7 dx corresponding to a level above the liver. Swedish military grade plastic explosive (M/46) was used for the blast injuries. M/46 consists of 86% penthyl (pentaerytritoltetranitrat, $C(CH_2ONO_2)_4$) and 14% mineral oil and has a detonation speed of 8400 m/s. One g M/46 was taped to a non-electric initiation system (2 g capsule and 9 meter

of cord snapline SL-0), connected to a Nonel Dynostart ignition box (Dyno Nobel). The explosive charge was taped to the skin and covered by ceramic plates from a military grade body armour, and blankets. The animals were covered with flexible freezer packs to generate hypothermia, and the ventilator was adjusted for hypoventilation by decreasing the respiratory rate to 5-10 per min and decreasing pressure support, so that pCO_2 would increase to >10 kPa within the 120 min observation period to establish respiratory acidosis. Lipo-polysaccharide (LPS) infusion was started (1 μg/kg/h) to induce a systemic inflammatory response.

4.7 RESUSCITATION

4.7.1 AAJT

After induction of hemorrhage shock the AAJT was tightened around the abdomen and fully inflated (300 mm Hg). Total occlusion of the aortic blood flow was verified by loss of pulse-wave and non-invasive blood pressure distal to the device. Application times of the AAJT did not succeed one minute in all animals. The AAJT was fully deflated at 240 min (I-II) or 60 min (III) respectively, with no attempt to a stepwise reperfusion with the purpose of maximizing the chance of detection of deleterious effects. No devices malfunctioned during the experiments.

4.7.2 REBOA

Study II: The REBOA balloon catheter (6F, 15mm diameter/50 cm shaft, REBOA Medical, Båstad, Norway) was inserted to the infra-renal (zone III) aorta via the prepositioned introducer, to a distance mark based on anatomical landmarks after completion of hemorrhage. The catheter-shaft was firmly fixed to the introducer with surgical tape to avoid balloon migration. The balloon was inflated with 8 mL normal saline (NS, NaCl, 0.9%) and left for 240 min. A zone III balloon position was confirmed in post mortem laparotomy. The balloons were promptly deflated after 240 min and left in situ for the remainder of the experiments.

Study III: After 30 minutes AAJT intervention a transition to zone III REBOA was performed in 6 animals. In these animals the REBOA catheter was advanced through the introducer until a resistance was felt corresponding to the AAJT pressure on the aorta. At that time, the AAJT was deflated simultaneously with partial inflation (4-6 mL, NS) of the REBOA balloon. The

REBOA catheter was carefully retracted until a distinct stop representing the level of aortic bifurcation. The balloon was re-advanced 2-3 cm to avoid iliac positioning and further inflated to a total of 8 mL NS for 30 min. After the 30 min REBOA intervention, the balloons were promptly deflated and left in situ for the remainder of the experiments.

Study IV: The REBOA catheter was introduced to a supra-celiac position based on topical measurement in a standardized procedure, and left in position after completion of hemorrhage. After post-hemorrhage data sampling, the balloon was inflated (8 mL, NS) under hemodynamic monitoring. Total aortic occlusion was verified by a combination of steep inflections in proximal BP, hemostasis of the free bleeding and loss of distal BP and pulse-oximetry. In this study one group (n = 6) was subjected to 60 min continuous full REBOA (cREBOA) and one group (n = 6) underwent a full balloon deflation of 180 s every 10 min for 60 min (5 deflations). The balloons were deflated promptly (typically 30 s) to ensure a uniform physiological response in the animals. During the intermittent reperfusions the balloon was re-inflated (rescue occlusion) if SBP decreased < 80 mm Hg.

4.7.3 Fluid Resuscitation

The intravenous fluid resuscitation consisted of RA and/or autologous whole blood. We used a protocol for crystalloid fluid infusion in accordance with current TCCC battlefield resuscitation guidelines (160).

Study I-II: Bolus infusions of 250 mL RA as needed, was given if MAP decreased < 60 mm Hg in all study groups. At the end of the 60 and 240 min interventions times autologous whole blood (450 mL) was transfused and additional RA until MAP approximated baseline. Rapid infusion of 2000 mL RA was continued throughout the reperfusion phase.

Study III: After inflation of the AAJT rapid infusion of RA was started until MAP reached ≥ 60 mm Hg, after which no further additional resuscitation fluids were administered.

Study IV: Simultaneously with inflation of the REBOA one bag of whole blood (450 mL) was administered. The animals were transfused with one additional bag of whole blood (450 mL) immediately prior to final deflation of the balloon and rapid infusion of 2000 mL RA were administered during the critical care phase. No resuscitation fluids were administered during the induction of hemorrhage shock or REBOA intervention.

Study V: Bolus infusions of 100 mL RA were given if MAP was less than 35 mm Hg.

4.7.4 Vasoactive support

Intravenous infusion of norepinephrine at 0.1µg/kg/min was used in the experiments. Infusion times were automatically recorded using a syringe infusion pump (B Braun, Hessen, Germany) and data were collected post-hoc. In study I-II and IV, infusion was started during the critical care phase after AAJT/REBOA deflation when MAP decreased < 60 mm Hg and stopped at MAP > 90 mm Hg. In study V, infusion was started if MAP decreased < 25 mm Hg during the whole experiment.

4.8 ASSAYS

4.8.1 Computer tomography (I)

Two animals from each AAJT group in study I underwent a standardized, contrast enhanced, CT scan of the thorax and abdomen before, and after 45 and 225 min AAJT application respectively. Image diagnostics were performed by a specialist radiologist using a questionnaire and an overall interpretation. The analyses consisted of blood flow patterns in major abdominal vessels and organs, anatomical description of organ affection and urinary outflow.

4.8.2 Microscopic diagnostics (I)

Tissue samples were collected from the aorta (between the bifurcation and most distal renal artery) intestine, kidney, liver, heart and the adductor muscle on hind leg post mortem. The specimens were fixed in formalin and stained with haematoxylin and eosin, blinded and analysed by a clinical pathologist.

4.8.3 Hemodynamics

Hemodynamic variables (HR, SBP, MAP, CO, CVP and RBF) was continuously monitored. RBF were expressed as relative changes from baseline values to adjust for individual

differences. EKG was monitored continuously and recorded hourly or at appearance of noteworthy changes in morphology or rhythm.

4.8.4 Blood chemistry

Arterial and renal vein (study IV) blood was collected at baseline, and at every 10-15 min during the experiments. pH, base excess, lactate, potassium, haemoglobin (Hb), hematocrit (hct), PaO_2, $PO_{2\ (renal\ vein)}$, PCO_2, $PCO_{2\ (renal\ vein)}$, glucose, ionized calcium and oxygen saturation (SaO_2, $SO_{2(renal\ vein)}$) were analysed.

4.8.5 Urine output

Urine output was collected continuously via the suprapubic catheter to a container and registered every hour by means of a custom made electronic weight scale.

4.8.6 Organ blood markers

Samples for troponin I and myoglobin were collected at baseline and post intervention and critical care phases and analyses were performed by the Karolinska University Hospital laboratory, Department of Clinical Chemistry, accredited by Swedac, Sweden's national accreditation body. Samples for creatinine (8L24, IDMS traceable enzymatic reagent) were collected at baseline and post intervention and critical care phases and were analyzed on a BS380 instrument (Mindray, Shenzhen, China) using reagents from Abbott Laboratories, (Abbott Park, IL, US). The total coefficient of variation for the creatinine method was 1.5% at 87 μmol/L.

4.8.7 Common femoral artery dimensions (III)

In study III, the diameter of the common femoral artery was measured in the animals not randomized to transition between interventions using a 7.5 MHz linear transducer (Sono Touch 20; Chison Medical Imaging CO Ltd, China) at baseline, hemorrhage completion and after 30 min AAJT application. The measurements were conducted perpendicular to the long axis

immediately distal to the inguinal crease corresponding to the site of REBOA catheter introducer.

4.8.8 Coagulation tests (V)

Tests for PT(INR) (photometry), aPTT (photometry), platelets (impedance and flow cytometry), fibrinogen (photometry), prothrombin (turbidimetry), aPC (enzymatic activity, photometry), D-dimer (turbidimetry) and ROTEM (INTEM, EXTEM, APTEM and FIBTEM) were performed by the Karolinska University Hospital laboratory, Department of Clinical Chemistry, accredited by Swedac, Sweden's national accreditation body. ROTEM was performed by the laboratory using a ROTEM Delta (Werfen), according to the manufacturer's instructions, and quantified for clot formation time, alpha angle, clotting time and maximum lysis time. Enzyme-linked immunosorbent assays (ELISA) were performed to quantify tPA (Innovative Research, Inc. Catalog No. IPTPAKT, lot No. 915) and PAI-1 (Innovative Research, Inc. cat. No. IPOPAIKT, lot No. 317), according to the manufacturer's instructions.

4.9 STATISTICAL ANALYSES AND CALCULATIONS

Statistical analyses were made using GraphPad Prism version 7.0.3-8.4.3 (GraphPad Software Inc., La Jolla, Ca) for windows. A $p < 0.05$ was considered significant. The following calculations were used for hemodynamic parameters: Stroke volume (SV) = CO/HR; Systemic vascular resistance (SVR) = 80 x (MAP-CVP)/CO.

I. For myoglobin, two-way analysis of variance with Sidak´s multiple comparisons test was used. For norepinephrine Student´s unpaired *t* test was used. For Ringer´s Acetate and hemorrhage, two-way analyses of variance with Tukey´s multiple comparisons test was used. The primary outcome was survival at AAJT release. Secondary outcomes were fluid requirements to maintain MAP and respiratory and hemodynamic variables associated with AAJT application. Error bars in figures represent SEM.

II. Two-way ANOVA with Sidak's multiple comparisons test was used for hemodynamic and metabolic parameters. Student's unpaired t-test was used for norepinephrine. Two-way ANOVA with Tukey's multiple comparisons test was used for iv fluids and hemorrhage volume. Primary outcome was cumulative

crystalloid fluid requirements to maintain MAP > 60 mm Hg. Secondary outcomes were hemodynamic and metabolic parameters. A power calculation for a continuous outcome superiority trial was performed and 12 animals were required to have an 80% chance of detecting a 2500 ml difference in the primary outcome ($\alpha = 0.05$). Standard deviations were calculated from pilot studies. For five animals in the AAJT group, a secondary analysis of previously published data was performed (160) and one animal was added according to the power calculation. All data are expressed as the mean ± SD.

III. A two-sample calculation was used for power analysis: ($n \geq (Z\alpha/2 + Z\beta)2 \times 2 \times Sp^2/C^2$). Assuming an effect size of 20 mm Hg (least clinically significant difference) and an $\alpha = 0.05$, a sample size of 6 animals/group were utilized to reach 80 % power. Pooled standard deviation was calculated from previously published data and pilot studies. Comparisons of hemodynamic and metabolic variables between groups were performed using repeated measures, two-way ANOVA. Sidak´s multiple comparisons test was applied for post hoc corrections. For SBP and MAP variability an unpaired, two-tailed t-test was used. For body weight, hemorrhage volume and IV fluids independent t-tests were used. Vascular diameter changes were reported as simple means.

Primary outcomes were arterial blood pressure variability and hypotension duration. Secondary outcomes of interest were cardiac output, systemic vascular resistance, pH, lactate, base excess, visible re-bleeding from the injury and common femoral artery lumen diameter.

IV. Comparisons of hemodynamic variables, urine output and metabolic changes were performed using repeated measures, two-way ANOVA. Sidak´s multiple comparisons test was applied for post hoc corrections. For hemorrhage volume, nor-epinephrine infusion time, total ischemic time and organ specific blood markers unpaired t-tests were used. We assumed an effect size of 0,67 (difference in survival between groups with death occurring during intervention or reperfusion phases) based on published data and our groups pilot studies on the present iREBOA protocol. An a priori sample size calculation using a two-tailed t-test family with an $\alpha = 0.05$, yielded a sample size of 12 animals to reach 80 % power (G*Power, version 3.1.9.4). Primary outcome of interest was mortality. Secondary outcomes

of interest were total ischemic time, renal blood flow (% of BL), urine output, hemodynamic- and metabolic changes. Error bars in figures represent SEM.

V. For temporal data sets, a mixed effects model with the Geisser-Greenhouse correction was used, comparing all groups to negative controls. For hemorrhage, Ringer´s Acetate and all coagulation tests an unpaired t-test was used, comparing all groups to negative controls at the corresponding time (0 min, 20 min, 120 min). Statistical analyses of ROTEM were performed using the R project for statistical computing (the R foundation) v.3.4.2. ROTEM-data were first analysed using a multi-variate ANOVA and visualized using principal component analysis (PCA), followed by an analysis of individual variables with Kruskal-Wallis test using Mann-Whitney U-test with a Benjamini-Hochberg correction for post-hoc evaluation. $p<0.05$ was considered statistically significant. Heteroscedasticity in ROTEM was evaluated by Bartlett's test for unequal variance between groups. The ROTEM data for CT, CFT, and alpha were found to be significantly heteroscedastic, unlike the data for MCF and alpha. The study assessed TIC by multiple coagulation tests. For calculation of power of the study, PT(INR) was chosen for a post-hoc power analysis for continuous endpoint, two independent sample study, and resulted in 81.5% power (group 1, negative control at 120 minutes: mean 1.00, SD 0.0707, group 2, hemodilution at 120 minutes: mean 1.35, SD 0.2646, alpha 0.05). Error bars represent SD.

4.10 ETHICAL CONSIDERATIONS

Trauma research is inherently afflicted by difficulties with the conduction of prospective and randomized human trials. Heterogeneities within clinical practice, demographics, predominant mechanisms of injury, collection of reliable data and the possibility and appropriateness to obtain informed consent are examples of limitations to this issue. Hence, there is a need for alternative strategies to conduct scientific research in order to improve the outcomes after major trauma. Laboratory animal studies can replace human studies and provide valuable knowledge in different aspects of medical research. For example, pre-clinical trials of novel pharmacological therapies and experimental surgical procedures are commonly performed on animals. Within the present research project, the use of laboratory animals is fundamental. The main objective with our study series is to significantly contribute to the development and introduction of novel treatment strategies for lethal, noncompressible hemorrhage. In order to

study the efficacy and safety of these interventions, a reproducible model with sufficient anatomical and physiological similarities with humans are required. Therefore, pigs of approximately 60 kg weight are utilized in our experiments. The complexity of the physiologic courses associated with the interventions and the need to study their effects on live tissue necessitates the use of living animals.

Our research group is actively and continuously working with the ethical aspects of the utilization of laboratory animals in our studies. All studies are approved and conducted in accordance with the legislation and board of animal research ethics. The experiments cannot be replaced by human experiments, computer animations or other methods. However, in order to refine and reduce the number of experiments within the project a series of actions are undertaken:

- Maximize the ability to publish results by development of well-defined hypotheses and research questions for each experiment including statistical calculations (when possible) to generate the minimal sample sizes required to reach statistical power (based on previously collected or published data).
- Maximize the utilization of each animal including post mortem training and collaboration with other groups.
- Minimize model development and pilot experiments by utilization of previously published and standardized procedures as far as possible.
- Utilization of highest proficiency and procedural skills to minimize losses due to poor practical conduction of experiments.
- Active dialogue with veterinary to ensure pre-trial animal welfare and that every single procedure is explicitly stated, understood and approved by the board of animal research ethics.
- Extensive and standardised collection of physiological parameters and experimental data for future needs (including baseline parameters, anaesthetic and pharmacological implications even if not relevant to the actual experiment).
- Ensure high quality data by abort experiments or exclude data if animals show signs of abnormalities.
- Continuously refine anaesthetic and experimental procedures to ensure high-quality data.

A second important aspect of ethical consideration within the present research project entails the protection of human rights during increased vulnerability in combat and disaster environments and the conduct of research during these circumstances. A significant amount of publications based on data collected in conflict or disaster environments have been used in the literature review for the present research project. Research in combat and disaster environments is governed by the same fundamental principles as in civilian biomedical research. Possible ethical issues that arises from this includes the utilization of data collected from trauma victims, survivors and demised, without information or any given informed consent to participate in research projects. Additionally, the decisional capacity may be affected by cultural or language barriers. To ensure that no part of the present research project contains unethical or questionable material, all references are carefully vetted for methods of data collection and if possible ethical aspects stated by the authors. Finally, references including laboratory animal research is carefully analysed for the generalisability according to methods and results and ethical approval by the local certification board.

5 RESULTS

5.1 INDUCTION OF HEMORRHAGE SHOCK

The protocols resulted in reproducible 25 – 60% of total blood volume hemorrhage. The hemodynamic changes during hemorrhage was a reduction in arterial pressures ranging between 50-80%. During the 10-15 min stabilization period after hemorrhage completion, MAP increased 10-15 mm Hg as a response to compensatory mechanisms. Splenic auto transfusion is a known phenomenon in swine models of hemorrhage shock and splenectomy is commonly used to reduce this compensatory effect. However, this procedure inherently causes an additional trauma from the laparotomy and also risks confounding of the results due to occult, ongoing bleeding from the surgical procedure. Furthermore, splenectomy have been shown to be avoidable in swine hemorrhage models utilizing pressure based protocols (161). We therefore did not perform this procedure.

In study III-IV, a free bleeding by transection of the femoral artery was utilized to simulate a realistic injury with a natural bleeding rate, and to allow for quantification of rebleeding during transition between aortic occlusion interventions and balloon deflation during REBOA. In study III, three animals died during the induction of hemorrhage shock. As compared to study IV, no previous controlled hemorrhage was conducted (in order to collect blood for later autologous transfusion) and it is likely that the increased rate of blood loss (average bleeding time 19 min) caused the circulatory collapse. One animal was included in study III after circulatory collapse with ventricular fibrillation following completion of hemorrhage, and return of spontaneous circulation within 30 seconds of chest compressions during inflation of the AAJT.

In study V, mean hemorrhage per kg was 15.9 mL (range 13.2-16.8) in pulmonary contusion with hemorrhage, 39.3 mL (range 29.1-40.2) in positive controls and 16.0 mL (range 0-17.5) in polytrauma groups respectively.

5.2 RESUSCITATION

5.2.1 Survival

The overall survival rate in the experiments were 93% (56/60 animals). In study III, 3 animals died during induction of hemorrhage shock and therefore mortality was related to the hemorrhage protocol and the experiments were reiterated. In study IV, one animal died in the cREBOA group after reperfusion. No statistically significant inferior outcomes in mortality were therefore shown with 240 compared to 60 min AAJT intervention or 60 min continuous zone I REBOA over intermittent reperfusion intervals. Hence, no survival benefit was proven according to the power calculations.

5.2.2 Hemostasis

Hemostasis was effective in all animals resuscitated with the AAJT and REBOA. CT examinations of blood flow in study I showed a total infrarenal occlusion of blood flow in the aorta and inferior vena cava and during intervention the pulse-oximetry and distal non-invasive blood pressure measurements were unmeasurable. The exposed and transected femoral artery in study III-IV showed no occurrence of quantifiable bleeding either during AAJT or REBOA intervention. Importantly, the transition procedure between AAJT and REBOA, even though conducted to minimize aortic overflow, and intermittent reperfusion intervals in study IV did not result in any rebleeding from the artery. These findings were unexpected and may not be applicable to humans.

5.2.3 Hemodynamic effects

In addition to hemostasis the aortic occlusion devices had a significant impact on the proximal arterial pressures. The principal mechanism for the improved blood pressures was a drastic increase in SVR and thereby cardiac afterload. This effect was observed to be greater in the animals resuscitated with the AAJT and zone I REBOA over zone III REBOA.

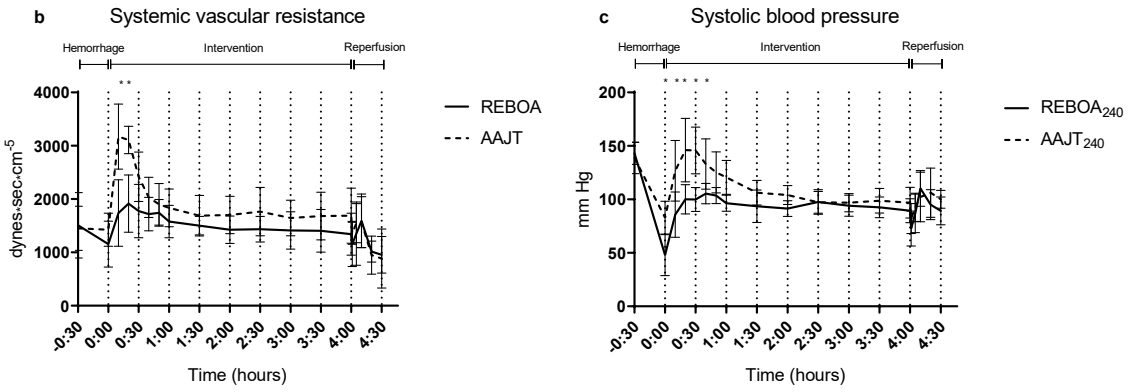


Figure 13. SVR was increased by the AAJT as compared to zone III REBOA (b) with associated increased systolic blood pressure (c) 40 min after application ($^{*}p<0.05$).

After AAJT application the aorta was occluded distal to the renal arteries, however, the significant compression of abdominal tissues cranially is likely to also have affected the abdominal circulation which impacted the SVR.

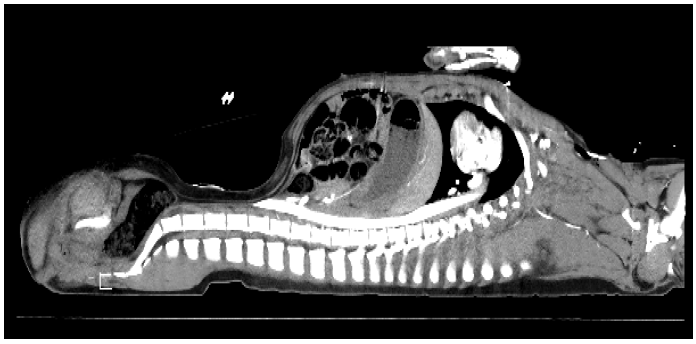


Figure 14. CT scan of a 60 kg pig with inflated AAJT. Note the contrast filled aorta with seized flow below the device and the cranially forced abdominal organs with the high standing diaphragm.

After zone I REBOA, the aortic pulse reflection is almost immediate and thus affects both cardiac output and coronary perfusion in diastole and systole. This is reflected by the steep, and supra physiologic increase in arterial pressure. Resuscitation with the AAJT and zone I REBOA caused a significantly increased heart rate to uphold cardiac output compared to zone III REBOA. A reduced venous cardiac return in combination with the substantial afterload reasons the tachycardia. Consequently, a compensation for contracted stroke volume to sustain cardiac output. Suggestively, stroke volume was also found to be significantly higher in the fluid resuscitation group (I) and during balloon deflations in the iREBOA animals (IV).

The transition procedure in study III caused a significant decrease in arterial pressure (mean 25 mm HG) during simultaneous deflation of the AAJT and inflation of the REBOA balloon. After transition to zone III REBOA these animals were hypotensive compared to the continuous

AAJT application. Our data suggested that the procedure can be safely performed with hemodynamic support such as a fluid bolus prior to deflation of the AAJT and vasopressor infusion during further zone III REBOA.

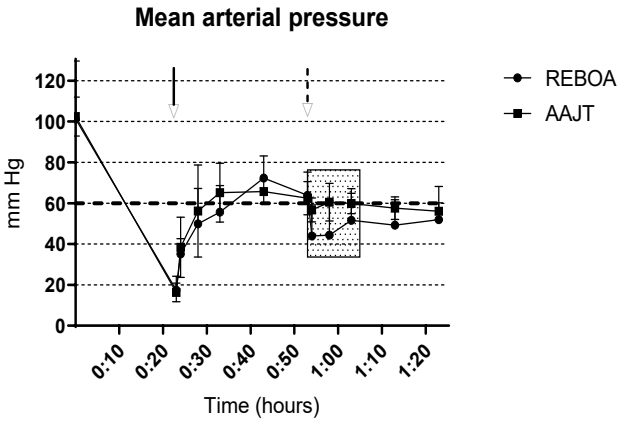


Figure 15. MAP was significantly decreased following the transition procedure after 30 min intervention (dotted arrow) between AAJT and zone III REBOA (grey area).

We showed that renal blood flow during intermittent REBOA was significantly higher during reperfusion intervals and after 60 min critical care. The renal aortic branch vessel flow during reperfusions corresponded to a decrease in proximal systolic blood pressure and increased blood flow distal to the balloon, however it was only restored to 27.5% of baseline values during the first 3 min reperfusion interval where after it decreased gradually to 13.5%. These results underline the complexity of the intrinsic mechanisms behind regulation of end organ blood flow. We did not perform measurements of blood flow in the carotid artery that could have revealed one possible explanation for the scarce return of RBF during balloon deflations. It is likely that preservence of blood flow to the brain in combination with endothelial hypoxia with vasoconstriction in the renal arteries have impacted the vaso-regulatory mechanisms and inferior blood flow in the renal arteries during full return of aortic blood flow. The interruption of worsening renal ischemia during iREBOA may therefore explain that the restoration of RBF after critical care reached 61% (iREBOA) compared to 39% (cREBOA).

5.2.4 Fluid requirements

In study I, we compared the crystalloid fluid requirements between animals resuscitated with the AAJT and a control group receiving crystalloids only. We found that the AAJT animals required a 64% smaller amount of fluids than the control group to keep a mean arterial pressure > 60 mm Hg. This was comparable to 3000 mL of crystalloid fluids. In a hypovolemic patient

with manifest or impending hemorrhage shock, such amounts of crystalloids only can be harmful. Considering that zone III REBOA and the AAJT are comparable regarding indications, we showed that the interventions are not equivalent as regards to fluid resuscitation requirements. In study two, the mean difference of crystalloid fluids to uphold a MAP > 60 mm Hg were 2079 mL (95% CI 627-3530 mL) and importantly, four animals of six in the AAJT group sustained a MAP > 60 mm Hg with no additional crystalloids.

After the uncontrolled bleeding with severe hypotension in study III, the animals required a mean total of 1600 mL RA in addition to inflation of the AAJT to restore MAP. This finding suggest that, in clinical scenarios with vessel injuries leading to rapid exsanguination, such as iliac and femoral artery injuries, even AAJT application solely may be insufficient in restoring critical proximal blood pressure.

In study IV, the animals were transfused with shed blood (450 mL) in parallel with inflation of zone I REBOA simulating a clinical scenario with access to whole blood. This amount was not sufficient to prevent severe hypotension episodes, necessitating rescue occlusions, during the intermittent reperfusion intervals. The total proportion of successful three-minute reperfusions were 63%. We suggest that intermittent reperfusion during zone I REBOA therefore only should be performed with invasive blood pressure monitoring capabilities. Furthermore, the decreasing rate of successful balloon deflations (83% to 33%) during the 60-minute iREBOA protocol also implies that this is a volume dependent method to avoid hemodynamic instability.

The amount of RA administered in the animals in study V was calculated by mean totals of mL/kg to compensate for differences in estimated total blood volume. The positive controls (hemorrhage and hemodilution) received a mean 103 mL/kg (range 92.3-117.5 mL/kg) to keep the acceptable MAP. The replacement of volume with crystalloid fluids only after a severe (60% of TBV) blood loss successfully established hemodilution coagulopathy, as shown by the coagulation tests.

5.3 ORGAN EFFECTS

5.3.1 Pulmonary

The mechanical intra-abdominal pressure towards the diaphragm from the AAJT caused a significant decrease in pulmonary compliance and tidal volumes (TV). Ventilator settings were adjusted accordingly to maintain oxygenation (by FiO_2 21%). REBOA did not cause any changes in pulmonary function demanding any changes in ventilator settings.

5.3.2 Heart

The hemodynamic changes associated with aortic occlusion caused a position dependent myocardial load. We showed a significant increase in troponin after continuous zone I aortic occlusion (666 ng/L) compared to intermittent balloon deflations (187 ng/L) in study IV. Furthermore, troponin increased during REBOA in both groups compared to baseline values and decreased after 60 min critical care with 32% (iREBOA) and 47% (cREBOA) respectively. These findings were in line with previous studies were pharmacological interventions on, cardiac selective, β–adrenergic receptors may have a therapeutic effect. Hence, the potential risk for myocardial infarction associated with thoracic aortic occlusion should be considered in patients during- and post intervention. We did not observe any EKG changes associated with type-2 myocardial injury during the experiments.

5.3.3 Liver and intestine

Microscopic signs of ischemic injuries were detected by focal necrosis around the central hepatic vein and small bowel necrosis after 240 min AAJT application. Obvious signs of ischemic injury were also detected in the small intestine on post mortem gross inspection.

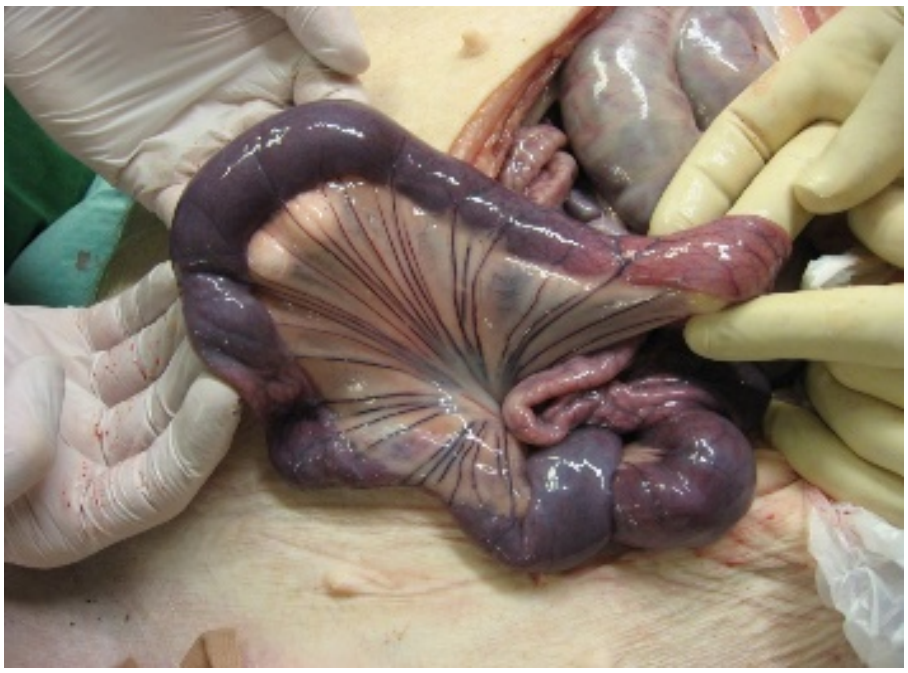


Figure 16. Post mortem laparotomy revealed ischemic bowel injury after 240 min AAJT application which was not observed after 60 min. Histologic examination confirmed bowel necrosis.

5.3.4 Kidney

No microscopic signs of kidney injury were detected after AAJT intervention. CT examinations of the AAJT animals showed bilateral contrast charging in the kidneys, with hydro nephrosis and hydro ureters developing during prolonged application, suggesting remaining renal perfusion, urine production and supra vesical obstruction. Prolonged AAJT application was associated with significantly increased myoglobin after reperfusion demonstrating rhabdomyolysis. Recognizing that kidney injury, and subsequent renal failure, can develop for up to seven days after trauma and exposure to toxic metabolites, the absence of acute tubular necrosis in microscopy-specimens should be carefully interpreted. Intermittent balloon deflations during zone I REBOA had a beneficial impact on urine output and restoration of renal blood flow implicating that mitigation of 60 min ischemia was beneficial, while the renal blood flow response did not correspond to restored aortic influx. P-Creatinine showed a two-fold increase after 60 min cREBOA compared to a half in iREBOA. Although this difference between groups did not reach statistical significance. After 60 min critical care, creatinine clearance was 27% in both study groups.

5.3.5 Homeostasis

Decreased hematocrit (hct) and hypothermia were associated with cumulative crystalloid infusion >2000 mL (I-II, V). In contrast, AAJT application was associated with an increase in hematocrit and core temperature. The significant differences in hct is likely explained by the proximal pooling of blood volume after AAJT application (I-II) which caused an extravascular shift of plasma in combination with hemodilution in the animals receiving larger amounts of crystalloids. After class II hemorrhage (I-II), pH remained within physiologic range during zone III aortic occlusion. Lactate increased (3-7 mmol/L) with a corresponding drop in base excess (+5-0) after hemorrhage and further during aortic occlusion with no differences between devices. Zone I aortic occlusion was associated with increased lactate and lower pH than zone III interventions. Interestingly, lactate levels were equal between proximal arterial- and renal vein blood. This implies 1) a multi organ ischemia and 2) a venous communication allowing for open distribution along the concentration gradient. In study V, the animals subjected to trauma with hemorrhage and severe hemorrhage only (positive controls) showed a significant increased lactate and lower pH. Arterial oxygenation was within physiologic range in all animals with standard ventilatory settings in the experiments during the intervention phases. Hypoxia were seen in the animals subjected to polytrauma and respiratory acidosis due to the limited ventilation.

5.3.6 Common femoral artery dimensions

In study three we assessed the feasibility of transition from AAJT to zone III REBOA. This procedure may be an alternative for continued care of patients resuscitated with the AAJT in the prehospital environment, by enabling surgery with maintained bleeding control and hemodynamic stability. A challenging step for REBOA insertion is vascular access to the femoral artery. We showed that femoral artery dimensions decreased with a mean 52% after hemorrhage and AAJT application and further that the pulsations (helpful for identifying the artery) disappeared with AAJT application. Surgical capability of femoral artery access may therefore be important during this procedure.

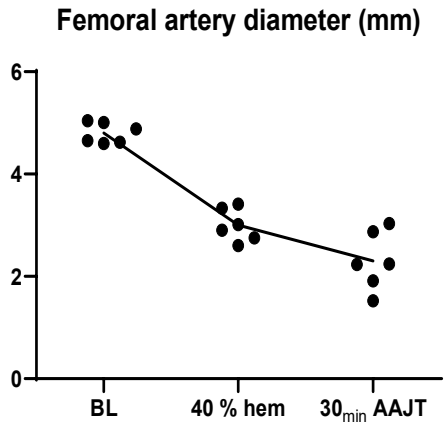


Figure 17. The major decrease in femoral artery dimensions occurred after the class IV hemorrhage but was further aggregated following aortic occlusion by the AAJT.

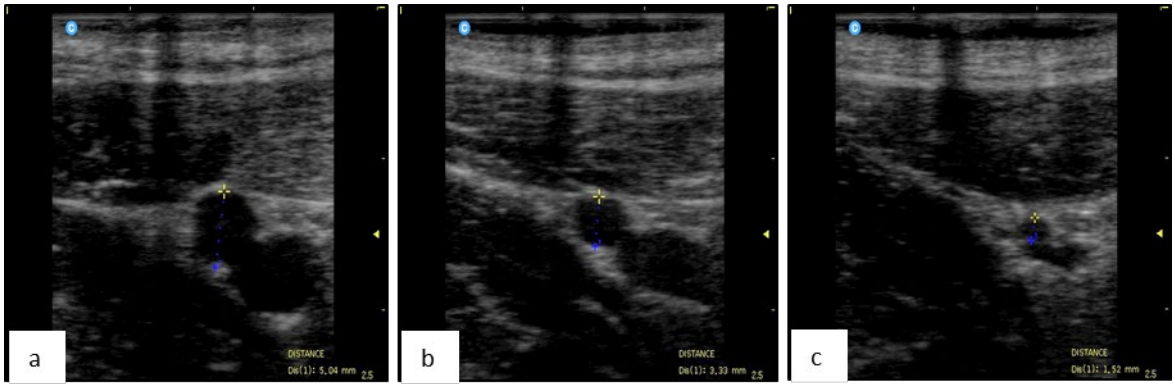


Figure 18. Preconditions for percutaneous arterial access (ultrasound) in a 60 kg pig at (a) BL: 5.04 mm, SBP 110 mm Hg; (b) 40 % blood-loss:3.33 mm, SBP 15 mm Hg; (c) 30 min AAJT: 1.52 mm, SBP 54 mm Hg. Measurements indicated by the dotted blue line between the yellow and blue marks.

5.4 REPERFUSION INJURY

We quantified reperfusion injury by metabolic acidosis, vasopressor requirements and in study IV measurements of renal vein PCO_2. Reperfusion after aortic occlusion was associated with considerable physiologic effects. All animals showed a sharp decrease in pH and lactate levels increased to between 8-12 mmol/L. Noteworthy, the lactate levels were not increased after prolonged AAJT intervention and peak levels were similar during 60 versus 240 min intervention. Infra renal aortic occlusion was associated with milder reperfusion injury which was expected, however this effect was observed even when comparing 240 min of zone III occlusion with 60 min zone I. Intermittent reperfusion during zone I REBOA resulted in decreased ischemic time and milder reperfusion injury as reflected by all variables. In study I, potassium levels (K^+) approximated 7 mmol/L which is associated with risk for lethal cardiac

arrhythmia. Peak levels did not differ between 60- and 240 min application times, however, after 30 min reperfusion, K^+ returned to baseline levels after 60 min application but showed no trend towards normalization after prolonged application. In study IV, significantly higher doses of norepinephrine were required to maintain central blood pressure.

5.5 COAGULATION TESTS

Physiologic preconditions, as depicted in figure 19, associated with coagulopathy after trauma were effectively induced in the polytrauma and positive control groups according to the protocol.

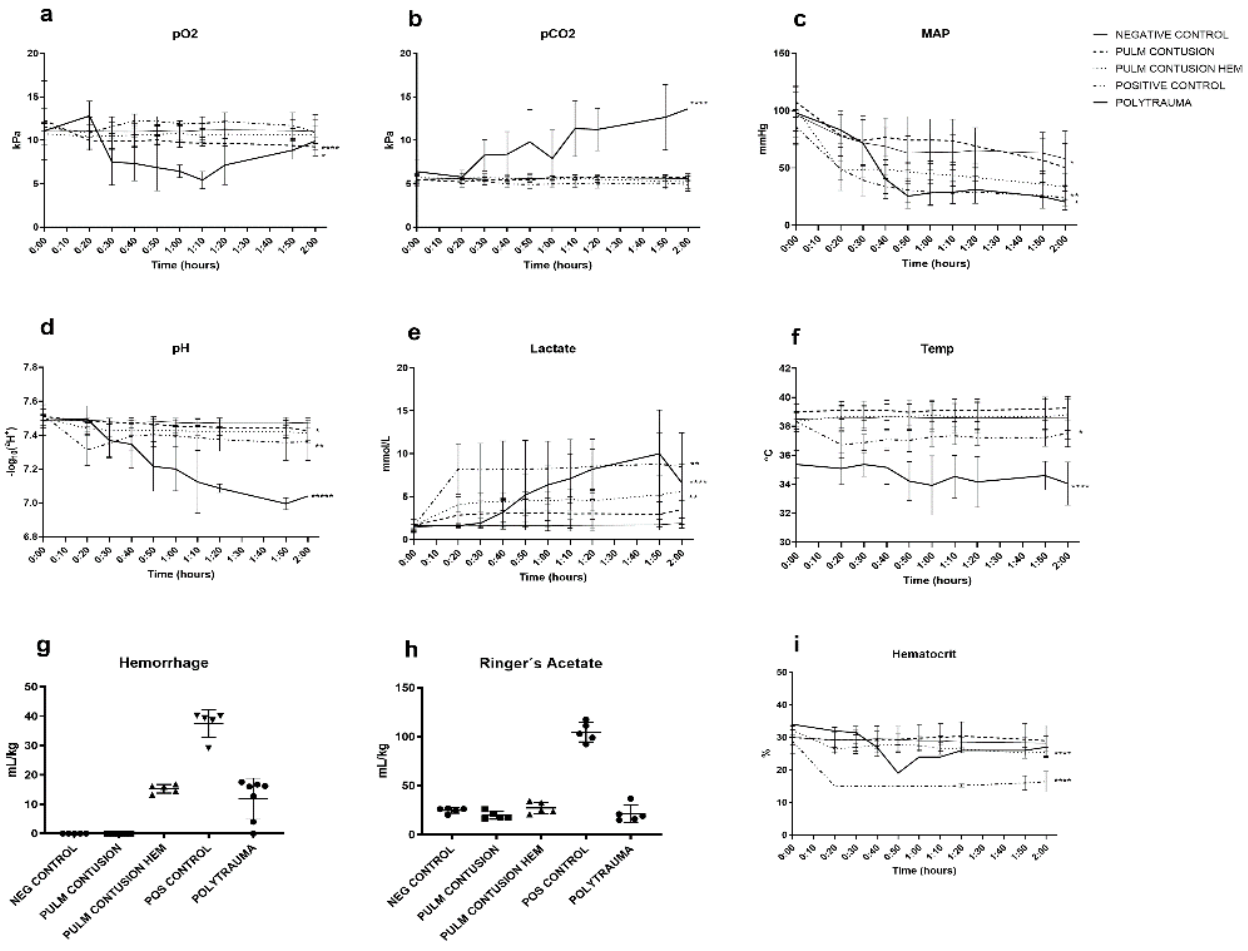


Figure 19. Systemic effects of the different trauma modalities (a-f, i). Hemorrhage and resuscitation fluid (RA) volume in the groups *pulmonary contusion*, *positive controls* and *polytrauma* (g-h). Comparisons were made with *negative controls* at the corresponding time. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.005$, $^{****}p<0.001$, $^{*****}p<0.0001$.

Deranged coagulation, as detected by ROTEM, a decrease in coagulation factors (platelets; prothrombin (fII), and fibrinogen (fI) and aPC) and an increased D-dimer was established by

the polytrauma model introduced in study V. The positive control group with hemodilution after 6180 mL crystalloids following a 2358 mL hemorrhage also displayed an increased PT(INR). The polytrauma model consequently established coagulopathy due to a negative impact in both primary hemostasis and plasma coagulation.

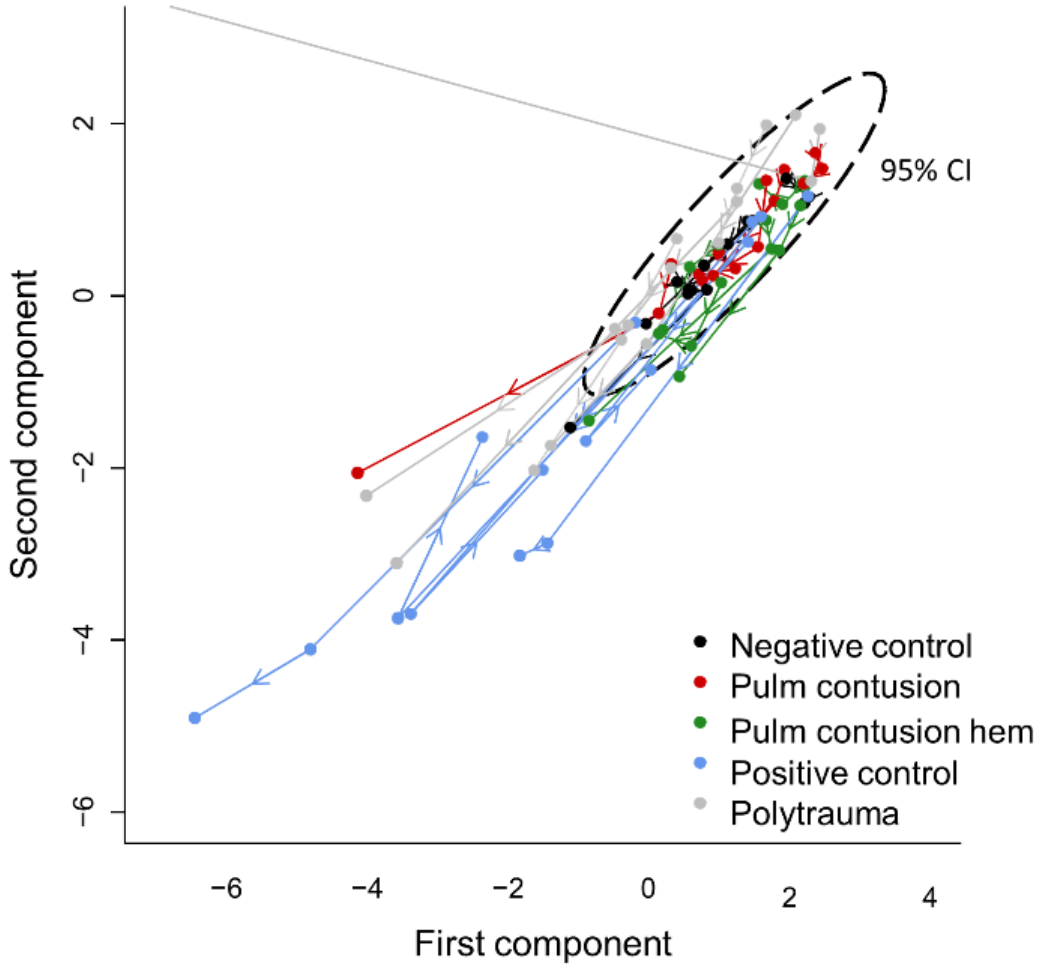


Figure 20. Principal component analysis of ROTEM presenting pathologic coagulation profiles in both polytrauma and positive control groups (visualized by the lines outside the dotted ring representing the 95% CI). Each dot represents an individual animal at a specific time point and the dots for the animal are connected with a line allowing for temporal visualization of the global coagulation performance at BL, 20 and 120 minutes.

6 DISCUSSION

Every health-care worker will encounter bleeding patients at some point during their career. Reflecting upon the fact that leakage of fluids from a system in almost any circumstance is fundamentally dealt with either by compression or wrapping directly to site of outflow or by upstream control of inflow to the damaged pipe, the invention of tourniquets and REBOA seems logical. Quite a few medical treatments used today were often preceded by historical interventions that sometimes were abandoned, not because they were proven useless, but rather due to lack of technical refinement and unexpected physiological consequences. This is certainly true regarding truncal tourniquets like the AAJT and even for endovascular techniques such as REBOA. This thesis has the privilege to discuss the fusion of ancient medical problems and their proposed solutions with the understanding of modern medicine. Further, the results of our studies may have direct implications for management of severely injured patients. This thesis shows that the application of today's technical possibilities and physiological knowledge can revitalize historical treatments.

The overall aim of this thesis was to provide physiological data on novel mechanical hemostatic adjuncts to facilitate their implementation into clinical practice and to contribute to the important research area of acute traumatic coagulopathy. We have contributed to the future management and possible rescue of severely injured patients with massive hemorrhage that historically, and today do not survive. We have investigated the efficacy and safety of a novel abdominal aortic tourniquet and to a certain extent novel aspects on the use of REBOA. Furthermore, we have provided new knowledge of the porcine research model for studies on the significantly important area of coagulation dysfunction associated with trauma. The establishment of a possible research model to enhance the understanding of TIC by use of viscoelastic tests that can be performed bedside, can possibly contribute to the understanding of the pathophysiology and development of new treatment options.

The porcine animal model used in this thesis is robust and comparable to the bulk of literature in the field of research addressing hemorrhage shock and trauma. In the studies of this thesis we used the two principally different ways of induction of hemorrhage shock by controlled or uncontrolled hemorrhage. Both was successful in achieving the desired amount of blood loss and state of shock. Numerous ways of inducing hemorrhage shock have been described and it seems likely that no single method can be regarded as a golden standard. Instead, each should be looked at in the context of the research aims of the study. In essence, the combination of a

tissue injury and hemorrhage from the same location may reduce bias from instrumentational blood loss in simulated rates and from different anatomical areas. In study III-IV this was aimed at by the surgical exposure and transection of the femoral artery, a model injury for use of the AAJT and zone III REBOA. Importantly however, the results of these studies may not be transferrable to humans due to unexpected differences in response to trauma and hemorrhage (vascular constriction and mechanistic differences in hemostasis and coagulation) between swine and humans. Thus, the important aspect of maintained hemorrhage control should be carefully read. In total, 60 animals were used in this thesis with a survival rate of 93%. This implies a robust and reproducible model in the context of potentially lethal hemorrhage and polytrauma, but also raises some concerns with the methodology. In studies I and IV we were not able to show any survival benefit in the primary outcome. It is possible that these studies were underpowered due to the small sample sizes yielded from the a priori calculations which were partly based on previously published external data. This underlines the importance of hypothesis development in small-scale animal research models.

The level of AO is fundamental for its physiological consequences as generally outlined in this thesis. In study I the finding of consistent infra-renal (zone III) AO by the AAJT was important with regards to the tolerance of application as well as the comparability with REBOA in study II-III. A possibly overlooked implication of the AAJT in this thesis (and the majority of reports of the AAJT) is the respiratory consequences. Endotracheal intubation and positive pressure ventilation is a potentially harmful intervention that should be avoided if possible in hypovolemic patients. On the other hand, the mechanical ventilation may have masked important aspects of respiratory function during application.

Anaesthetic management of the animals in hemorrhage shock research is fundamental, and surprisingly often not discussed in detail, in terms of the interference with the biological response to trauma and hemorrhage. Anaesthesia of the pigs in the hemorrhage shock literature is generally maintained with inhaled agents such as isoflurane. This affects both baseline hemodynamic variables and the response to hemorrhage and trauma. We consistently used ketamine as the principal anaesthetic agent with adjuvant benzodiazepines and fentanyl in accordance with the ethical permit. This strategy both resembles the likely anaesthetic management of human patients in situations of hypovolemic shock and introduces less bias to the animal model by reduced interference with autonomously sympathetic-mediated responses to trauma and hemorrhage.

We have shown that the previously reported correlation mismatch between aortic flow rate and aortic branch vessel flow in part may be explained by IRI. As indicated by the peak levels of

metabolic variables and clearance rate we confirmed that IRI is developing in a dose-effect relationship to ischemic time and level of AO. The milder reperfusion injury after zone III AO is logically explained by the higher tolerance to ischemia by the skeletal muscles in the lower extremities than abdominal organs.

A major limitation of this thesis (and many other animal models in trauma research) is the general lack of longer post-intervention observation times and lack of functional outcome assessment. Studies have been conducted underlining the benefits of such methodology if ethically possible (162). This is especially limiting study IV were development of AKI could not fully be assessed due to the definition and the fact that it may develop up to 7 days after trauma and therefore not were detected. However, indices of organ function such as urine output and significantly higher return of RBF were indicative of the possibility to mitigate IRI by efforts to allow circulation to distal organs to some extent during AO. The possible mechanism behind this observation may be the combination of low grade oxygenation and avoidance of accumulating total hypoxic conditions and decreasing the impact of recirculation of large amounts of ischemic metabolites after uninterrupted AO.

The possibility to detect TIC by ROTEM in pigs showed that this assay may be useful in parallel with its beneficial use in clinical scenarios. The traditional diagnostic criteria of TIC (using the platelet derived variables collected from plasma) has not yet been replaced by viscoelastic parameters. PCA is a computational model for analysing data sets with high dimensionality with preservence of information that can be presented in a way that is easier to interpret. In study V, PCA was used to combine all 16 parameters of ROTEM for each time-point of measurement into one for all animals (BL, 20 120 minutes). We were not able to assess the individual components of the polytrauma model, including systemic inflammatory response from LPS infusion, in relation to the development of TIC in our animal model. However, as an initial study it showed promise as a “proof of concept” and further studies may include PCA of ROTEM data to elucidate more specific contributions from each component used for inducing TIC. We showed a decrease in coagulation factors and aPC and an increased D-dimer after polytrauma indicating that the model includes the contribution of both disrupted clot-formation and fibrinolysis which are necessary for TIC.

Finally, the possible implementation of results from our studies conducted on laboratory animals into clinical practice warrants discussion. The limitations of the experimental work and its conclusions must be thoroughly considered and clearly identified to avoid inappropriate or excessive use of potentially harmful procedures in clinical practice, this includes both too much or too little credence to the results.

7 CONCLUSIONS

In this thesis we were able to confirm the efficacy of novel, mechanical hemostatic adjuncts such as the AAJT and REBOA for hemorrhage control in severe NCH. We further elucidated important aspects of fluid resuscitation in conjunction with these adjuncts. We were not able to determine a specific application time beyond which irreversible organ damage develops with AAJT application, however, we did provide novel and important findings of specific ischemic organ injuries associated with the device. We have shown that the AAJT and REBOA can be used to maintain hemostasis in the same patient during different levels of care and also provided important procedural and physiological aspects of changeover between the interventions. We have also confirmed that management strategies such as intermittent reperfusion during aortic occlusion may impact the dose-dependent development of ischemic reperfusion injury and direct organ effects of REBOA.

I The AAJT causes ischemic injuries to abdominal organs beyond 60-minute application, including the small intestine and liver, both due to mechanical compression and decreased visceral perfusion. Further, the intra-abdominal pressure causes decreased pulmonary compliance. Release of the AAJT should only be performed with the capability to counteract severe hypotension and metabolic acidosis.

II In comparison with the AAJT, resuscitation with zone III REBOA may require fluids to restore hemodynamic function, comparable to 2000 mL of crystalloids.

III Percutaneous access to the common femoral artery for REBOA insertion may be challenging after severe hemorrhage, and further complicated with external AO by the AAJT. The procedure was performed with preserved hemostasis but resulted in mild hypotension.

IV Intermittent REBOA was associated with significant hemodynamic fluctuations including decreased heart rate and blood pressure with associated lower levels of troponin I, used as an indicator of cardiac injury. We confirmed that aortic blood flow during hemorrhage shock and REBOA deflation did not correlate with renal artery blood flow. However, the degree of renal perfusion during balloon deflations resulted in increased urine output and thus improved organ function.

V We established a porcine research model fulfilling the Berlin criteria of polytrauma and introduced principal component analysis for viscoelastic tests

(ROTEM), which may improve the detection and differentiation of TIC-phenotypes in trauma populations.

8 POINTS OF PERSPECTIVE

Prospectively collected human data are necessary to consolidate the findings from the increasing data gathered from translational research models. Such efforts are underway and by the formation of the AORTA registry in the US, a solid platform for data collection has been built. With the rapid development of endovascular techniques and adjuncts it is likely that REBOA in clinical practice will be seen as a more dynamic tool for controlling hemorrhage with increased focus on mitigation of IRI by pREBOA. This will also influence its use in the early phases of care including pre-hospital and battlefield environments.

The major findings in the present thesis that may be transferrable to clinical management of human patients are considered as follows:

- AAJT and REBOA are effective for control of severe NCH and should be further considered for prehospital use.
- The AAJT may be considered as a point of care intervention due to the ease of use and absence of malfunction.
- A 60 min application time should not be exceeded with the AAJT.
- Deflation of the AAJT or REBOA balloon should not be performed in the prehospital environment.
- Patients resuscitated with the AAJT should be considered for zone III REBOA in higher levels of care and the procedure can be performed safely with caution regarding hypotension.
- Zone III REBOA should be assisted with fluid resuscitation to restore effective proximal circulation after massive hemorrhage.
- Weaning from AO should be monitored several hours with regards to acidosis, electrolyte abnormalities, distal perfusion conditions including development of compartment syndrome after IRI.
- Further refinement of balloon catheters or timings in protocols for intermittent reperfusion should be explored.
- Intermittent reperfusion during REBOA may be considered in relatively stable patients to counteract IRI and possibly to decrease the risk of organ damage to the heart and kidneys.
- Cardiac function should be closely monitored during zone I REBOA.

9 ACKNOWLEDGEMENTS

I would like to express my *sincere* appreciation to:

Dr Mattias Günther, for being an inspirational principal supervisor and talented researcher with impeccable balance of mentorship, guidance and demand of a student. You really transformed all the work into science worthy of publication.

Dr. Ulf Arborelius and Prof. Mårten Risling, my co-supervisors and the grand old men of the institution. For education in the art of navigation through the academic world and overcome the sometimes frustrating toughness to transform an idea into practical research.

Dr. Katarina Silverplats, my co-supervisor, for being there in times of need.

Dr Louis Riddéz, who first saw the potential in this and helped me get started.

Jenny Gustavsson. I realize that without you, this thesis would not have been written. You are an amazingly competent and loyal person. You made *me* feel confident when *you* often where the reason that the experiments worked. I really hope to get to work with you again.

Dr. Torbjörn Roos at the clinic of anaesthesiology and intensive care at Falu lasarett, for your exceptional way of leadership and simply believe in this when hiring me. This would not have been possible without your support.

Dr. Lisa Hårdstedt at the clinic of anaesthesiology and intensive care at Falu lasarett, for making my life liveable during stressful times. Most of the time, you (thankfully) had a better control of my day-to-day life than me.

Dr. Karin Leifland, for the (secret) access to the computer tomography and the technician Merhaj at the radiology department at Södersjukhuset, it was a game changer.

My resident- and consultant colleagues in Falun, for your support.

My brilliant wife Josefin, my deepest love and respect for your understanding and encouragement even when I did not deserve it. You remind me every day to try to be a better man.

My parents Lars and Maria for the trip to Norway and raising me like the way you did.

My daughter Molly and son Theo for reminding me of what is really important in life.

Anders Brunskog and Christina Strömbeck for nice company, inspiring discussions and housing during the practical work-up for this thesis.

Lars-Gunnar Olsson, for facilitating the studies with excellent technical support, your sharing of important lessons in life and for - you know why.

At last,

To my former colleagues and friends in the Swedish Special Operations Group – I really miss your natural capability to simplify, without dumbify, a really hard task and accomplish it with relentless enthusiasm. The “make it happen” – approach to life you have in common, has brought me invaluable perspectives on what you can accomplish, when you believe in yourself and your team and are willing to roll up your sleeves. There are so many moments that I will treasure for the rest of my life. It was a privilege to serve with you.

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