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BLEEDING AND HEMOSTASIS DURING NORMO- AND HYPOTHERMIA

STUDIES ON PORCINE AND RAT MODELS

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I have no special talent. I am only passionately curious.

Albert Einstein

ABSTRACT

Background: Numerous animal studies have shown protective effects of hypothermia (HT) on hemorrhagic shock. These findings do not correlate with clinical findings, were studies on trauma registers have shown that HT is an independent factor of death when associated with trauma. HT affects hemostasis, but is just one of many factors that cause the coagulopathy often seen in trauma patients with uncontrolled bleedings. To what extent HT *per se* contributes to the hemorrhage and hence, the deterioration of shock is virtually unknown. In this thesis we investigate HTs impact on uncontrolled hemorrhage, but also if rebleeding volumes could be affected by hemostatic drugs or different resuscitation regimes.

Methods: I: 18 pigs were randomized to HT (n = 10) or normothermia (NT) (n = 8). A volume controlled hemorrhagic shock was induced by a 40 % exsanguination of estimated blood volume (EBV). HT animals were cooled to 32.5 °C and rewarmed again after 2 hours. The observation time (OT) was 420 minutes. **II:** 23 pigs were randomized to receive tranexamic acid (n = 11) or placebo (n = 12). Uncontrolled hemorrhage was induced by lacerating the aorta, producing an exsanguination estimated to 35 – 40 % of EBV. These animals were not actively cooled. Rebleeding events were monitored by ultrasonic probes. OT was 130 minutes. Thrombelastography (TEG) was used to evaluate coagulation changes in study I and II. **III:** 40 rats were randomized to HT (n = 20) or NT (n = 20). Uncontrolled hemorrhage was induced by puncturing the femoral artery, producing an exsanguination estimated to 24 % of EBV. HT animals were cooled to 30 °C and rewarmed again at 90 minutes. The incidence, on-set time, duration and volume of rebleedings were followed. OT was 180 minutes. **IV:** 60 rats, all cooled and processed according to the protocol of study III, were randomized to 3 different resuscitation groups; Low (LRe), Medium (MRe) or High (HRe) or Medium resuscitation + Desmopressin (MRe + D) (n = 4 x 15).

Results: I: HT induced a coagulopathy apparent at temperatures < 35°C, and reversible upon rewarming. There were no differences in hemodynamics, blood chemistry and mortality between groups at the end of the study period. **II:** There were no differences in rebleeding or mortality between the tranexamic acid and placebo groups. Non- survivors had significantly higher rebleeding volumes compared to survivors. At the end of observation, there was a strong correlation between an aggravated coagulopathy, as measured by TEG, and total bleeding volumes. **III:** HT animals had significantly higher rebleeding volumes (HT = 43 % *vs.* NT = 3 % of EBV). Rebleeding volumes were larger in the HT group even at temperatures > 35 °C. Much higher blood pressure, induced by cooling, was seen in the HT group. **IV:** There were significantly higher rebleeding volumes in the HRe group and a trend towards higher mortality in the LRe group. No significant differences in the number or volume of rebleeding and no difference in mortality between the MRe + D and MRe groups was seen.

Conclusions: HT induces a coagulopathy that is reversible upon rewarming. During trauma and uncontrolled hemorrhage, other factors than HT contribute to this coagulopathy. Hemodynamic changes provoked by cooling and HT, *i.e.* a rise in blood pressure, contribute to repeated rebleeding and hence, continuous hemorrhage. An MRe resuscitation regime seems most beneficial for outcome during HT and uncontrolled hemorrhage. Tranexamic acid at NT and desmopressin at HT conditions does not reduce rebleeding in penetrating trauma with uncontrolled hemorrhage.

List of Publications

This thesis is based on the following papers, which will be referred to in the text by their roman numerals:

I. **Heinius G, Wladis A, Hahn R, Kjellström BT.**
Induced Hypothermia and Rewarming after Hemorrhagic Shock
J Surg Res 108(1): 7-13, 2002.

II. **Drobin D, Sjostrand F, Piros D, Hedin A, Heinius G, Hahn R.**
Tranexamic Acid Does Not Prevent Rebleeding in an Uncontrolled Hemorrhage Porcine Model
J Trauma 59(4): 976-83. 2005

III. **Heinius G, Hahn R, Sonden A.**
Hypothermia Increases Rebleeding in a Novel Uncontrolled Hemorrhage Rat Model
Submitted

IV. **Heinius G, Hahn R, Sonden A.**
Effects of Desmopressin and Different Intravenous Fluid Regimes on Uncontrolled Bleeding During Hypothermia
Manuscript

CONTENTS

LIST OF ABBREVIATIONS

ACoTS	Acute Coagulopathy of Trauma-Shock
APTT	Activated Partial Thromboplastin Time
BE	Base Excess
BMR	Basal Metabolic Rate
CO	Cardiac Output
DDAVP	Desmopressin
DIC	Disseminated Intravascular Coagulation
DO_2	Oxygen delivery
DO_{2crit}	The critical lowest level of oxygen delivery
EBV	Estimated Blood Volume
ER	Emergency Room
FFP	Plasma (Fresh Frozen Plasma)
HR	Heart Rate
HT	Hypothermia, Hypothermic
INR	International Normalized Ratio
ISS	Injury Severity Score
LR	Lactated Ringers solution
MAP	Mean Arterial Pressure
NT	Normothermia, Normothermic
OER	Oxygen Extraction Ratio
pRBC	packed Red Blood Cells
PT	Prothrombin Time
RBC	Red Blood Cells
ReFVIIa	Recombinant Factor VIIa
ROTEM	Rotation Thromboelastometry
RCTs	Randomized Controlled Trials
SEM	Standard Error of Mean
TEG	Thrombelastography
TF	Tissue Factor
VHA	Viscoelastic Hemostatic Assays
VO_2	Oxygen consumption

1 INTRODUCTION

1.1 BACKGROUND

Worldwide, more than 16,000 people die from traumatic injuries every day, and it is estimated that injuries causes 16 % of the global burden of disease. In high-income countries, road traffic injuries, self-inflicted injuries, and interpersonal violence are the three leading causes of death among people aged 15 to 44 [1]. Uncontrolled bleeding contributes to nearly 40 % of these deaths and pass for the leading cause of potentially preventable deaths among trauma victims [2, 3].

Accidental hypothermia (HT) is common among trauma victims and, in the prehospital setting or upon arrival at the Emergency room (ER), has been described to be as high as 50 % when defined at a temperature < 36 °C [4] and between 2 – 20 % when defined at a temperature < 35 °C [5-7]. HT is associated with higher mortality among trauma victims, but is also typically apparent in more severe trauma. Some authors state that HT is a predictor of adverse outcomes and not a detrimental factor by itself [7, 8]. On the other hand, studies on trauma registers in the USA, which investigated between 38,000 – 700,000 trauma patients, have shown that HT is, by itself, an independent factor of death [5, 6, 9].

Induced HT has been used during cardiovascular and neurosurgical surgery since the 1950s to reduce cerebral and spinal cord damage [10]. It has also been used as neuroprotection, both for patients with traumatic head injuries [11] and after cardiac arrest [12]. In 1956, Postel et al. [13], in a volume-controlled hemorrhagic shock model for dogs, showed a dramatic prolongation of survival among animals exposed to HT. Since then, numerous animal studies have presented similar findings, and there are some physicians that propose induced HT as a treatment for trauma victims with hemorrhagic shock. However, most of the animal studies mentioned above were done on pressure or volume-controlled hemorrhagic shock models; thus, possible bleeding caused by HT could not be evaluated.

HT affects hemostasis on several levels [14, 15], but is just one factor that causes the coagulopathy often seen in trauma victims with hemorrhagic shock [16]. To what extent HT by itself contributes to the bleeding volume and, hence, to the deterioration of the shock during uncontrolled hemorrhage is virtually unknown.

This thesis is primarily focused on bleeding and coagulation during hemorrhagic shock with uncontrolled hemorrhage, both during HT and normothermic (NT) conditions. It also analyzes the impact of different intravenous fluid regimes and pharmacological treatments on such bleeding. The hemodynamic and metabolic changes during hemorrhagic shock, induced HT and rewarming are also studied.

1.2 HISTORICAL NOTES

The word *iatros*, which means physician in modern Greek, originally meant “extractor of arrows,” and the word τραυμα, meaning “wound” in ancient Greek, has led to the use of the word trauma to describe a wound or injury.

In the 17th century, William Harvey was the first to describe the circulation of the blood, thus providing a more solid base for understanding the effects of trauma. During the same century, Christopher Wren discovered that medicines could be administrated to animals intravenously, and Richard Lower demonstrated that homologous blood could be transfused between animals. Transfusions between humans were also tried; however, numerous fatal transfusion reactions led to legal proscriptions against blood transfusions, and the problems with these reactions were not solved until the first years of the 20th century.

In the 18th century, Steven Hale was the first to measure blood pressure by inserting a long glass tube into the artery of a horse, measuring the height of the blood column, and Antoine-Laurent Lavoisier showed that respiration was necessary for the oxidation of living tissue.

During the 19th century, the introduction of anesthesia in the middle of the century and the development of antiseptic surgery introduced by Joseph Lister in 1867 greatly improved the outcome for trauma victims, but still hemorrhage and its consequences remained a nemesis for the surgeon dealing with this group of patients.

In the 20th century, major progress in the understanding of hemorrhagic shock was made. In the first decade of the century, George Crile showed that low blood pressure was an essential characteristic of chock. The discovery of human blood groups by Karl Landsteiner in 1901 made it possible to transfer blood in a safe way, and the technique to store blood by cooling and adding sodium citrate developed. In 1916, the first successful transfer of stored blood was made. However, during World War I, the understanding of hemorrhagic shock was still inadequate, and few casualties were given adequate resuscitation, which led to high mortality figures among the soldiers with hemorrhagic shock.

In the 1930s, the works of Alfred Blalock [17] showed that blood and plasma volume deficit was the cardinal feature of hemorrhagic shock. As a result of this knowledge, whole blood, plasma and colloids were used to treat hemorrhagic shock during World War II and during the Korean conflict. This drastically improved early survival, but many casualties later died of acute renal failure. It was, by the works of Shires [18, 19] and others, in the beginning of the 1960s, that we understood that the extracellular fluid space has to be repleted during hemorrhagic shock. This led to the usage of crystalloid solutions in large volumes, instead of colloids, for initial shock resuscitation.

Much has happened over the last 50 years in trauma care, and some of these issues will be discussed in the following chapters, but one may state that infusion of large quantities of crystalloids is still considered the initial treatment of choice for patients suffering from hemorrhagic shock.

1.3 SHOCK

1.3.1 Defenition

Shock could be defined as situations in which the perfusion to vital organs is not sufficient enough for the metabolic demands of the cells in these organs. Various classifications of shock have been proposed, and most of them fit into one of

three general types: 1) decrease in circulating blood volume; 2) pump failure; 3) increase in the capacity of the vascular tree that exceeds the circulating blood volume [20].

The first type, which should be addressed as hypovolemic shock, results from a reduction in the circulating blood volume, either by whole blood, plasma or extracellular fluid, or a combination of the three. By definition, hypovolemic shock could develop without any bleeding at all. In trauma, for example, such a situation is common among patients afflicted with extensive burns. However, in the following text, hypovolemic shock caused by hemorrhage will be discussed and, thus, the term hemorrhagic shock will be used.

1.3.2 Hemorrhagic shock

According to Baskett [21], hemorrhagic shock could be divided into four different stages (or classes) depending on the magnitude of the blood loss. This is also the classification used in the ATLS concept for trauma care [22] and is therefore accepted worldwide, even thought it has actually never been evaluated [23]. The definition and physical reactions of a young and otherwise healthy person are briefly as follows (the blood volume described below is for a 70-kg man):

1. **Stage I, bleeding < 15% of estimated blood volume (EBV) (<750 mL of blood).**
   This bleeding corresponds to the amount of blood given during a blood donation. Clinical symptoms are minimal with no changes in blood pressure, pulse pressure or respiratory rate. Vasoconstriction is the major mechanism by which arterial pressure is maintained. Through transcapillary refill, 0.5 – 1.0 L of fluid can be mobilised into the intravascular space [24], and blood volume will be restored within 24 hours.

2. **Stage II, bleeding 15-30 % of EBV (750 – 1500 mL of blood).**
   The symptoms seen are dependent on whether the bleeding is rapid and ongoing or slower and stopping, but in either case clinical symptoms of inadequate perfusion are obvious. Typically, tachycardia is seen, but this could also be followed by a transient period of bradycardia when blood pressure falls [25-27]. Pulse pressure is decreased due to an increase in diastolic pressure. The decrease in systolic blood pressure usually follows with ongoing bleeding. The release of catecholamines and other vasoconstrictors decrease the blood flow to skin, muscles and parenchymal organs, whereas the blood flow to the heart and brain is maintained [20].

3. **Stage III, bleeding 30-40 % of EBV (1500 – 2000 mL of blood).**
   When the blood loss exceeds 30 % of EBV, the compensatory mechanisms activated by the bleeding can no longer maintain the circulation, and classic signs of inadequate perfusion are always present. Tachycardia and tachypnea are seen, there is a significant decrease in systolic blood pressure, and the patient is mentally

affected. The lowest critical level for oxygen delivery ($DO_{2\,crit}$) is reached and anaerobic metabolism increases [28] (Fig 1).

4. **Stage IV, bleeding more than 40 % of EBV (> 2000 mL of blood)**

When the blood loss exceeds 40 %, the risk for irreversible shock and death is immediate if resuscitation is not started. The skin is cold and pale, there is apparent tachycardia, the pulse is threadlike and systolic blood pressure is markedly depressed. Blood loss exceeding 50 % will result in loss of consciousness, pulse and blood pressure and an early death.

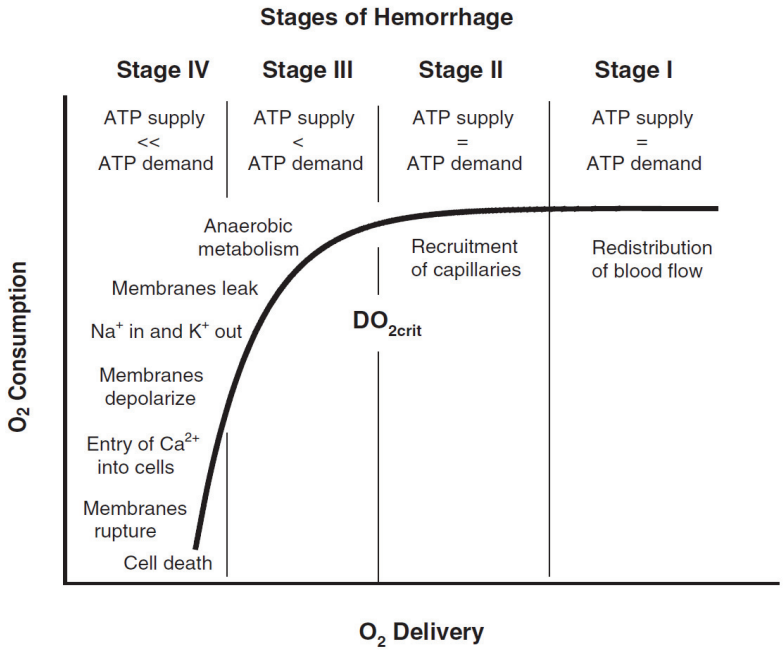


Figure 1 Changes in oxygen consumption as a function of oxygen delivery are shown. The hypothetical relationships of these parameters to the stages of hemorrhage and changes in cellular membrane integrity are also shown. DO2crit = critical oxygen delivery. *From: Gutierrez, G., H. D. Reines, et al. (2004). "Clinical review: hemorrhagic shock." Crit Care **8**(5): 373-81*

1.3.3 Changes on microvascular and cellular levels during hemorrhagic shock

During and after resuscitation, there are changes on the microvascular and cellular levels due to hemorrhagic shock. Swelling of the capillary walls is seen [29] as well as occlusion of capillaries due to leukocyte trapping [30]. An activation of these leucocytes by damaged endothelia cells probably contributes to increased capillary leak [31].

A cell membrane dysfunction develops in the Na/K/APTase pump of the cells [32, 33], which leads to intracellular gain of sodium and loss of potassium to the extracellular space. This facilitates cellular swelling and transfusion of extracellular fluids into the cells. A calcium influx into the cells is also seen, which may cause irreversible damage to cellular function [34].

In a study on rats, Ke-Seng Zhao et al. [35] showed that during hemorrhagic shock, only 20 - 30 % of capillaries were open. A few hours after resuscitation, the survivors reached 40 - 50 % of normal circulation in their arterioles, while the non-survivors only regained 15 - 20 % of normal microcirculation. These findings indicate that changes on the microcirculatory level play a vital role in the development of irreversible shock.

1.3.4 DO_2 , VO_2 , inadequate ATP production and the development of acidosis

Systemic oxygen consumption, defined as VO_2, is much lower than the oxygen delivery, defined as DO_2, during normal conditions. During hemorrhagic shock, DO_2 rapidly decreases with little change in VO_2. When oxygen delivery falls below a critical value (DO_{2crit}), VO_2 will start to decrease linearly with ongoing decrease in DO_2, and an oxygen depth with anaerobic metabolism will develop. If this condition is not rapidly reversed, the risk of death is imminent. (Fig 1). DO_{2crit} varies somewhat according to circumstances, but has been measured to be at a level of around 8 – 10 ml O_2/min/kg both in man and in laboratory animals [36, 37].

For energy, the cell is dependent on the hydrolysis of ATP to ADP, a process by which energy and an H^+ ion are produced. During normal conditions, the H^+ ion is reclaimed in oxidative phosphorylation (aerobic metabolism) of ADP back to ATP. When DO_2 declines, reaching critical levels for oxygen delivery, ATP can only be synthesised by anaerobic glycolysis. This is a slow reaction, reaching just 5 – 10 % of normal production. As a result of this, H^+ is not reclaimed. The H^+ concentration in the extracellular fluid is therefore a quantitative indicator of the magnitude of the shock on the cellular level [38].

1.3.5 Treatment of hemorrhagic shock

There seem to be three phases of hemorrhagic shock: a) compensated hemorrhagic shock; b) uncompensated hemorrhagic shock that is reversible; and c) irreversible hemorrhagic shock [39]. The transition from a reversible to an irreversible stage is insidious and is often defined in retrospect. With continual bleeding and/or inadequate volume resuscitation, continued hypoperfusion due to low cardiac output, as well as regional perfusion defects, will create a vicious cycle of further tissue and microcirculatory changes that at one point will be irreversible [20]. Consequently, the treatment consists of two parts; to stop the bleeding and to provide sufficient resuscitation without any delay. However, a real challenge to the best treatment will arise when trying to resuscitate a patient with uncontrolled bleeding, before surgical hemostasis has been achieved.

Uncontrolled bleeding should be suspected in all penetrating torso trauma patients, but it is common even in blunt trauma. Lechleutner et al. [40] showed that 25 % of all blunt trauma victims in a cohort of 353 patients had uncontrolled inner bleeding. The mortality rate among this group was 39 % compared to 16 % in the group with controlled hemorrhage.

As mentioned earlier, the works by Shires and others demonstrated the importance of compensating extracellular, as well as intravascular, fluid losses. Hence, since the mid-1960s, the initial resuscitation of choice during hemorrhagic shock has been large volumes of intravenous crystalloids. This therapy, since then, has been challenged by many authors in favor of colloids. However, three meta-analyses looking at crystalloid vs. colloid treatment for hemorrhagic shock have all shown improved survival in the crystalloid groups [41-43].

On the other hand, animal models, designed for detecting uncontrolled bleeding, have shown increased bleeding and higher mortality rates in treatment groups with high intravenous crystalloid infusion regimes [44-49]. Other factors, like hypothermia

as well as traumatic coagulopathy, will also interact with resuscitation and affect outcomes during uncontrolled bleeding. Some of these issues will be discussed in the following chapters.

1.4 HYPOTHERMIA

1.4.1 Thermophysiology

Man is homeotermic, which means that we need to maintain a body temperature close to 37 °C, regardless of the surrounding temperature. We are, as a matter of fact, more adapted, as a species, to life in the tropics with temperatures around 27 °C than to the climate we have in northern Europe [50]. Thus, at our latitudes we usually have to protect ourselves from the surrounding climate. Heat loss from the body occurs because of four mechanisms [51].

1. ***Radiation*;** such losses could be substantial if we do not wear some sort of insulation in cold weather. As an example, at a temperature of 4 °C, one can lose 50 % of the body's total heat production from one's unprotected head.
2. ***Conduction*;** which means transfer of heat by direct contact. Water conducts heat 32 times better than air!
3. ***Convection;*** best described as when particles of air or water, which have been heated by the body, are swept away. For instance, when a cold wind blows right through ones sweater!
4. ***Evaporation;*** when water, e.g. sweat, evaporates, energy is needed and, hence, the skin is cooled.

The best way to describe the body from a thermoregulatory point of view is by dividing it into a core protected by outer layers. The superficial layer - skin, subcutaneous tissue and the thermoreceptors that are situated here - is the most important part in the regulation system. Skin temperatures can drop to near-environmental temperatures in the attempt to conserve heat [51]. The intermediate zone (skeletal muscles) is only activated when the core temperature is in danger of falling and can produce a high amount of heat by shivering, thus providing warm blood to the core. The regulation of this interplay is controlled from the preoptic anterior hypothalamic area, which reacts to the temperature of the blood when it flows through this part of the brain [52]. It is also important to emphasise that behavior itself, e.g. seeking shelter from a cold wind, should be considered an integral part of the thermoregulatory system [52].

1.4.2 Definitions of hypothermia

HT is most often defined as a temperature < 35 °C. One common classification is as follows: Mild HT (35 °C – 33 °C), moderate HT (33 °C – 30 °C) and severe HT (< 30 °C) [53, 54]. This classification is convenient for understanding physiological changes as the temperature drops. As an example, shivering is most intense between 35- 33 °C, and heart arrhythmias start at temperatures < 30 °C. Moreover, at temperatures below 30 °C, the body loses its ability to spontaneously rewarm itself (i.e. the patient becomes poikilothermic); thus, active rewarming must be performed [54]. However, because of the harmful effects that HT is considered to have when it is associated with trauma, another classification has been recommended for

trauma victims. According to this classification, all patients with a core temperature < 36 °C should be considered hypothermic, and patients with temperatures < 32 °C should be considered severely hypothermic [22, 55].

1.4.3 The body's reaction to cooling

Sudden cold exposure to the body stimulates, among other things, the secretion of catecholamines, which give rise to rapid hemodynamic changes. These changes are immediate and appear before the decrease in core temperature. In a study by Johnson et al. [56], where healthy human test subjects were exposed to 10 °C by water immersion, plasma norepinephrine increased twofold after only two minutes, preceding the drop in body temperature by 15 minutes. The plasma norepinephrine concentration then continued to increase during the 60-min study period and correlated to the increase in metabolic rate and to the decrease in temperature.

Raven et al. [57] examined 11 young men who were exposed to a 5 °C room temperature over two hours. Cardiac output (CO) increased over time to nearly 100 % above baseline values and mean arterial pressure (MAP) increased by 20 %. Only minor changes in heart rate (HR) were seen. The increased CO was explained by a rise in stroke volume. Similar findings have been reported by others, with a rise in CO between 30 - 60 % and a rise in MAP by 5 - 25 % [58-61]. These changes seem to be more pronounced in men than in women [60, 61].

1.4.4 Physiological changes during hypothermia

Major physiological changes will appear in relation to the decrease in temperature. Some of the most important are listed below.

1. **Metabolic rate**
   If shivering is absent, the basal metabolic rate (BMR) falls by 6 - 8 % per °C and will be approximately 75 % of the baseline at 33 °C and 60 – 65 % at 30 °C. However, under normal conditions, shivering starts when the body temperature has dropped by 0.7 °C and will increase the metabolic rate by up to five times the basal levels. Usually, the shivering is most pronounced at around 35 °C and declines at a body temperature < 33 °C [54].

2. **Hemodynamics**
   As mentioned above, there could initially be a considerable increase in CO and MAP that may persist even at moderate HT, but that finally declines at lower temperatures. HR may initially rise somewhat, but usually decreases early. Stroke volume is increased and will be unchanged even at low temperatures. Bradycardia and atrial fibrillation are seen below 30 °C, and ventricular fibrillation may occur at temperatures < 28 °C [62].

3. **Respiratory function**
   Transient tachypnea is common as a reaction to cold exposure. The respiratory rate will, however, soon decline when temperature decreases and can be extremely low at temperatures < 30 °C. Bronchorrhoea, probably due to deficient ciliary function, is common [54] and bronchospasm could be seen [63]. However, gas exchanges seem to be sufficient even at low temperatures [64].

4. **Central nervous system**
   Drowsiness is commonly observed at temperatures around 33 °C. As the temperature falls, the patient will be lethargic and often falls asleep; however, unconsciousness rarely occurs at temperatures above 28 °C, so a cause other than HT should be considered if comas exist above this temperature [54].

5. **Renal function and fluid balance**
   Urine flow increases with decreasing temperature. This is caused by depressed oxidative tubular activity, which causes reduced water and sodium resorption [51]. Other temperature-dependent mechanisms will, in addition to this, cause a translocation of fluids to the interstitial space and, hence, there is a risk that the HT patient develops hypovolemia.

6. **Blood chemistry**
   Usually, HT induces hyperglycemia, but if the glycogen stores are depleted, a hypoglycemia can develop, which may inhibit shivering [54]. Hematocrit rises as a consequence of the above-mentioned fluid shifts. Granulocytopenia and thrombocytopenia are seen because of sequestration in the liver and spleen. A reversible hypokalemia develops due to an intracellular redistribution of potassium from the extracellular compartment [65]. The mechanism behind this effect is not fully understood and could only partly be explained by the increased levels of catecholamines seen during HT [66]. PH and PCO_2 alter with temperature; the reason for this change is that the partial pressures of dissolved gases decline as the temperature drops, with a decrease in $PaCO_2$ and consequently a rise in pH. This rise in pH is considered not to reflect a "true" alkalosis, as it will be automatically corrected when the temperature rises. Because of this, it is generally advocated that blood gases should be measured at 37 °C irrespective of the temperature of the patient [67]. An induction of HT usually creates a respiratory alkalosis, which is followed by a mixed respiratory and metabolic acidosis as the body temperature falls [54].

1.4.5 Treatment of the hypothermic trauma patient

The current recommendation is to rewarm the hypothermic trauma patient as soon as possible. However, some physicians have proposed continuous HT as treatment for this group of patients [68-70]. The rationale for this is convincing results in animal studies, where hemorrhagic shock in combination with HT seems to increase survival in rats [68, 70, 71], pigs [72, 73] and dogs [13, 74]. However, the impact of rebleeding was inadequately, or not at all, investigated in these studies.

Studies addressing the issue of uncontrolled bleeding during hemorrhagic shock in combination with HT have mostly been done on rats, using the "tail cut model" [75-77]. The conclusion from these studies was that HT did not increase the rebleeding volume. However, the authors themselves had comments on the method, and one of them, Takasu, addressed peripheral vasoconstriction in the tail as a reason for the small rebleeding volumes seen.

As mentioned earlier, clinical studies show that HT in combination with hemorrhagic shock predicts a more detrimental outcome [7, 78, 79]. There are a number of

reasons that could explain these findings, and it is hard to analyze the cause and effect of HT in relation to other factors, but the known coagulation defect that HT induces may be one important factor in the outcome.

In a randomised trial with 57 patients, Gentilello et al. [80] compared slow and fast rewarming for critically injured hypothermic trauma patients. He showed improved short-term survival and reduced fluid resuscitation requirements in the "fast rewarming" group, but no impact on long-term survival. In the "slow rewarming" group, 46 % of the patients died during the first 24 hours, compared to 14 % in the "fast rewarming" group. Among those who died, the resuscitation requirements were five times higher than among the survivors. These findings support the assumption that rebleeding caused by e.g. coagulation disturbances could be an important cause for outcome among hypothermic trauma victims in hemorrhagic shock.

1.5 THE HEMOSTASIS PROCESS

1.5.1 Primary and secondary hemostasis

The hemostasis process is divided into *primary hemostasis* and *secondary hemostasis* (the coagulation process). A brief description of the different steps in the process is listed below [81, 82]:

1. **Primary hemostasis**
   Primary hemostasis consists of vascular constriction and the establishment of a platelet plug.
   a) **Vascular constriction**
      When the endothelial cells in the vessel are damaged, collagen is exposed and platelets are attached to the damaged area by the von Willebrand factor. The platelets release substances such as thromboxane A2 and serotonin, which will give rise to a local vascular constriction.

   b) **Establishment of a platelet plug**
      The platelet plug is formed by platelet adhesion. This process is initiated by platelets that "roll" on the vessel wall in an injured vessel.
      The rolling process is an interaction between the red blood cells and the platelets and will not work properly with an Hb < 100 g/L.
      During the adhesion process, when the platelets get "sticky," a lot of different factors are released that are part of the aggregation process, but also in the latter coagulation process. The bonds that are activated in the adhesion process are not strong and need to be stabilised by fibrin during the coagulation process.
      When the platelet plug "reaches" undamaged endothelial cells, the process is stopped by substances on those cells' surface, such as prostacyclin (PGI_2), nitrogen monoxide (NO) and heparin sulphate.

2. **Coagulation (secondary hemostasis)**

The classic coagulation model describes the coagulation system as a cascade or "waterfall" of reactions ending with the conversion of prothrombin to thrombin [83]. However, this theory has been replaced by a "cell-based" model, where the different reactions take place on cell surfaces in three overlapping steps; initiation, amplification and propagation [84]. With this model, coagulation disorders *in vivo*, as well as factor VII important role in the coagulation process, is better understood.

Normally, inactivated coagulation factors are present in circulating blood, as well as a small amount of activated FVIIa (1-2 %).

a) Initiation

Coagulation is activated by a Tissue Factor- (TF) carrying cell. A lot of cells can express TF (e.g. fibroblasts, machrophages and endothelial cells), but the TF factor is not exposed to the blood until injury or inflammation occurs. FVII is activated into FVIIa, which binds with TF factor. This TF/FVII complex will activate FIX into FIXa and FX into FXa. FXa activates FV into FVa. FXa/FVa creates a complex on the TF-bearing cell and starts to convert a small amount of prothrombin to thrombin.

b) Amplification

The small amount of thrombin mentioned above will start the amplification process.

It converts FV into FVa, FVIII into FVIIIa and FXI into FXIa. FXIa also converts FIX into FIXa. It also "activates" the platelets that have assembled on the injury site as described above (primary hemostasis).

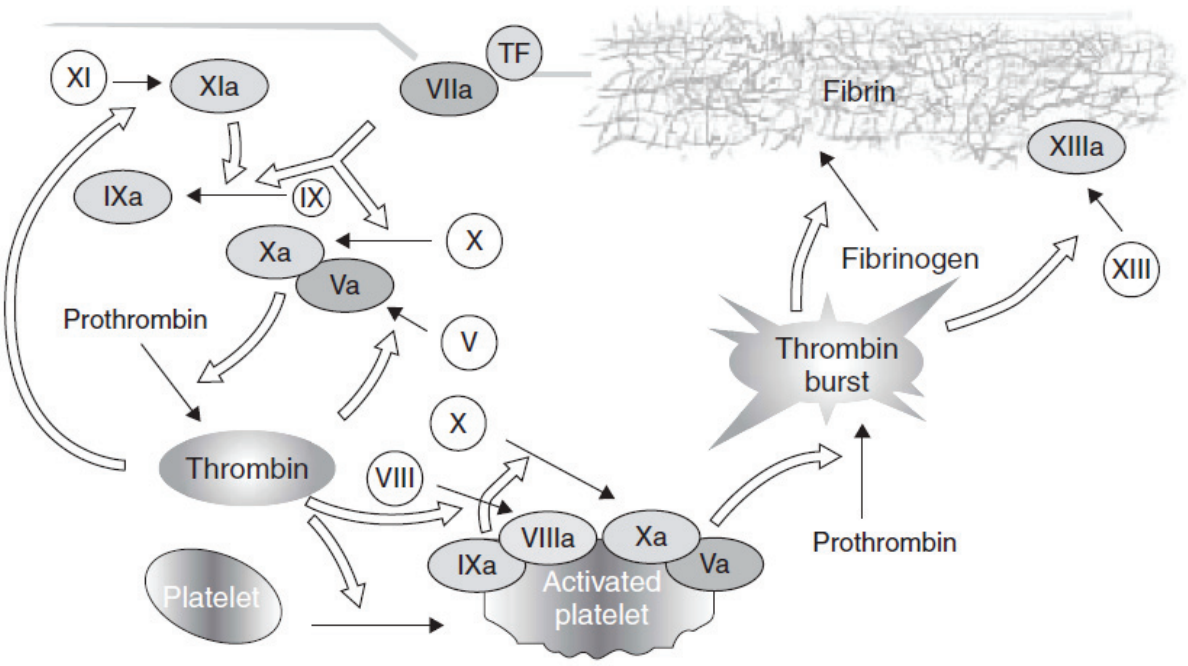


Figure 2 The coagulation process.

From: "Coagulopathy and blood component transfusion in trauma" by D.R. Spahn, British Journal of Anaesthesia 95 (2): 130–9 (2005).

c) Propagation

During propagation, FIXa/FVIIIa and FXa/FVa create complexes on the activated platelet. The former complex activates the latter to convert large amounts of prothrombin to thrombin (the "thrombin burst"). Thrombin divides fibrinogen to fibrin monomers. Thrombin also converts FXIII to FXIIIa, which is needed in the final cross-linking of the soluble fibrin monomers to form a stable fibrin clot.

1.5.2 Regulation of the hemostatic process

The coagulation system is regulated by several inhibitors, e.g. antithrombin, protein C and tissue factor pathway inhibitor. These inhibitors bind to coagulation factors that have been activated and prevent them from reaching the bloodstream.

The activation of protein C is maybe the most important. This activation starts when free thrombin binds to thrombomodulin, which is a receptor on the endothelial cell. This receptor activates protein C (APC) and its cofactor protein S. These factors inactivate FVa and FVIIIa, which lead to a dramatic decrease in the production of thrombin.

The fibrinolytic system regulates the formation of fibrin. This process is controlled by the conversion of plasminogen to plasmin. Plasmin dissolves fibrin into fibrin degradation products (FDP). Plasminogen is produced in the liver and circulates in the bloodstream. It binds to a fibrin clot and is activated by urokinase plasminogen activator (u-PA) or tissue plasminogen activator (t-PA). The latter is the most important activator of plasminogen and the main regulator of fibrinolysis. T-PA is continually released from the endothelial cells, but is deactivated by plasminogen activator inhibitor 1 (PAI-1). The level of PAI-1 usually exceeds that of t-PA, preventing active t-PA from reaching the bloodstream. Other plasmin inhibitors are TAFIa, PAI-2 and α2-Antiplasmin.The last acts together with FXIII and protects the fibrin clot from being dissolved too early. α2-Antiplasmin is also the primary physiological inhibitor of plasmin in the bloodstream [82, 85].

When operating in balance, the coagulation process and the regulation processes interact to ensure hemostasis by clot formation, simultaneously ensuring revascularization and a maintained blood flow. However, in trauma patients with major bleeding, the capacity of the coagulation process will be stretched to the limit and may cause coagulopathy even in patients with previously normal hemostasis.

1.5.3 Bleeding abnormalities during hemorrhagic shock

Even though a majority of trauma patients are actually hypercoagulable after an injury [86-88], the more severely injured patients are frequently hypocoagulable, and this condition seems to be more common than previously believed. Several studies have shown that between 10 - 34 % of all trauma patients have a coagulopathy already upon arrival at the ER [89-94]. This coagulopathy has been shown to be an independent factor in a detrimental outcome and increases the risk for death by as much as four times [95].

Earlier descriptions of traumatic coagulopathy have focused on the changes that appear later in the process of resuscitation. They are as true today as they

were almost three decades ago when they were first described [96] and can be explained as follows:

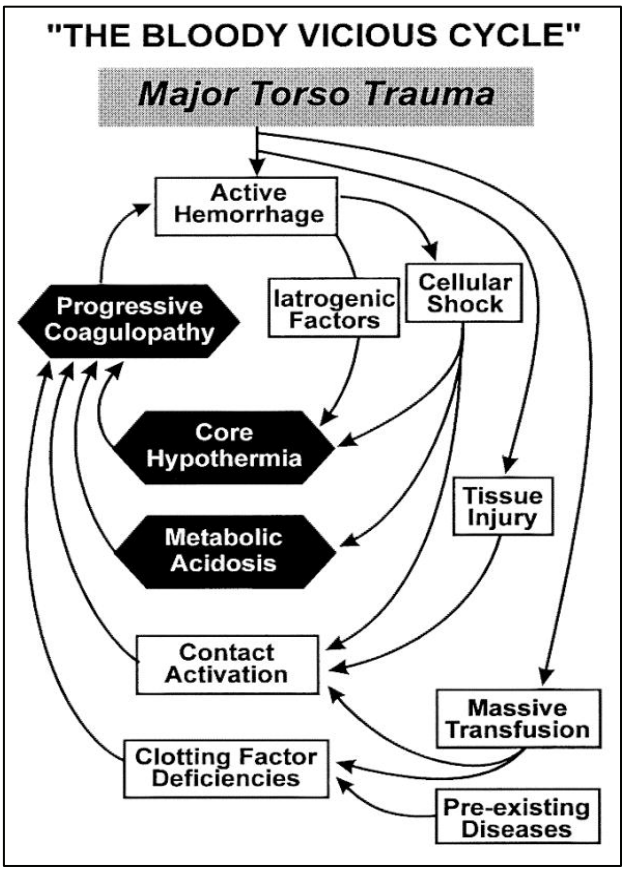


Figure 3 The pathogenesis of the bloody vicious cycle after severe injury
From "Staged Laparotomy for the Hypothermia, Acidosis, Coagulopathy Syndrome" by EE Moore, The American Journal of Surgery, Vol. 172, pp. 405-410. Copyright 1996 by Excerpta Medica, Inc.

With an ongoing uncontrolled hemorrhage, a point of no return will be reached, where it will be impossible to stop the bleeding because of a refractory coagulopathy. This dreaded condition has been named the "bloody vicious cycle" [96] and is described as a triad of persistent metabolic acidosis, progressive hypothermia and, as a result of these and other factors, a progressive coagulopathy (Fig. 3). However, the early coagulopathy described above probably has other causes. To distinguish it from other forms, as dilution- or HT-induced coagulopathy, the name *Acute Coagulopathy of Trauma-Shock* (ACoTS) has been proposed [16]. This name will be used in the following text. Definition, incidence and possible causes of ACoTS, as well as the impact of acidosis, dilution/consumption and hypothermia on the coagulation system are briefly described below.

1. **Acute Coagulopathy of Trauma-Shock**
   A definition of ACoTS is a PT > 18 seconds (INR >1.6) or an APTT > 60 seconds measured on arrival at the ER. These limiting values have been taken from national British and American guidelines that define coagulopathy during ongoing bleeding [89]. ACoTS is associated with a more severe trauma as measured by ISS. In one study, 11 % of patients with ISS <15 were coagulopathic, compared to 62 % of patients with ISS > 45. In the latter group, mortality was 46 % among the patients with normal coagulation, compared to 84 % for the patients that fulfilled the criteria for ACoTS [91].

In one study transfusion of red blood cells (RBCs) was five times higher among ACoTS patients during the first 24 hours after admission, compared to patients with normal coagulation [97]. Shock, and the hypoperfusion that it creates, seems to be the most important factor in the development of ACoTS. Base Excess (BE) also seems to correlate well with the Coagulopathy. IN the same study, no patients with normal BE on arrival at the ER had prolonged PT/APTT, regardless of ISS score [97]. A theory that has not yet been confirmed is that systemic anticoagulation through protein C activation and hyperfibrinolysis is the key factor behind ACoTS [16, 97].

2. **Coagulopathy caused by acidosis**
   Acidosis has a significant effect on the coagulation process. *In vivo* studies show that activation of FVIIa and generation of thrombin are reduced to 50 %, at a BE of -15 mmol/L [98, 99]. In other studies, both prolongations of clotting time as well as a reduction of clot strength have been seen during acidosis [100, 101]. However, in animal studies where the acidemia has been corrected with buffer solutions, the coagulopathy was not corrected [101, 102]. These findings indicate that acidosis may start other processes in the coagulation and regulation cascades of hemostasis and probably is more than just a physical reduction of the protease activity.

3. **Coagulopathy caused by dilution/consumption**
   Activation of the coagulation system results in consumption of coagulation factors. The most extreme form is disseminated intravascular coagulation (DIC), defined as a coagulation defect related to exaggerated generation of thrombin and fibrin and, at the same time, excessive consumption of platelet and coagulation factors [88]. Just as with ACoTS, DIC is triggered by tissue trauma and shock/tissue anoxia [103]. Even if it has been suggested that DIC and ACoTS are two different entities [16], there is an ongoing debate as to whether ACoTS is just an expression of one of two phenotypes of DIC, namely the fibrinolytic (hemorrhagic) phenotype [104, 105], and some good arguments for this hypothesis have been presented by Gando[106]. However, most of the articles published on the issue of early coagulopathy in trauma patients have been chosen to present ACoTS as an individual entity.
   Transfusion will also cause a coagulopathy by dilution. Maegele et al. [94] showed, in a study of 8,700 trauma patients, that 50 % of the patients were coagulopathic on arrival at the ER if they had received > 3 L of fluid in the prehospital phase. Resuscitation with packed red blood cells (pRBC) will also create a dilution of coagulation factors if it is not supplemented with plasma (FFP) and platelets. There is no consensus on the optimal ratio between pRBC/FFP, and recommendations have differed from a ratio of 10:1 to a ratio of 3:2 [81]. Mathematical models have even suggested a ratio of 1:1:1 for pRBC/FFP/platelets for optimal resuscitation during trauma with ongoing hemorrhage, and there are clinical data that support this hypothesis [107].

4. **Coagulopathy caused by hypothermia**
   HT affects both primary and secondary hemostasis. Wolberg et al. [108] showed changes in primary hemostasis as reduced platelet adhesion and aggregation already at a temperature of 33 °C. On the contrary, enzyme activity and platelet activation in the coagulation cascade were not affected at this temperature, and significant changes could be seen only at temperatures < 33 °C.
   Johnston et al. [109] showed that at a temperature around 33 °C, the activity in most of the coagulation enzymes was reduced to a level that could be compared to 50 % of clotting factor deficiency. These changes had a minor effect on clotting time, as measured by PT or APTT (it is not until a clotting factor deficiency of about 80 % that significant change in PT and APTT occurs) [109], but could be important when superimposed by, e.g., dilution of clotting factors.
   In animal studies, an activation of the fibrinolytic process has been seen when the animals were cooled to very low temperatures (20 °C) [110]. However, at moderate HT, the fibrinolytic process was not activated [86, 111].
   In a study by Valeri et al. [14], local cooling of the skin in baboons to 27 °C more than doubled the bleeding time. However, if the baboon's body temperature was lowered to 32 °C, and if the skin temperature was normal, the bleeding time was also normal. Similar findings were done by Reed et al.[112] in a study on rats, where HT rats had normal coagulation if the blood test was analyzed at 37 °C, and NT rats had a coagulopathy if their blood samples were analyzed at low temperatures. These findings support the assumption that HT acts "locally" on the coagulation process, and that these changes are reversible upon rewarming, but also that analysis for HT-induced coagulopathy must be performed at the actual body temperature of the patient.

1.5.4 Interactions that aggravate an induced coagulopathy

It is important to emphasise that the causes for coagulopathy, some of which are described above, interact in an intricate way. As an example, there is an additive effect by HT´s slowing of the enzyme speed of the coagulation process, as described above, and dilutional coagulopathy [113]. Other interactions are more complex as that between red blood cells and platelets. Usually, platelets flow at the vascular margin, and this radial transport is important for their adhesion to damaged sites on the vessel wall. However, when Hb falls below 100 g/L, the marginalization of platelets will be interrupted, and this will affect primary hemostasis [98]. Moreover, a fresh unit of pRBC has a BE of -20 mmol/L; after 6 weeks BE has changed to -50 mmol/L. This is usually not a problem, but in a trauma patient, with ongoing bleeding and hypoperfusion, repeated transfusions with such blood can boost an acidosis that, in the end, will aggravate the coagulopathy [98].
To summarise, there seems to be some key factors to account for in order to avoid traumatic coagulopathy. The most important is probably to avert uncompensated hemorrhagic shock and the hypoperfusion that it creates. However, to reduce the amount of fluids/transfusions given and at the same time keep an acceptably high Hb is also crucial. Again, these goals will be a challenge to reach when treating trauma patients with uncontrolled hemorrhage.

1.6 HEMOSTATIC DRUGS IN THE TREATMENT OF TRAUMATIC BLEEDING

In 1999, Kenet et al. [114], in a brief case report, described the rescue of a 19-year-old soldier. After a severe high-velocity rifle injury to the vena cava and adjacent paravertebral muscles, the soldier developed the classical signs of "the bloody vicious cycle" with acidosis, hypothermia and dilution/consumption coagulopathy. The vena cava was ligated successfully, but diffuse bleeding continued at a rate of 300 mL/min. Repeated attempts with packing were unsuccessful, and a fatal outcome seemed inevitable. In a desperate attempt to control the bleeding, recombinant factor VIIa (ReFVIIa) was given, and 10 minutes after injection bleeding decreased to 10 - 15 mL/min. After a repeated dose of the drug one hour later, the bleeding stopped promptly, and the coagulation test returned to normal values.

This report was the start of an extended use of ReFVIIa as a "rescue" drug for refractory coagulopathy in trauma patients and maybe also the start of a growing interest in pharmacological treatment of trauma-induced coagulopathy overall.

ReFVIIa has probably rather few side effects. There are, however, reports on thromboembolic complications that cannot be ignored [115]. Moreover, the drug is expensive and, hence, there has been growing interest in investigation as to whether other hemostatic drugs have any place in the treatment of traumatic bleeding.

1.6.1 Tranexamic acid

Tranexamic acid is a synthetic lysine analog and acts as an effective inhibitor of fibrinolysis by blocking the lysine-binding sites on the plasminogen molecule. This blocking will inhibit the formation of plasmin and therefore inhibit fibrinolysis [116]. Numerous studies on tranexamic acid have shown its effect on reducing bleeding during acute or elective surgery without any serious adverse effects, and these findings were confirmed in a cochrane analysis in 2007 [116]. However, few studies have been done on the effect of tranexamic acids on uncontrolled bleeding during hemorrhagic shock. A cochrane analysis in 2004 [117] concluded that only 2 RCTs had been done, with a total of less than 100 participants. None of these studies were high-quality enough for them to be used for the analysis.

1.6.2 Desmopressin

Desmopressin (DDAVP) is a synthetic vasopressin analogue. It is a selective V_2 receptor agonist, thus having antidiuretic effects, but lacking the vasopressor effects of vasopressin [118]. DDAVP acts via endothelial V_2 receptors to increase plasma concentrations of FVIII and the von Willebrand factor. It also activates the release of t-PA and has a vasodilatory effect [119]. DDAVP seems to have little effect on platelet count or aggregation, but enhances platelet adhesion to the vessel wall [120].

DDAVP has been used since the mid-1970s to treat mild hemophilia type A, von Willebrand disease and congenital or acquired platelet dysfunctions [121, 122]. It has also been used on patients without preexisting bleeding disorders. In some studies, reduction of blood loss and transfusion requirements during elective surgery has been seen [123]. However, a cochrane database analysis in 2004 [124], which reviewed 18 trials on this issue, could not confirm these findings. There are no studies on the use

of DDAVP for traumatic bleeding disorders [125]. There is, however, a theoretical benefit of DDAVP for the HT-induced impairment of primary hemostasis and one in the *in vitro* study by Ying et al. [126] that have shown promising results in reversing this coagulopathy.

The side effects of DDAVP are few and usually mild. Fluid overload and hyponatremia have been reported in < 0.01 % of patients treated [118]. The risk for thrombosis seems to be small [119].

2 AIMS OF THE STUDIES

The overall aim of this thesis is to investigate coagulation changes during HT as well as bleeding patterns during uncontrolled hemorrhagic shock both at normothermic and hypothermic conditions. We wanted to investigate in what way HT provokes rebleeding and if it affects bleeding size, but also observe rebleeding patterns overall using a "one-vessel penetrating injury" model of uncontrolled bleeding in pigs and rats. In addition, we wanted to investigate if pharmacological treatment – other than recombinant factor VIIa – has any place in the acute treatment of uncontrolled hemorrhage in penetrating trauma. More specifically, the following questions were addressed in these studies:

I. In what way is coagulation affected by HT, as measured by thrombelastography, during volume-controlled hemorrhagic shock, and are these changes reversible during rewarming? In addition, we wanted to study hemodynamic and metabolic changes during hemorrhagic shock superimposed by HT with special interest in the rewarming and post-HT period.

II. Can tranexamic acid reduce the size and number of episodes of rebleeding, induced by a penetrating injury, followed by uncontrolled hemorrhage? We also had an interest to investigate if the number of episodes and size of rebleeding are an important factor for patient outcome, and if these bleeding can be correlated to coagulation changes as measured by thrombelastography.

III. In what way do cooling and HT affect bleeding pattern in a penetrating injury, followed by uncontrolled hemorrhage? We had a special interest to investigate when, how often and at what temperature rebleeding occurred and in what way these findings differed from bleeding during normothermic conditions.

IV. In what way do different resuscitation regimes with crystalloids during HT affect rebleeding in a penetrating injury followed by uncontrolled hemorrhage? We also wanted to investigate if treatment with desmopressin could reduce the number of episodes and size of rebleeding during HT conditions.

3 METHODOLOGICAL CONSIDERATIONS

In this section, brief principal comments on material and methods used will be given. For a detailed description of experimental procedures and relevant technical details, the reader is referred to the M&M section in each separate paper.

However, some methods used will be described more comprehensively, with special consideration of thrombelastography. The development and remodeling of the rat model used in papers III and IV will also be discussed in detail.

3.1 PAPERS I AND II

3.1.1 Animals

Porcine models are well-established for studying HS. Most circulatory functions in the pig are fully developed already at birth, and hemorrhage causes reactive defense mechanisms that are similar to the ones seen in humans [127]. The pigs used in papers I and II were all Swedish landrace; they were about three months old, with a weight of around 21 kg each.

3.1.2 Anesthesia

All animals were premedicated with an intra muscular dose of ketamine. Pentobarbital and atropine were given before tracheotomy. The animals were ventilated on room air, and for the remainder of the experiments, anesthesia was maintained by continuous infusion of ketamine at a rate of about 40 mg/kg/h. Ketamine was used to avoid the vasodilatory effects of general anesthesia [128], but also to mimic acute trauma care, since ketamine is the most widely used anesthetic agent in trauma.

3.1.3 Hemorrhage models and models for cooling and rewarming

3.1.3.1 Paper I

During pilot tests for paper I, a combination of the aortic tear model, as described by Bickel et al. [129], and a placement of flow probes (proximal aorta, distal aorta, renal artery and portal vein) to measure blood flow and to calculate bleeding, as described by Riddez et al. [46, 130], was tested. However, the combination of a traumatic shock caused by the rather long surgical preparation and the hemorrhagic shock that the aortic tear produced, led to such a severe shock that just three of the eight pilot animals lived long enough to be rewarmed after the HT period. Because of these test results, the model was redesigned as a volume-controlled hemorrhage shock model that mimicked the aortic tear model in bleeding pattern. Bleeding volume and the duration that the animals were bled were calculated from earlier studies using the aortic tear model [44]. The cooling/rewarming protocol included a special HT bed with a Plexiglas hood. This bed could best be described as a big incubator, allowing air at any given temperature to circulate around the animal placed in it. Adjacent rewarming was performed by instillation of warm Lactated Ringers solution (LR) in the urinary bladder. Body-warm LR was also given as intravenous infusion.

3.1.3.2 Paper II

In paper II, the aortic tear model was used (for a detailed description of the model, see M&M paper II). The blood flow was measured by two flow probes placed proximal and distal to the "tear" in the aorta. The probes placed at the renal artery and portal vein, mentioned above, were not used in this study, thus decreasing the time for the surgical intervention.

The blood flow probe uses a "transit time flow meter system." Briefly, the system can be described as follows; each probe has two ultrasonic transducers; an ultrasonic beam is sent upstream and one beam is sent downstream; the difference in time for the signals to come back determines the blood flow. This system has been evaluated both *in vivo* and *in vitro* and has low variability and small errors in measurement [131]. The difference in blood flow between the two probes was used to calculate the size of the initial bleeding, to detect rebleeding and to calculate additional blood loss. Blood flow was recorded every 15 seconds during the first five minutes and whenever rebleeding was suspected. At other times, the recordings were done every minute.

The animals in paper II were not actively cooled, but kept at normal room temperature, and their bodies were covered with a blanket during the experiment. However, if the body temperatures of the pigs declined, no active rewarming was performed.

3.1.4 Hemodynamic measurements and blood sampling

A flow-directed thermodilution Swan-Ganz catheter was placed in the pulmonary artery for measurement of cardiac output (CO) and pulmonary artery pressure. A catheter was also placed in the left common carotid artery for arterial blood samples and pressure monitoring. This catheter was also used for exsanguination in paper I. Hemodynamic parameters were recorded every 10 minutes. Blood gases were recorded every 20 minutes in paper I and every 10 minutes in paper II and were measured at 37 °C. Before exsanguination, the blood volume was estimated to be 65 mL/kg. The hemoglobin dilution method, as described by Hahn [132], was used to estimate changes in blood volume over time.

For calculations of oxygen content in arterial (CaO_2) and venous (CvO_2) blood as well as oxygen delivery (DO2) and oxygen consumption (VO2), the following formulas were used:

$$CaO_2 = (1.39 * Hb * SaO_2) + (0.223 * PaO_2)$$
$$CvO_2 = (1.39 * Hb * SvO_2)$$
$$DO_2 = CO * CaO_2 / \text{body weight}$$
$$VO_2 = CO * (CaO_2 - CvO_2) / \text{body weight}$$

The oxygen tension is expressed in kilopascals (kPa) and Hb is expressed in g/L.
SaO_2 = saturation of arterial blood in centesimal.
SvO_2 = saturation of mixed venous blood in centesimal.

Oxygen extraction ratio (OER) was calculated with the formula VO_2/DO_2.

3.1.5 Thrombelastography

Thrombelastography (TEG) was used to investigate changes in coagulation during the experiments. This method was developed by Hartert in 1948 and remained largely a research tool for nearly 40 years. However, during the last 20 years, there has been increasing interest in the method in the clinical setting.

A more modern version of TEG has been developed, called rotation thromboelastometry (ROTEM). This new version has a slightly different structure, but the values measured are essentially the same as in TEG even though they have been given new names. However, ROTEM is easier to manage than TEG and is also more robust and thus more suitable to use in the clinical setting. These devices are, with a mutual name, called viscoelastic hemostatic assays (VHA) [133]. In the following text, only the TEG function and nomenclature will be explained.

In essence, TEG consists of two mechanical parts: a heated cup, where the temperature can be changed as needed, and a pin that hangs freely from a torsion wire. Freshly drawn blood (0.35 mL) is placed in the cup, which moves slowly. When the clot starts to form, a fibrin strand will transfer the movement from the cup to the pin, and these movements will be depicted in a "TEG trace," in which the wideness of the curve reflects the strength of the clot at any given time (fig 4). A width of 0 mm reflects a liquid with very low viscosity (as water) and a width of 100 mm reflects a totally congealed clot.

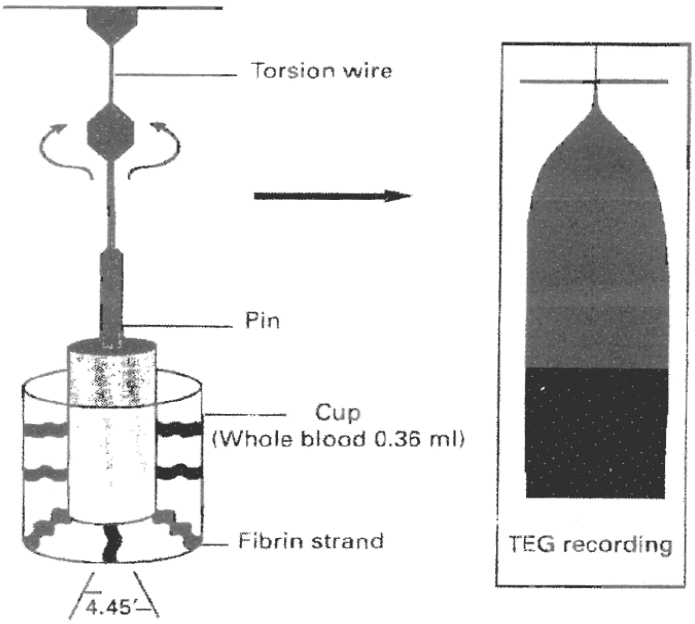


Figure 4 Principles of Thrombelastography
From Mallett, S. V. and D. J. Cox (1992). "Thrombelastography" Br J Anaesth ***69****(3): 307-13.*

The values that are analyzed from the TEG curve will be described below.
The normal range for each value described is for man. The pig blood is hypercoagulable [134], and thus the normal ranges could not be interpreted as similar to the values described in the TEG tests in papers I and II.

r = Reaction time.
The time from sample placement in the cup until the TEG trace amplitude reaches 2 mm. (normal range in man, 6-8 minutes). This time corresponds to the *initiation* phase of the coagulation process.

K = Clot formation time
The time from when the TEG trace has reached 2 mm until it has reached 20 mm. (normal range in man, 3 – 6 minutes). This time corresponds to the *amplification* phase of the coagulation process.

Alpha angle
Angle formed by the slope of the TEG tracing from the r to the K value (normal range in man, 50 - 60°). This angle corresponds to the "thrombin burst" in the *propagation* phase of the coagulation process.

Maximum amplitude (MA)
The greatest amplitude (or width) of the TEG trace (normal range in man, 50-60 mm). This value is a reflection of the absolute strength of the fibrin clot.

A_{60}
Amplitude (or width) of the TEG trace 60 minutes after MA was reached. The clot lysis index (CL60) is derived as A_{60}/MA * 100 (%) (normal range in man, >85%). This value reflects "remaining" strength of the clot after 60 minutes. Another way to define clot lysis is by "Ly60," where 0 % represents no lysis at all and 100 % represents total lysis of clot.

Coagulation Index (CI)
An index derived from r, K, MA and Alpha angle. Normal values in man are between -3.0 and +3.0. Positive values over +3.0 indicate that the sample is hypercoagulable; lower values than -3.0 indicate hypocoagulability.

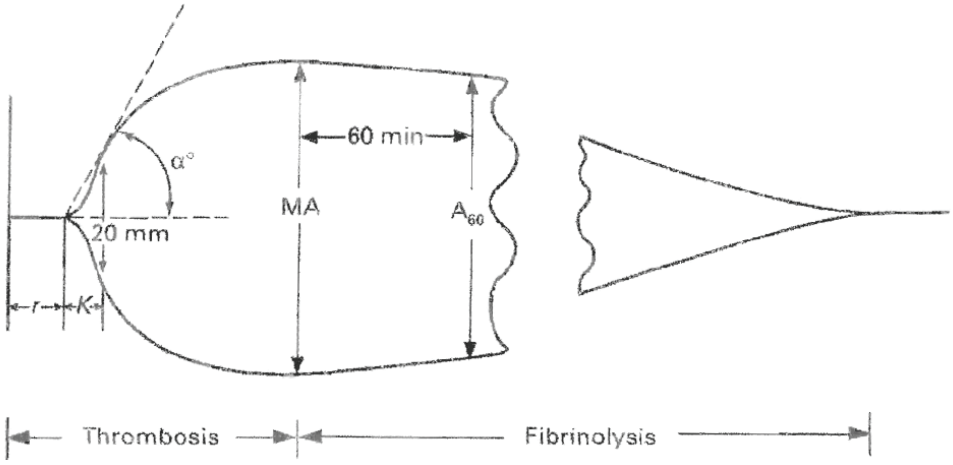


Figure 5 Analysis of the TEG curve. For explanation see text.
From Mallett, S. V. and D. J. Cox (1992). "Thrombelastography"
Br J Anaesth ***69****(3): 307-13*

Investigations with electron microscopy have demonstrated that the *r-phase* correlates with the *initiation* phase in the coagulation process and that the *K-phase* correlates to the *amplification* phase [135]. Other studies have demonstrated that the alpha angle corresponds to the "thrombin burst" during the *propagation* phase and determines the strength and stability of the clot [136, 137]. Moreover, enhanced fibrinolysis, which could contribute to bleeding during traumatic coagulopathy, could be identified by VHA. However, more sophisticated mathematical methods to evaluate clot lysis than those available from the thrombelastographs used in papers I and II (TEG 3000, TEG 5000) are probably needed to get precise information on the fibrinolytic process [138].

It is important to emphasise that VHA does not measure primary hemostasis; thus, factors that influence platelet aggregation and adhesion to the endothelium cannot be detected by this method.

A "celite" activator was added to the blood samples during TEG tests in paper I and paper II. This is a reagent similar to those used for the APTT tests, but much less potent. This activator speeds up the coagulation process, and the results are obtained in half the time of a normal test. There is no difference in interpreting the results. The range for normal values is, however, different from normal tests.

3.2 PAPERS III AND IV

When setting up the experiments for papers III and VI, we wanted to be sure to have a reproducible uncontrolled bleeding model to work with. We also consider it important that the cooling and rewarming process could be controlled easily. Because of the rather disappointing results from the pilot test done in paper I and the considerations mentioned above, the decision was to use rats as laboratory animals in these experiments.

3.2.1 Animals

Rats have been used extensively in hemorrhagic shock studies, and hemodynamic and other data from these studies have been accepted as relevant for scientific use. Studies have also shown that the immune response to trauma and hemorrhagic shock seen in rats in many parts resembles those seen in man [139]. Rats are also preferable from a practical and ethical point of view and have less generic variability than larger animals. However, detailed monitoring of cardiovascular and metabolic changes is harder to achieve compared to larger animals.

The animals used in papers III and IV were all male Sprague-Dawley rats; about three months old and weighing around 300 grams each.

3.2.2 Anesthesia

Fentanyl/droperidol, or fentanyl /fluanisone, just as ketamine/xylazin, are common rat anesthetics. The combination ketamine/diazepam or ketamine/midazolam is more seldom used. However, when tested, all common rat anesthetics but the combination of ketamine and a benzodiazepine have detrimental effects on the cardiopulmonary system, the most important of which is maybe profound hypotension that will be induced at higher doses [140, 141]. Because of these findings, we decided to use

the combination ketamine/midazolam as anesthetics. The analgesic effect of this combination is weaker, and the ketamine dose has to be higher than with the combination ketamine/xyazin. To be able to titrate the right dose of ketamine, a continuous intravenous infusion was chosen. This approach is even more important during hemorrhagic shock, as pH changes in the blood, from the upper to the lower normal level (7.45 to 7.20) can change the required dose of ketamine with over 100 %, to achieve a sufficient anesthesia [142].

One of the side effects with ketamine is increased salivation from the airways. In paper III, animals received atropine just once upon induction of anesthesia. However, this approach proved to be insufficient. When we performed the animal experiments presented in paper IV, we changed from atropine to a more potent and long-lasting anticholinergic drug, glycopyrron (Robinul®), which was given on three different occasions during the ongoing administration of anesthesia.

During the observation period, animals breathed spontaneously without the addition of oxygen. However, in experiments presented in paper IV, a low dose (<0.2 L/min) of oxygen was added.

3.2.3 Model for uncontrolled hemorrhage

The most widely used model for uncontrolled hemorrhage in rats is the "tail amputation model." During the pilot tests, we tried this approach on three animals. However, there was no possibility to detect rebleeding events, as a large clot rapidly formed on the tail stump. Our impression was also that the total bleeding volume was limited from this injury. Some of the authors who have used this model have reported similar problems [76, 143]. Others, like Kentner et al. and Capone et al.[77, 144], used heparin topically to increase the bleeding. Because of these reports and our own findings, the decision was to use a different model for uncontrolled bleeding.

When considering the details in a new uncontrolled hemorrhage model, the possibility to detect and exactly measure rebleeding events was the most important factor to make allowance for. This decision excluded a model with injury to a major vessel in the abdomen, as an open abdomen would have been very difficult to handle during cooling and rewarming procedures. On the contrary, an opening over the femoral vessels in the left groin was easy to protect without interfering with the visual inspection of the vessel. The use of a needle made the injury standard in size, especially as we allowed the needle to pass through the vessel.

3.2.4 Pilot tests

The pilot test consisted of puncture of the femoral artery and/or the femoral vein, with needles of varying diameters. The puncture was done before cooling was initiated or when rats had reached the desired temperature of 30 °C. Rebleeding never occurred from a vein puncture in the eight pilot animals on which this technique was tested. When puncture was done with a 0.5-mm ∅ needle on the animals that were hypothermic (n = 8), the initial bleeding was high (approximately 35 - 40 % of EBV), but total rebleeding volume was small (5 % of EBV). All these animals died during the observation period. With a puncture with the same needle size, before HT was induced (n = 7), the initial bleeding was modest (approximately 20 % of EBV), but the rebleeding volume was high (25 % of EBV). Seventy percent of these animals survived. The observation period differed across some during pilot tests, but was

usually not more than two hours; the resuscitation volume also differed between the animals.

Other approaches were also tested, such as repeated punctures with needles of smaller diameter both before and after cooling. However, the most important findings, described above, indicated that the cooling process seemed to be an important factor in the reoccurrence of rebleeding. Moreover, the approach to start cooling at the same time as the inflicted injury was relevant from a clinical point of view.

3.2.5 Cooling and rewarming

Popular methods to induce HT in rats have been e.g. to cover animals with crushed ice, or soaking the fur with alcohol and then using a fan to increase the evaporation [145, 146]. We tried both these methods, but found out that by placing a thin gauze bandage over the animal and moistening it with water, the evaporative heat loss produced, was enough to create the desired level of HT at 30 °C, in less than 40 minutes. In that way we avoided possible unwanted side effects from the alcohol vapor produced, in the method described above. Rewarming was elementary and normal body temperature at 37.5 °C was achieved in about 30 minutes with the help of a heating pad and a heating lamp.

3.2.6 Hemodynamic measurement and blood sampling

Blood pressure and HR was measured continuously by a pressure transducer connected to a catheter placed in the carotid artery. Blood samples for blood gas analysis (including base excess and serum lactate) were taken as baseline values and thereafter in 45 minutes intervals during the study period. Cardiac output was not measured.

There are several established methods for measuring cardiac output in rats. However, some of these are complicated [147] and may also have an impact on survival or may affect outcome in other ways [148]. Important information is lost when not having these data and DO_2 and VO_2 is not possible to calculate. However, base excess [149-151] and serum lactate [152, 153] are considered good markers for tissue hypoxia. It is our believe that these and other laboratory data received from blood gases, together with basal data from arterial pressure measurement gave us sufficient information to judge possible development and deterioration of hemorrhagic shock.

We considered the use of Thrombelastography in the experiments presented in paper III and IV. However, 4 blood samples for this test correspond to almost 10 % of the EBV in a 300 gram rat and would have nearly tripled the amount of blood taken for blood tests. Our belief is that such a large blood loss might have affected mortality or at least the time for death, especially for the animals that developed hemorrhagic shock during the study period.

3.2.7 Improvement of the rat model

When setting up the rat model, the initial planning was to have an observation period of 2 hours divided in 4 parts, each 30 minutes long (cooling, steady state HT, rewarming and post HT). At the end of each part, blood samples were to be taken and at the beginning of the part called “rewarming,” rewarming should have started. Because of this plan, most of the pilot animals were observed for just two

hours. However, cooling usually took a little more than 30 minutes and because of this observation and other factors a decision was taken that each part of the observation period should be 45 minutes, and hence, the observation time was extended to 3 hours.

During the experiments in paper III, some animals developed breathing problems during the second half of the observation period. Among the animals affected, the fist symptoms, transient whining, could be seen from around 1 hour and 30 minutes. After 2 hours some rats had recurrent symptoms of upper airway obstruction. These changes were observed mainly among animals not cooled and not having major rebleeding. Our guess was that the breathing problems observed contributed to the mortality, especially in the normothermic group. Because of this suspicion, no calculations on mortality were done in paper III. (These problems are also described in the discussion section in paper III).

When preparing for experiments in paper IV, a series of 10 sham animals were performed. These animals were not cooled and not bled, but otherwise the anesthetic procedure described in paper III was used. Seven of the animals had some sort of breathing problems which started on an average of 1 hour and 55 minutes from start of observation. Two of these animals died after an average of 2 hours and 13 minutes.

A series of another 10 animals were performed and during these test it became apparent that the lower parts of hypopharynx had to be cleaned from mucus at regular times. This procedure could only be done in a standardised way, to be able to reach the area that had to be cleaned (Fig 6.) and the blood pressure usually rose intermittently during this intervention. Because of this, cleaning was performed at two set times, just after preparation had been finished and at a second time, 1 hour and 45 minutes into the observation period. If this intervention provoked rebleeding, which happened once, the animal was deleted from the experiment.

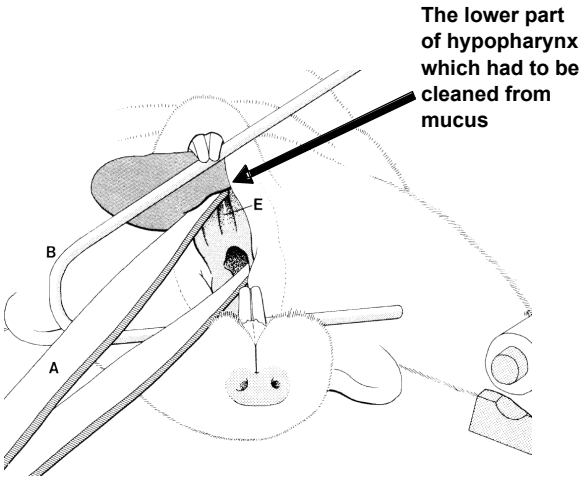


Figure 6 Procedure for suction of mucus from lower hypopharynx
A = Surgical forceps for lifting the tongue
B = Modified paperclip to fix the jaws in an open position
E = Vocal cords
From van Dongen J.J. et al. (1990) "Manual of microsurgery on the laboratory rat"

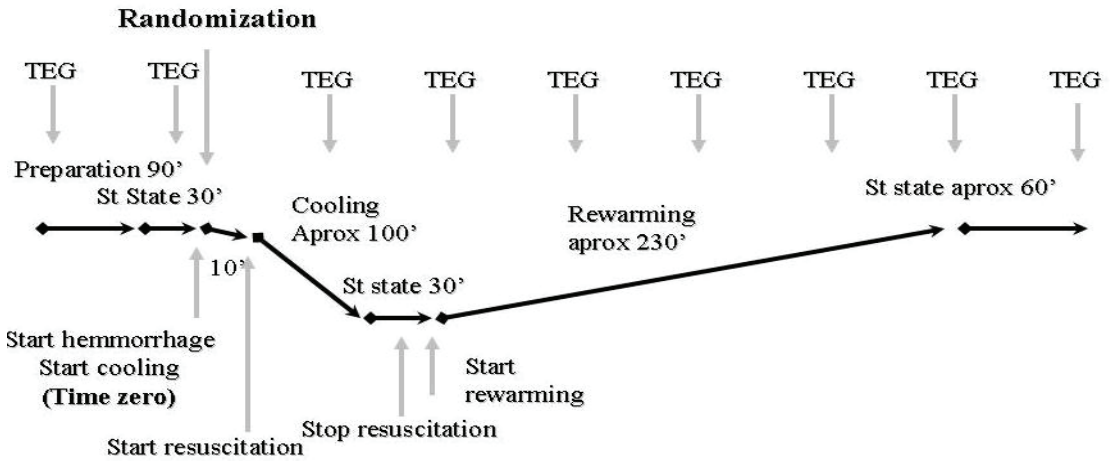
In conclusion, three modifications were done to solve the problem with accumulation of mucus in lower hypopharynx.

1. Suction of lower hypopharynx at regular times as described above.
2. Atropine was replaced with glycopyrron as mentioned earlier.
3. Oxygen was added and was provided in an open catheter at a low dose (< 0.2 L/min). This dose was titrated to ensure that no effect on blood pressure was seen [154].

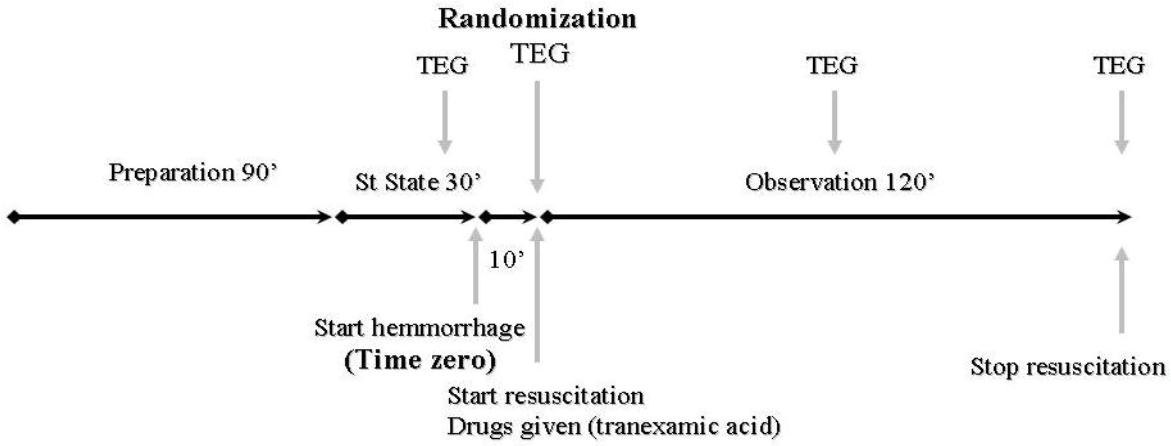
Sham animals (n = 8) were included in the randomization process during experiments for paper IV. No or minimal breathing problems were observed and none of the rats in this group died during the observation period.

3.3 STUDY DESIGN PAPER I – IV

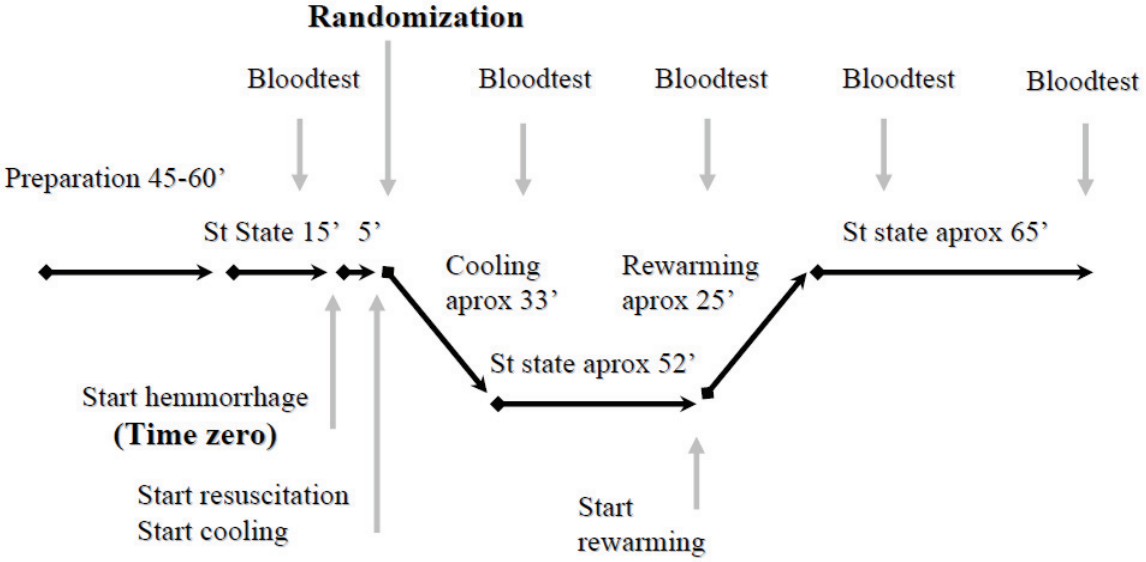
Study I Pigs randomised to HT (n = 10) or NT (n = 8). Volume controlled hemorrhage to 40 % of EBV during 5 minutes. Resuscitation with lactated Ringers solution (LR) 3 times the bled volume was given for 2 hours. HT animal were cooled to 32.5 °C and rewarmed again after 30 min steady state HT. The observation time was 420 minutes.
(TEG = time when blood test for thrombelastography was taken).



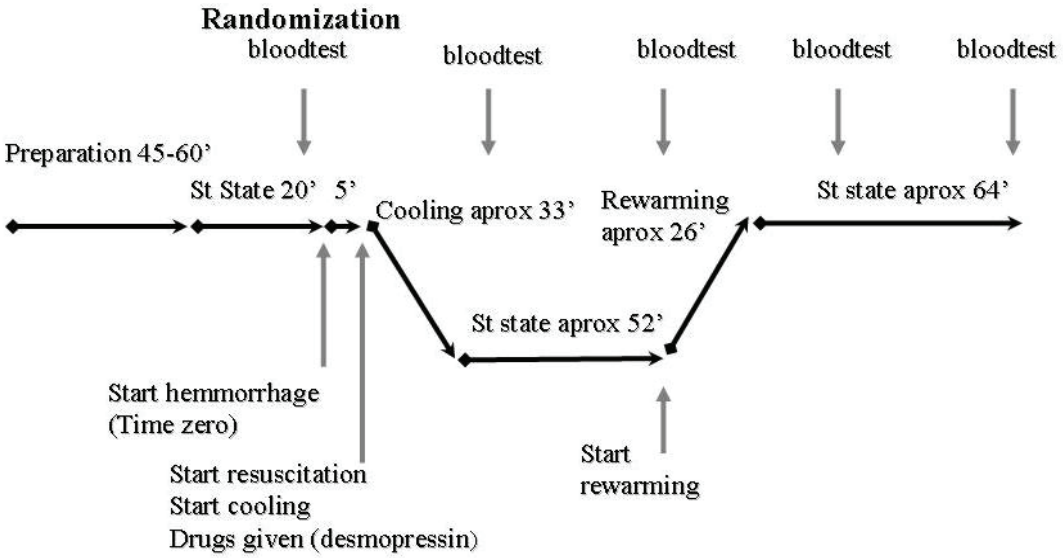
Study II Pigs randomised to tranexamic acid (n = 11) or placebo (n = 12). Uncontrolled hemorrhage was initiated by the "aortic tear" with an initial bleeding of approximately 25 - 30 % of EBV during 4 minutes. Drugs or placebo was given 10 minutes after aortic tear. At the same time resuscitation started. LR 100 mL/kg (approximately 2000 ml) was given during 110 minutes. The observation time was 130 minutes.



Study III Rats randomised to HT (n = 20) or NT (n = 20). Uncontrolled hemorrhage was initiated by puncture of the femoral artery with an initial bleeding of approximately 25 % of EBV during 2 ½ minutes. Resuscitation started at the same time as cooling. LR up to 4 times calculated bleeding was given at a rate of 15 mL/hour. Maintenance dose before and after resuscitation was 3 mL/h. HT rats were cooled to 30 °C, rewarming started at 90 minutes. The observation time was 180 minutes.



Study IV Uncontrolled hemorrhage was performed in the same way as in study III. Rats (n = 4 x 15) were randomised to 4 resuscitation groups, receiving intravenous infusion of LR as follows: *HRe* (15 mL/h, 4 times calculated bleeding), *MRe* (8 mL/h, 2 times calculated bleeding), *MRe + desmopressin* and *LRe*(1 mL/h for the whole study period). Maintenance dose of LR before and after resuscitation was 1 mL/h. All animals were cooled to 30 °C. The observation time was 180 minutes.



3.4 STATISTICS

Paper I
For statistical evaluation in and between groups, analysis of variance was used. Results are expressed as mean and standard error of mean.
Paper II-IV
The results are expressed as mean and SE or as median and 25^{th} through 75^{th} interquartiles when appropriate. Categorical data were analyzed using χ^2 test. Difference between variables and the influence of time were studied by analysis of variance in paper II.. In case of a skewed distribution the Mann-Whitney test was used. When comparing 3 or more groups, Kruskal-Wallis test was used to analyze differences between variables having a skewed distribution and one-way ANOVA followed by the Scheffe´s test was used for data with normal distribution. The linear correlation between parameters was studied by simple regression.
In all 4 papers, $p < 0.05$ were considered statistically significant.

4 PRINCIPAL RESULTS

In this section, key findings of each paper are presented with particular attention given to the questions posed in the "aims of the studies" section. Figures and tables are referred to by the roman numeral of each paper. For access to this information and other results, the reader is referred to the "results" section of each paper. However, a few figures will be reproduced in this chapter. Some additional results (including new figures and tables), not included in the separate papers, will also be presented.

4.1 PAPER I: INDUCED HYPOTHERMIA AND REWARMING AFTER HEMORRHAGIC SHOCK

In this study, pigs in two groups were tested against each other: HT (n = 10) and NT (n = 8).

4.1.1 Hemodynamic and metabolic changes

Exsanguination of 40 % of the EBV between three and five minutes produced a significant rise in HR and depression of MAP (Fig. **I:** 2, 3), indicating the induction of a profound shock. In both groups, DO_2 decreased ($p < 0.01$) from normal baseline values, to low values 10 minutes after exsanguination (HT 7.9 ±2 vs. NT 9.4 ± 4 mL O_2/min/kg). During resuscitation, DO_2 slowly increased in both groups with a maximum at two hours, when fluid resuscitation was stopped, thereafter decreasing again, reaching low values, with no difference between groups, at the end of the study period (HT 8.5 ±3 vs. NT 9.4 ± 3 mL O_2/min/kg).
Induction of HT produced a decrease ($p < 0.01$) in VO_2 among the HT animals, with the lowest level reached at 80 minutes after exsanguination. This change was not seen in NT animals and resulted in a significant difference between groups. Among HT animals, VO_2 increased when rewarming started and nearly reached baseline values at the end of the rewarming procedure. VO_2 remained stable among NT survivors, while decreasing among the three animals in the NT group that later died. No difference between groups was seen during the last hour of the experiment (Fig. **I:** 6).

Because of the decrease in DO_2 directly after exsanguination, the OER more than doubled in both groups ($p < 0.001$) compared to baseline values. However, the decrease in VO_2 led to a nearly normalised OER among HT animals at 120 minutes. These changes were not seen in the NT group and led to a difference between groups during the first two hours after hemorrhage ($p < 0.05$). During rewarming, OER increased among HT animals, following the increasing oxygen demand in this group. During the last three hours, the OER values were nearly doubled from baseline values, without any differences between groups (Fig. **I:** 7).

No significant differences between groups were seen in BE during the entire observation period; however, non-survivors among NT animals had a significantly lower BE during the last half of the observation period (Fig 7 a). Cooling-induced hypokalemia in the HT group led to a difference between groups during the first two hours ($p < 0.01$). During the last three hours of observation, the difference between groups was related to the three non-survivors in the NT group. During the last

hour of the observation period, hyperkalemia had developed in both groups (Fig. **I:** 9). Three animals in the NT group and one animal in the HT group died during the observation time.

Figure 7a)
Base Excess
Time in minutes on x-axis,
mmol/L on y-axis

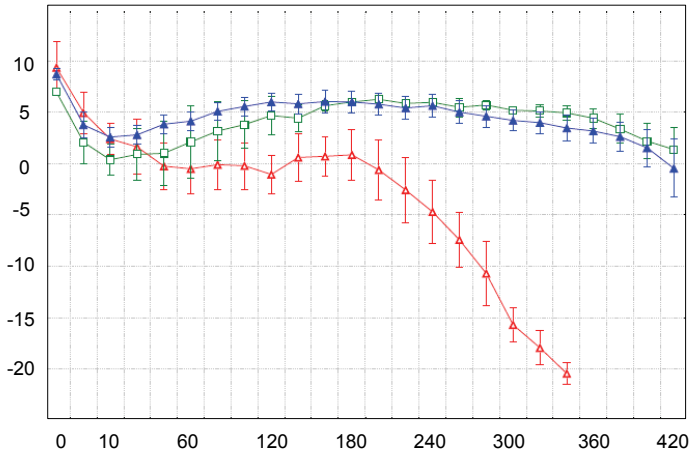


Figure 7b)
Alfa angle
Time in minutes on x-axis, degrees on y-axis

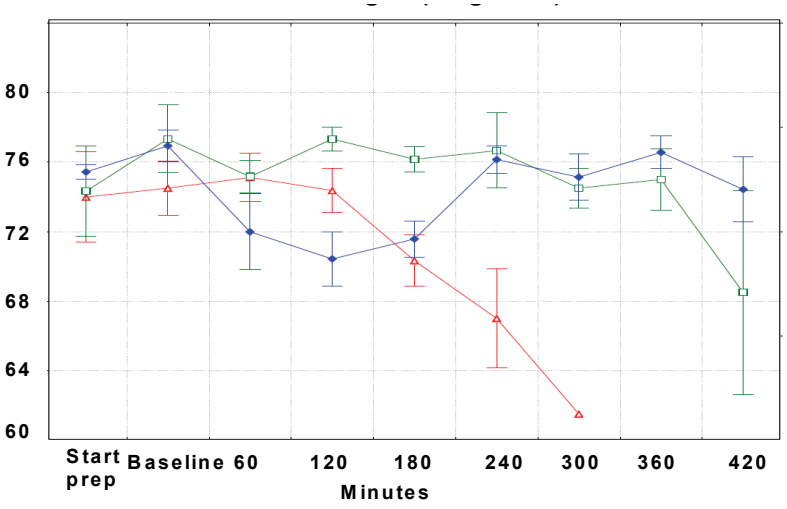
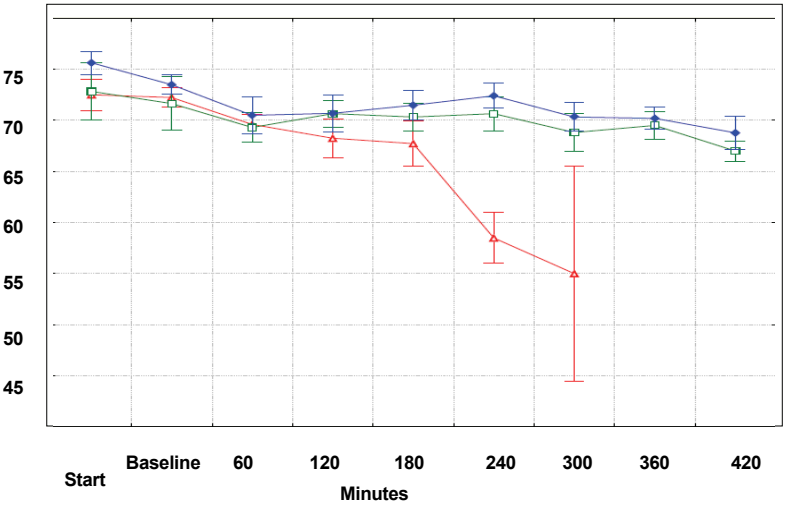


Figure 7c)
Max amplitude (MA)

Time in minutes on x-axis, mm on y-axis



In chart 7 a-c, lines represent:
HT (blue),
NT survivors (green)
NT non survivors (red)
Bars represent SEM

4.1.2 Coagulation changes

HT induced a prolongation of clot formation time as measured by thrombelastography values r-time, K-value and alpha-angle ($p < 0.01$). These changes became significant when the animals had reached a core temperature < 35 °C and were reversed during rewarming (Fig. **I:** 10). In the alpha angle value, there was a difference between groups, when the non-surviving NT animals were excluded ($p < 0.05$) (Fig. 7 b).

The strength of the fibrin clot, as measured by MA, was not affected by HT and no differences between groups were seen (Fig. **I:** 11). Similarity between groups became even more obvious when non-surviving NT animals were excluded (Fig. 7 c). There was a significant reduction of clot lysis as measured by Ly 60 among HT animals. This change was not seen in the NT group, and the difference between groups was significant before rewarming started. (Fig. **I:** 12). Platelet count decreased gradually without any difference between groups.

4.2 PAPER II: TRANEXAMIC ACID DOES NOT PREVENT REBLEEDING IN AN UNCONTROLLED HEMORRHAGIC PORCINE MODEL

In this study, pigs in two groups were tested against each other: Tranexamic acid group (n = 11) and placebo group (n = 12).

4.2.1 Hemodynamic and metabolic changes

The “aortic tear” produced an initial bleeding, which amounted to 35 – 40 % of EBV (as measured by the gravimetric method). This bleeding stopped within four minutes and led to a raised HR and a nearly 50 % decrease in MAP and CO in both groups. These changes resulted in a reduction of DO_2 by more than 50 % ($p < 0.001$ for all changes). After resuscitation started, 10 minutes after hemorrhage, CO improved and HR decreased, but DO_2 remained at sub-baseline levels, mainly because of a continual decline in Hb (Fig **II:** 1,3). During the first 20 minutes of the observation period, BE decreased equally in both groups, with a significant change from baseline values. During the remainder of the observation period, BE was restored somewhat among surviving animals in both groups.

Three animals in the tranexamic acid group and two animals in the placebo group died during the observation period.

4.2.2 Rebleeding and coagulation changes

Sixteen of the 23 animals (70 %) had one or more episodes of rebleeding. Nine of them had their first rebleeding before resuscitation began. Of the pigs that died, 100 % had these early episodes of rebleeding compared to 22 % of the survivors ($p < 0.01$). These episodes of rebleeding were small and because of this, calculations were performed only on rebleeding size and number of episodes of rebleeding detected after resuscitation started (referred to as "late rebleedings" in paper II).

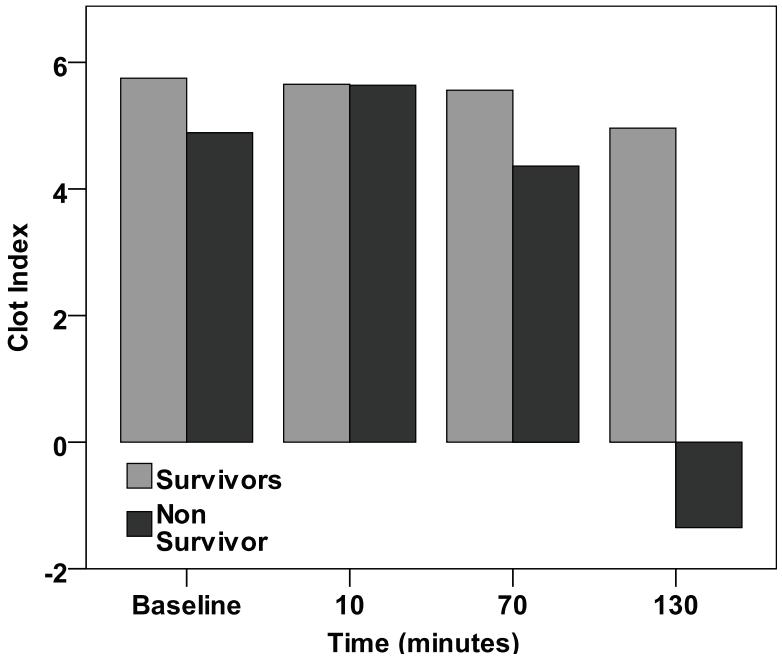
There were no significant differences between the tranexamic acid and placebo group with respect to animals with rebleeding ($p = 0.19$), total number of episodes of rebleeding ($p = 0.18$) or total rebleeding volume ($p = 0.22$) (Table **II:** 1). Moreover, no significant difference between groups could be detected in clot formation time, the stability of the clot or clot lysis. However, in analyzing the clot index, a significant decrease compared to baseline values was seen at the end of the study period among survivors in the tranexamic acid group ($p < 0.05$). This change was not seen in the placebo group ($p = 0.21$). The clot *lysis* index at the end of the observation period was 81 ± 6 % in the tranexamic acid group and 81 ± 2 % in the placebo group ($p = 0.44$) (Fig **II:** 2).

4.2.3 Non-survivors *vs.* survivors

Non-survivors had a higher temperature at the baseline compared to survivors (39.0 °C (range 37.5 – 39.2 °C) vs. 37.2 °C (range 36.8 – 37.9 °C), $p < 0.04$), but no such difference was detected at the end of the observation period (35.2 °C (range 32.0 – 35.7 °C) vs. 35.4 °C (range 34.9°C – 35.9 °C), $p = 0.33$). The hemodynamic profile and blood chemistry, as measured by CO, MAP, Hb and BE, clearly differed between groups from 60 minutes onwards (Fig. **II:** 4). The number ($p < 0.02$) and total volume ($p < 0.02$) of rebleeding was higher among non-survivors compared to survivors (Table **II:** 2). Comparing the last TEG value taken (just before death in non-survivors and at 130 minutes in survivors), there was a prolongation of clot formation as measured by r-time, K-value and alpha angle (all these values $p < 0.01$) in the non-survivors group. There was also a weakening of clot strength (MA) ($p < 0.001$) among those who died, but clot lysis was not affected ($p = 0.40$). These differences between groups could not be detected at the baseline or during the first hour of the study period (Fig. 8). Moreover, there were strong correlations between clot index (correlation coefficient -0.75, $p < 0.001$) as well as total strength of clot (MA), (correlation coefficient -0.83, $p < 0.001$), when these parameters were plotted against total hemorrhage as measured by the gravimetric method, indicating a weaker clot strength and a prolongation of clot formation with higher total bleeding volumes.

Figure 8 Clot index
(Index derived from r, K, Alpha angle and MA values)

There was a significant difference between groups at 130 minutes ($p < 0.01$)



4.3 PAPER III: HYPERTHERMIA INCREASES REBLEEDING IN A NOVEL UNCONTROLLED HEMORRHAGE RAT MODEL

In this study, rats in two groups were tested against each other: HT (n = 20) and NT (n = 20).

4.3.1 Hemodynamic and metabolic changes

Puncture of the femoral artery produced bleeding that amounted to 24 % of the EBV. This bleeding stopped within 2 ½ minutes and led to a decrease in MAP by 60 % in both groups (Table **III:** 2). Start of cooling caused a rise in MAP among HT non-bleeders, and MAP leveled off at higher-than-baseline values in this subgroup of animals. These changes were not seen among non-bleeders or rebleeders in the NT group and caused a significant difference between groups that persisted until rewarming started. Due to recurrent bleeding, there was also a difference between HT rebleeders and HT non-bleeders during the same period ($p < 0.05$) (Fig **III:** 3).

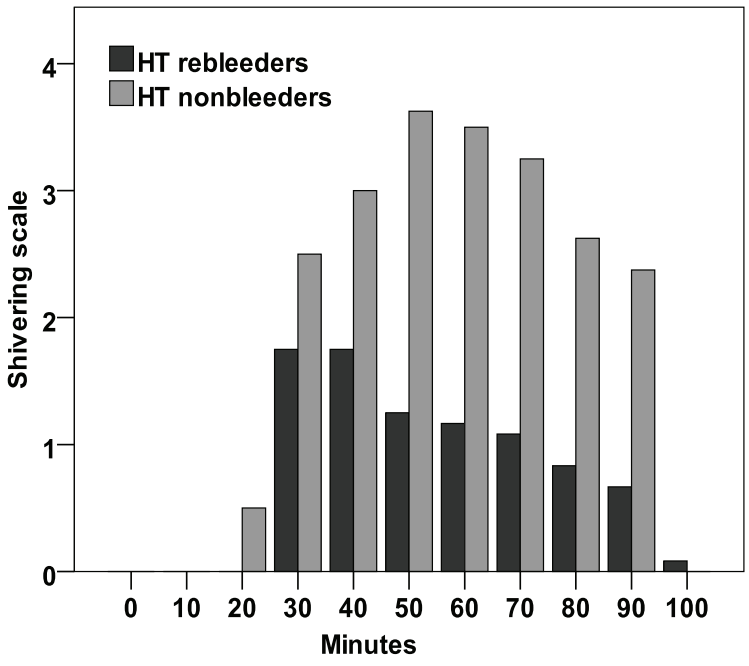
During steady-state hypothermia, the HT animals had higher oxygen saturation and reduced serum potassium values compared to NT animals ($p < 0.01$). These changes disappeared after rewarming.

Shivering was observed in 16 HT animals, but in none of the NT animals. A subjective evaluation of the degree of shivering was done by one person (the author of this thesis) in all experiments. This assessment was measured on a six-degree scale every 10 minutes as follows: 0 = absent, 1 = very little, 2 = little, 3 = medium, 4 = much, 5 = very much. Typically, shivering was more pronounced in animals without rebleeding. Shivering rapidly faded away in all animals as soon as rewarming started (Fig 9).

Figure 9 Shivering observed during the study period
0 = no shivering
5 = maximal shivering

Shivering was only seen among HT animals. Typically, animals with large rebleeding had less pronounced shivering.

Bars represent medium value for all animals in respective subgroup (HT rebleeders and HT non-bleeders).



4.3.2 Rebleeding events

Twenty-five animals (60 %) had one or more episodes of rebleeding. (HT, n = 12; NT, n = 13). The first rebleeding started < 10 minutes from time zero in 22 of these 25 animals (88 %). The HT animals had more episodes of rebleeding ($p < 0.001$), and these cases were more voluminous ($p < 0.01$) and had a longer duration ($p < 0.001$) compared to those in the NT group. This resulted in a higher rebleeding volume in the HT group. (41.3 ± 22.9 % *vs*. 2.5 ± 3.2 % of the EBV ($p < 0.001$)) (Fig 10) (Table **III:** 3).

When studying rebleeding episodes occurring at a temperature > 35 °C (first 15 minutes of study period), there was still a higher rebleeding volume ($p < 0.05$) among HT animals. This observation was, however, only explained by a higher bleeding rate for each bleeding episode in the HT group ($p < 0.05$) (Table **III:** 4). Looking at the entire observation period, duration of bleeding for each bleeding episode was longer in the HT group compared to the NT group ($p < 0.001$); however, bleeding episodes longer than three minutes were only seen at temperatures < 33 °C (Fig **III:** 5).

Rebleeding was much more frequent during the first half of the study period, and only 5 % of all rebleeding cases started later than 90 minutes from time zero (Fig 11). Forty-five percent (9/20) of the animals in the HT group had a total bleeding volume of two-thirds or more of their estimated blood volume. All these animals died between 86 and 127 minutes from start of hemorrhage. A total of 11 HT and 12 NT animals died during the observation period (Fig 11).

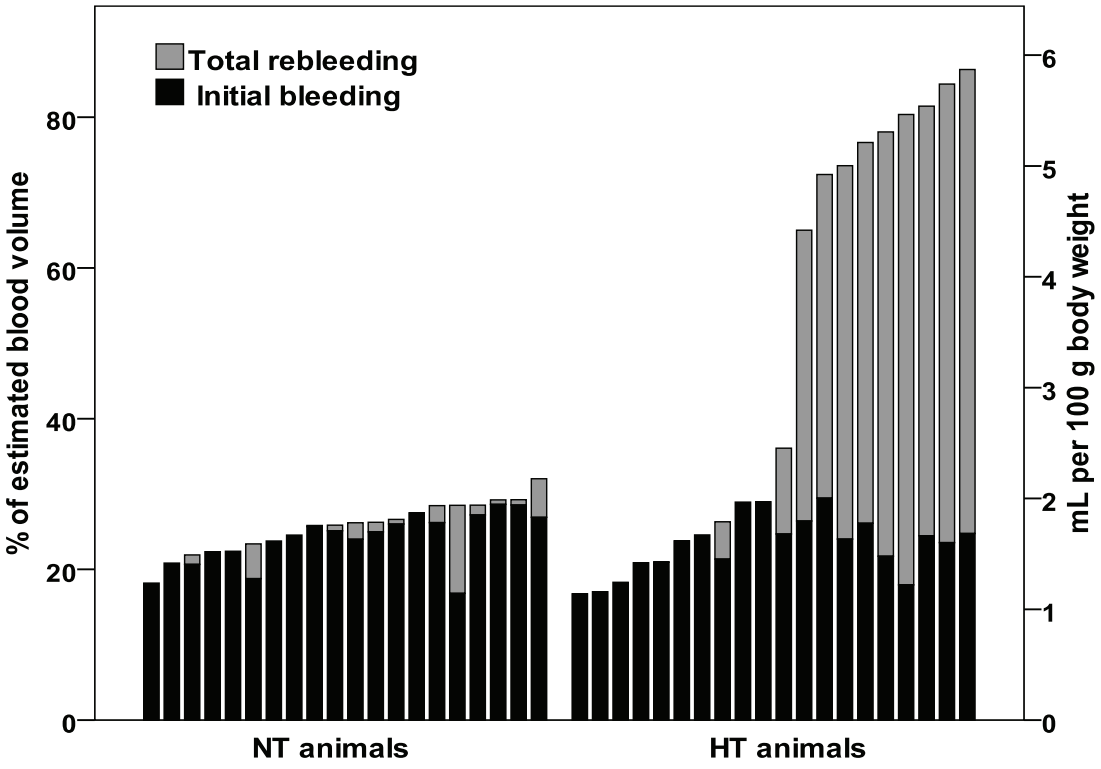


Figure 10 Total bleeding volume in all NT and HT animals divided into initial bleeding (black) and total rebleeding (grey). Each bar represents one animal.

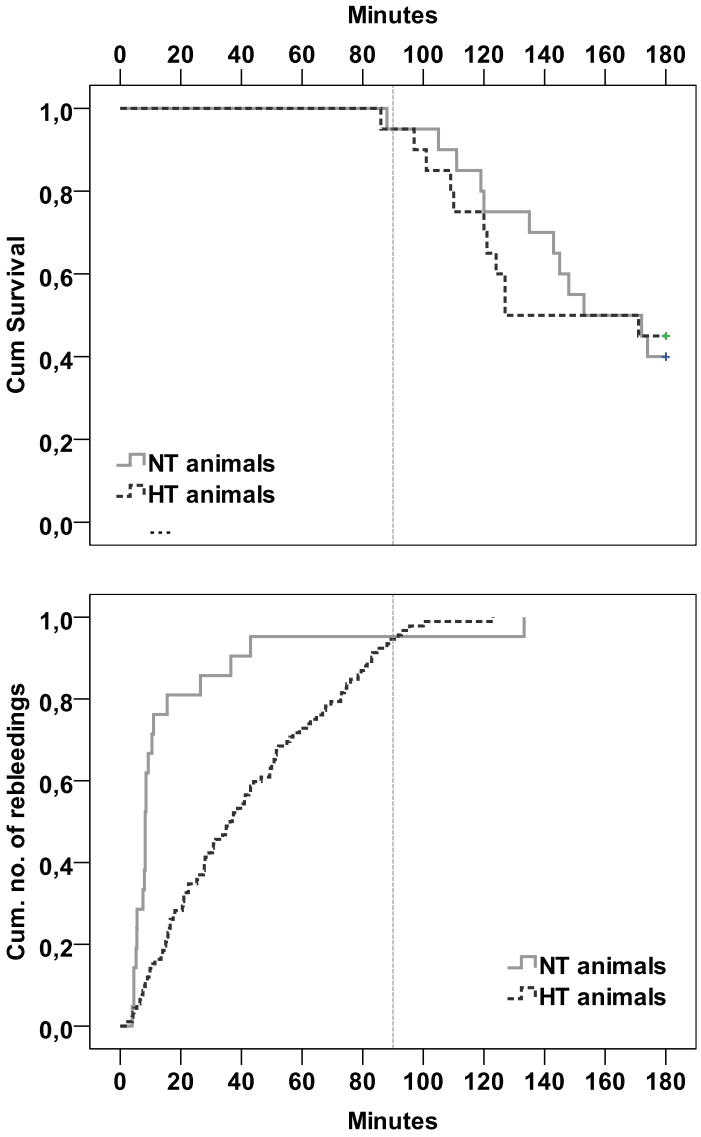


Figure 11 Kaplan-Meier curve for cumulative survival compared with the cumulative number of rebleeding events. There were a total of 113 rebleeding events, 92 in the HT group and 21 in the NT group. Ninety - five percent of all rebleeding started during the first half of the study period.

(Dotted vertical line marks the time for start of rewarming at 90 minutes)

4.4 PAPER IV: EFFECTS OF DESMOPRESSIN AND DIFFERENT INTRAVENOUS FLUID REGIMES ON UNCONTROLLED BLEEDING DURING HYPOTHERMIA

In this study, rats in three groups were tested against each other: low resuscitation (LRe) (n = 15), medium resuscitation (MRe) (n = 15) and high resuscitation (HRe) (n = 15). An additional group, medium resuscitation + desmopressin (MRe + D) (n = 15), was also tested against the MRe group mentioned above.

Puncture of the femoral artery produced bleeding that amounted to an average of 24 % of estimated blood volume. This bleeding stopped within 2 ½ minutes and caused a drop in MAP by 65 %. There were no significant differences between the four study groups with respect to this bleeding.

4.4.1 Desmopressin *vs.* placebo group

The MRe + D group was matched against the MRe group. No statistical differences between groups could be detected in MAP, BE or lactate values over time.

Approximately 50 % of the animals had one or more episodes of rebleeding (Fig **IV:** 6). There was no difference in the number (p = 0.39), size (p = 0.73) or duration of bleeding (p = 0.88). Hence, no difference in total rebleeding volume (p = 0.37) could be detected (Table **IV:** 3). When analyzing rebleeding episodes starting at a temperature > 35 °C, only 14 such episodes were recorded. Again, no significant differences between groups were seen, even though a trend towards higher rebleeding volumes was seen in the treatment group (Table 1).

Table 1.
Rebleeding Characteristics in MRe + D and MRe Animals
Rebleeding Occurring at a Temperature > 35 °C (first 15 minutes of study period)

	MRe+D	MRe	Statistics
No. of animals	6	4	
Numbers of rebleeding cases per animal	1.0 (1 - 2)	1.0 (1 – 2.5)	*p* = 1.0 NS
MAP at start of each rebleeding episode (mmHg)	49 (37 - 60)	46 (39 - 73)	*p* = 0.90 NS
Bleeding volume for each rebleeding episode (mL)	0.36 (0.19 - 0.45)	0.22 (0.15 - 0.26)	*p* = 0.05 NS
Duration of bleeding for each rebleeding episode (min)	1:00 (1:00 - 1:22)	0:45 (0:30 - 1:00)	*p* = 0.08 NS

Figures are expressed as median values and interquartile ranges
NS = Non-significant

4.4.2 Hemodynamic and metabolic changes in the three fluid regime groups

Low (LRe), medium (MRe) and high (HRe) fluid resuscitation groups were compared against each other. Among nonbleeders in each group, MAP was significantly higher in the HRe group compared to the LRe and MRe groups, during steady-state HT (40 - 90 minutes) and post-HT (130 – 180 minutes). Among rebleeders, however, MAP only differed between groups during post-HT, with a significantly lower MAP in the LRe group compared to the two other groups (Fig **IV:** 4).

BE was significantly lower in the LRe group compared to the other two groups at 135 and 180 minutes, and Hb was significantly lower in the HRe group compared to the other two groups from 45 minutes onwards (Table **IV:** 1).

4.4.3 Rebleeding events in the three fluid regime groups

Approximately 60 % of the animals in the three fluid regime groups had one or more rebleeding events, with no significant differences between groups (p = 0.14). Comparing rebleeders only, the total rebleeding volume was larger in the HRe, compared to the LRe group (p < 0.05). This difference was explained by a longer duration of bleeding in each bleeding episode in the HRe group

($p < 0.001$) (Table **IV:** 2). Even though numerically higher, the rebleeding volume in the HRe group did not reach statistical significance when compared to the MRe group ($p = 0.15$), (Fig 12). There was a trend towards higher mortality in the LRe group ($p = 0.07$) (Fig **IV:** 5).

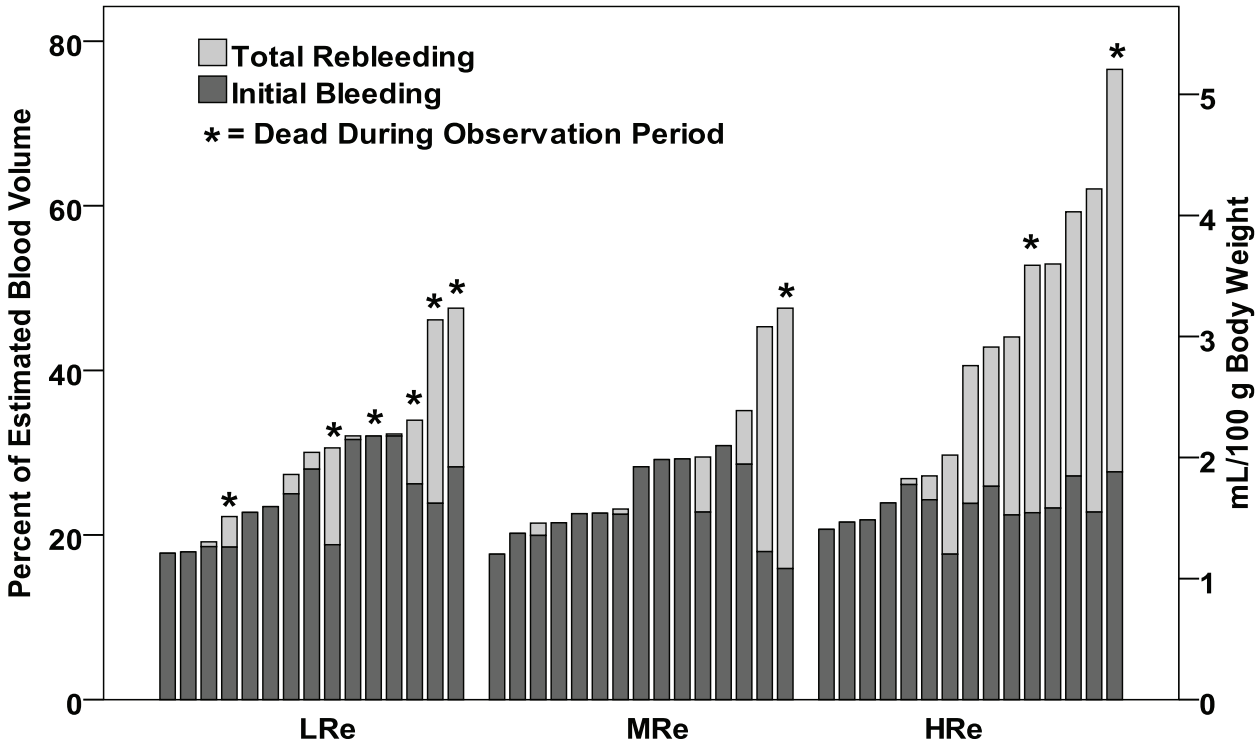


Figure 12 Total bleeding volume in all LRe, MRe and HRe animals, divided into initial bleeding (black) and total rebleeding volume (grey). Each bar represents one animal.

4.4.4 Non-survivors *vs.* survivors

Comparing non-survivors and survivors in all four groups (LRE, MRe, MRe + D and HRe), there was a higher risk of death if the animals had one or more cases of rebleeding ($p < 0.05$). Non-survivors had lower MAP and BE, as well as higher serum lactate levels compared to survivors. These differences were already significant during steady-state HT and became more pronounced over time (Fig **IV:** 7).

Comparing rebleeders only, non-survivors had higher bleeding volumes compared to survivors. The only factor explaining this difference was the number of episodes of rebleeding, which differed significantly between groups (Table 2).

Table 2.

Bleeding Characteristics in Animals With at Least one Rebleeding, From All Groups (LRe, MRe, MRe + D and HRe), Divided into Survivors (n=25) and Non-survivors (n=10)

	Survivors	Non- survivors	Statistics
No. of animals	25	10	
Initial bleeding percent of estimated blood volume	25 (22 - 28)	25 (19 - 27)	p = 0.48 NS
Rebleeding cases per animal	2 (1 - 5)	7 (5 - 11)	p < 0.01
Bleeding volume for each rebleeding episode (mL)	0.40 (0.16 - 0.93)	0.49 (0.24 - 0.91)	p = 0.51 NS
Duration of bleeding for each rebleeding episode (min)	1:30 (1:00 - 2:07)	1:30 (1:00 - 2:30)	p = 0.33 NS
Bleeding rate for each rebleeding episode (mL/min)	0.25 (0.17 - 0.49)	0.30 (0.16 - 0.51)	p = 0.81 NS
Total rebleeding volume percent of EBV	7 (1 - 19)	26 (11 - 38)	p < 0.01
Total bleeding volume (initial + all rebleeding cases) percent of EBV	32 (28 - 45)	48 (33 - 65)	p < 0.05

Figures are expressed as median values and interquartile ranges
NS = Non-significant

5 DISCUSSION

Since the beginning of the 1990s, there has been an ongoing discussion about the risk of excessive early fluid resuscitation in trauma patients with possible uncontrolled bleeding. The debate has been mainly focused on hemodynamic changes, as a rise in blood pressure or blood flow as the main cause for rebleeding and the adverse outcome among such patients [44-46, 155-157]. During the last seven or eight years, there has, however, been a growing interest in the traumatic coagulopathy named Acute Coagulopathy of Trauma Shock (ACoTS). This coagulopathy seems to affect a relatively high proportion of seriously injured trauma patients [16]. ACoTS arises early after trauma and could thus contribute to early rebleeding and to an ongoing hemorrhage that will ultimately lead to the progressive coagulopathy, described by Kashuk et al. [96] as "the bloody vicious cycle." In this trauma triad of death, metabolic acidosis, dilutional coagulopathy and hypothermia are considered to be the main factors for an ongoing bleeding that, in the end, is not possible to stop.

Hypothermia has been described by many as a "double-edged sword." HT´s most desirable effect during hemorrhagic shock is probably the fall in basal metabolism, which will preserve cellular energy and lower the oxygen demand [158]. However, on the other side of the "sword," several side effects emerge, and when treating victims with a possibly uncontrolled hemorrhage, the HT-induced coagulopathy must be considered as one of the most important.

5.1 EFFECTS OF HYPOTHERMIA

In paper I, the induction of HT to 32.5 °C led to a significant reduction of VO_2. This change was reflected by a nearly normalised OER among the HT animals during the HT period. This observation was made despite the fact that shivering was not prevented. One has to consider that the higher saturation of the returning venous blood, at least partially, could be explained by the HT-induced left shift of the oxyhemoglobin dissociation curve. However, Gutierrez et al.[159] studied this problem in a model on dogs cooled to a temperature of 31 °C and could see no impairment in oxygen delivery in the HT group. Another observation that supports the assumption of a "true" decreased oxygen demand among the HT animals is the BE values, which were unchanged until the last hour of the study period. However, unchanged BE values were also observed in all but three animals in the NT group. Those three animals, like the one animal in the HT group, developed low BE values during the second half of the study period and later died. The observed inter-individual difference could be explained by the fact that individual animals react differently to the same insult. Crowell et al. [160] showed in a study on 100 dogs that the estimation of accumulated oxygen dept over time (the difference between basal oxygen consumption, recorded before the insult, and the "suboptimal" oxygen consumption recorded during hemorrhagic shock) is a very exact method with which to predict mortality. Moreover, he observed that different animals had to be exposed to the same insult (in his study, pressure-controlled hemorrhagic shock) for a highly different period of times to reach the desired accumulated oxygen dept, thus explaining why there is a difference between individuals exposed to the same insult during equally long periods. The use of accumulated oxygen dept as an exact

predictor for mortality has later been confirmed in other canine and porcine models [161, 162]. Moreover, studies including more than 8000 trauma patients have shown that BE and, to a lesser extent serum lactate, correlates well with oxygen dept and can be used to predict outcome among trauma patients in hemorrhagic shock [163].

Even if the observed lower oxygen consumption among HT animals in paper I may have prevented some deaths in this group, it is important to emphasise that there was no statistical difference in mortality between groups. It is also interesting to notice that there seemed to be no difference between groups considering hemodynamic and metabolic changes after rewarming, including the BE values. Hence, for surviving animals in the HT group, there seemed to be no short-term advantages or any obvious disadvantages with the hypothermic period.

5.2 COAGULATION CHANGES MEASURED BY THROMBELASTOGRAPHY

Over the last two decades, viscoelastic hemostatic assays (VHA) (i.e. TEG & ROTEM) have been used rather extensively in the peri- and postoperative setting to detect nascent coagulopathy. More than 20 studies, including over 4,500 patients, have been published on this matter. All these studies have reported VHA to be as good as or better than the standard coagulation tests in identifying coagulopathy and to guide transfusion therapy [133]. VHA has also been evaluated in trauma patients, resulting in about 10 studies on a total of 700 patients. In several of these studies, about 60 % of the moderately injured trauma victims have been shown to exhibit *hyper*coagulability, a state that the standard coagulation test could not detect [87, 164]. When detecting *hypo*coagulability, some studies have found consistency between VHA and standard coagulation tests [93], whereas others indicate that VHA maybe is better to detect fatal traumatic hypocoagulability [165].

It is, however, important to emphasise that VHA does not measure the primary hemostasis; thus, factors that influence platelet aggregation and adhesion to the endothelium are not detected by this method.

In paper I, a significant reduction of clot formation time was seen among HT animals at temperatures < 35 °C. These changes were reversible during rewarming. No effect on clot strength, as measured by MA, was seen during HT. These findings correspond with results from other animal studies [111]. Similar changes have also been seen during cardiac operations [166] and in trauma victims. Watts et al. [86] examined 112 hypothermic trauma patients with TEG and concluded that ≤ 34 °C was the temperature where coagulation enzyme speed significantly decreases. The final clot strength, as well as the fibrinolytic activity, was, however, not affected by HT in any of these studies. Moreover, Bernabei et al. [167] measured perioperative blood loss in 122 trauma victims and found that a body temperature < 35 °C was associated with significantly higher bleeding volumes, even when the same ISS scores were compared.

In paper I, there were indications that clot lysis was prevented by HT. We measured the fibrinolytic activity with a simple index (Ly 60). However, this method seems to be rather insensitive [138], and analysis of the results should be done with some caution.

In paper II, when including all animals and comparing survivors and non-survivors, animals body temperature spontaneously decreased and reached 35 °C at the

end of the study period, without any differences between groups. Even among survivors, a significant reduction in clot index could be seen, compared to baseline values. Whether this, at least partly, was a result of the acquired HT is difficult to estimate. However, clot strength, as measured by MA, was affected in both groups, and this observation indicates that factors other than HT contributed to the coagulopathy.

The most pronounced coagulopathy developed among non-surviving animals in both studies. These changes seemed to correlate well with low BE values (Fig 7a-c, Fig **II:** 4), indicating that a metabolic acidosis contributed to the coagulopathy seen among these animals.

Acidosis affects the coagulation system in a different way from HT. As an example, HT inhibits fibrinogen synthesis, whereas acidosis accelerates fibrinogen degradation [168]. There also seems to be an additive effect of the two parameters [169, 170]. Moreover, animal studies have shown that coagulopathy, generated by induced acidemia in otherwise healthy animals, is not reversible by just correcting the acidemia [102]. These findings indicate that acidosis starts other processes that are not fully understood in the coagulation system. If the coagulopathy mentioned above could be related to ACoTS, which has been described to be associated with decreasing BE values, could neither be confirmed nor rejected by our findings.

A dilution coagulopathy was certainly added to the changes mentioned above. A dilution, as measured by the Hb value, was most obvious among the non-surviving animals in paper II. These animals also had the most excessive bleeding. Studies show that dilution impairs coagulation both by platelet reduction and reduced concentrations of clotting factors. The first coagulation factor decreasing to pathologically low levels seems to be fibrinogen [171, 172], and it is plausible that this deficit interacts with the changes caused by HT and acidosis, described above, to further deteriorate the impaired coagulation.

In a state of uncontrolled hemorrhage, it seems obvious that the development of an aggravated coagulopathy as described above interact in a vicious cycle with ongoing bleeding, which will result in larger bleeding volumes in the end. The strong correlation, showed in paper II, between total bleeding volume and impaired coagulation as measured by the clot index and MA supports this assumption.

5.3 THE HEMOSTATIC EFFECTS OF TRANEXAMIC ACID AND DESMOPRESSIN (DDAVP)

In paper II, the effect of tranexamic acids on rebleeding was tested. There were no significant differences over time, between the tranexamic acid group and the placebo group, in any of the TEG values. These findings were reflected by an insignificant difference in bleeding volume between groups.

Tranexamic acid has proven to be an effective drug to prevent bleeding during acute and elective surgery, and this statement was confirmed in a cochrane analysis in 2007 [116], analyzing 211 randomized controlled trails, with over 20,000 participants. On the other hand, tranexamic acid's impact on traumatic bleeding has been sparsely investigated [117]. However, the theoretical benefit with an inhibitor of fibrinolysis should be obvious, as hyperfibrinolysis seems to be an important factor in early traumatic coagulopathy [97]. There have also been case reports showing that tranexamic acid is beneficial during such circumstances [173]. There are, however, other

reports showing that hyperfibrinolysis is present in only 3 to 6 % of trauma patients arriving at the ER [165, 174], and it is possible that tranexamic acid is effective only in this subgroup of patients.

In paper II, no pigs in the placebo group showed signs of fibrinolysis, as measured by clot lysis index, and the results of this index were surprisingly similar between groups ("CL60(%)" Fig. **II:** 2). Even if these figures should be interpreted with some caution [138], they indicate the absence of fibrinolysis among the studied animals and may give an explanation as to the lack of efficacy in the treatment group. It is also possible that tranexamic acid is more effective to stop low-pressure bleeding, hence having a better effect in blunt trauma. Nevertheless, a large RCT investigating tranexamic acid effects on death and transfusion requirements in adult trauma patients was started in 2005 [175]. The aim was to recruit 20,000 participants (sic!), and this goal will be reached in January, 2010, when the study will be closed. Hopefully, this study will give a final answer on the question as to whether tranexamic acid has a place in the treatment of trauma victims with uncontrolled hemorrhage.

In paper IV, we tested DDAVP, instead of tranexamic acid, as a hemostatic drug. The difference in this study, besides the animal model, was the induced HT, creating a rapid decrease in body temperature, followed by steady-state HT and, at the end, rewarming. There were no significant differences between the DDAVP and control groups with respect to bleeding pattern or rebleeding volumes, indicating the drug's inefficiency to decrease the hemorrhage during HT conditions.

DDAVP's lack of effect is maybe less surprising than the results from the tests with tranexamic acid, as DDAVP's impact on surgical bleeding is less convincing in the literature [124]. However, the drug's theoretical benefit for the HT-induced impairment of primary hemostasis is apparent [14, 108, 176], and the results from a recent *in vitro* study have strengthened such a hypothesis [126]. At temperatures between 37 °C and 35 °C, where mainly the primary hemostasis is affected by HT, and where the effect of the drug should be most obvious, we found, somewhat surprisingly, a trend towards higher rebleeding volumes in the treatment group. Even if this result should be interpreted with caution, a possible explanation for the observation could be DDAVP's vasodilatory effect [119]. This and other side effects may have been more pronounced by the high doses given, as ten times the normal dose was administrated. This dose was chosen to secure maximum effect in the shortest possible time and to ensure high enough concentrations even during ongoing rebleeding. The dose given was based on results from two previous studies on rats where these doses have been tested without any obvious side effects [177, 178].

Having said this, the conclusion is that the two drugs tested, tranexamic acid and DDAVP, seem to have no beneficial effects in reducing bleeding volume or rebleeding events under the conditions described above.

5.4 UNCONTROLLED BLEEDING DURING HYPOTHERMIA

A new model for uncontrolled hemorrhage in rats was used for the experiments presented in papers III and IV. The advantage with this model was its high reproducibility and the possibility to detect and exactly measure all rebleeding events.

Rebleeding occurred in about 60 % of all animals in paper III as well as in paper IV, without any significant differences between the groups, indicating that

none of the treatments given to the groups (HT and/or different fluid regimes) induced the first rebleeding. The reasons for this regularity remain unclear.

5.4.1 Paper III

When rebleeding had occurred once, there was a highly significant difference in rebleeding volume between the NT and HT animals in this study. HT increased both the number of cases of rebleeding as well as the bleeding volume in each bleeding episode. The prolongation of bleeding time, seen at temperatures < 33 °C, was not unexpected and can, at least partly, be explained by HT-induced coagulopathy [86, 108, 179].The significantly higher bleeding volumes in the HT group, seen at temperatures between 37 °C - 35 °C, were, however, more surprising. Since only bleeding rate and not the duration of rebleeding was affected, it is likely that hemodynamic changes caused these bleedings.

There was quite a dramatic effect on blood pressure among non-rebleeders in the HT group in this study. This rise in MAP was probably due to a combination of increased CO and peripheral vasoconstriction [180]. Similar changes have been reported by others [143, 145], but not to the extent seen in our study. However, the choice of anesthetics seems to be crucial when trying to mimic a course of events during the first hours in the prehospital setting. Ketamine (which was used both in paper III and IV) is less depressive on MAP than other anesthetics, which may explain the difference between our results and others [181, 182]. Even better than using Ketamine would have been to use non-anesthetized animals, but this is an ethical issue. However, HT´s impact on non-sedated rats in a volume-controlled hemorrhagic shock model has been published [145]. The result from this study showed that cooling, in combination with oxygen, induced quite a dramatic rise in blood pressure.

It seems obvious that hemodynamic changes also contributed to the rebleeding, in the HT group, at temperatures < 35 °C. When looking at separate blood pressure curves in the HT group, there was quite often a distinct rise in blood pressure after rebleeding had stopped. Such changes were not seen in the NT group (data not shown). Our conclusion is also supported by the significantly higher start MAP for each rebleeding episode, as seen in the HT group.

5.4.2 Paper IV

Rebleeding volume was significantly higher in the high resuscitation group (HRe), compared to the low resuscitation group (LRe). No significant differences in relation to the medium resuscitation group (MRe) were seen. A correlation, however not strong, gave some support to the assumption that higher resuscitation volume also gives higher rebleeding volumes. Interestingly, the higher rebleeding volume in the HRe group was essentially explained by an extended rebleeding time and not by an increase in the number of rebleeding cases. The reason for this is unclear, but can maybe be explained by a dilutional coagulopathy [94]. The LRe group did worse when compared with the other two groups. This was reflected by lower BE values and higher serum lactate values, as well as a trend towards higher mortality. Even if the changes in BE and serum lactate were discrete, the observation supports these test's reliability to predict a detrimental outcome [163].

The rise in MAP, especially in the LRe group, was quite impressive and unexpected, as no resuscitation was given to this group. MAP even rose above baseline

levels among LRe non-rebleeders, showing the strong effect of cooling and HT on the hemodynamic system. As a matter of fact, the rise in MAP was much more pronounced in the LRe group receiving minimal fluid resuscitation than among the NT animals in study III, which were given a high resuscitation regime.

Higher bleeding volumes in the HRe groups and a more detrimental outcome in the LRe group indicated that the most favorable regime for resuscitation during HT conditions seems to be the MRe alternative.

When survivors and non-survivors in all groups (including the group that was given desmopressin) were compared, non-survivors had much higher rebleeding volumes than survivors. Interestingly, the only reason for this huge difference in bleeding volume between groups was the number of rebleeding cases. Moreover, there was a significantly higher risk of death if animals had at least one rebleeding. These results, together with the results from paper II, strengthen the assumption that rebleeding, especially when repeated, is detrimental to the outcome during uncontrolled hemorrhage.

5.5 CLINICAL IMPLICATIONS

Results from animal studies should always be interpreted with caution. However, some of the questions asked in this thesis are not possible to investigate on humans, and hence studies in animals had to be done.

The most interesting finding in this thesis, and also the most unexpected, is the HT-induced hemodynamic changes and the impact these changes had on rebleeding. However, an important question to answer is if these findings are relevant for man.

Many studies have investigated cold exposure on non-sedated human test objects (for details, see "The body's reaction on cooling" in the introduction chapter), and most of them show a significant increase in cardiac output and/or blood pressure both during coldwater and cold air exposure [57-59, 61]. A transient rise in blood pressure has been noted even when only the hand or the face has been exposed to cold [183]. These changes seem to be related to increased levels of catecholamines [56] and are expressed with different strengths according to gender and age [60, 61, 184, 185].

Results from most of these studies mentioned above indicate that exposure to cold, at least in a subpopulation of individuals, could have the same, or maybe even worse, impact on the risk for rebleeding, as early high-fluid resuscitation regimes seem to have [46, 186, 187].

Other results in this thesis support the current knowledge that acidosis and dilution seem to interact in an intricate way with HT to worsen an acute coagulopathy. Even if HT-induced coagulopathy seems to be mild, at least at temperatures ≥ 34 °C, and if also reversible on rewarming, its interaction with a developing acidosis and dilution will worsen a coagulopathy and, hence, aggravate the possibility of stopping an ongoing bleeding.

Moreover, our results indicate that recurrent rebleeding is detrimental for outcome. This finding is maybe not so surprising, as it seems logical that a "new" hemostatic plug, forming after every case of rebleeding, will probably be weaker in structure if an acute coagulopathy caused by, e.g., dilution and/or acidosis develops.

To summarize, the findings in this thesis support the current recommendations to rewarm trauma patients with possible uncontrolled hemorrhage as

soon as possible. This statement is even more relevant in the prehospital setting where trauma patients, exposed to cooling and with possible inner bleedings, must be treated as high-risk patients to develop rebleeding. The possible benefits of hypothermia on hemorrhagic shock could not compensate for the risk to develop a traumatic coagulopathy; hence, if trauma patients with uncontrolled hemorrhage arrive at the ER in a hypothermic state, ongoing bleeding must be excluded and rewarming must be initiated. If rewarming is not possible during the prehospital setting and uncontrolled hemorrhage is suspected, a medium infusion regime with crystalloids (< 30 mL/kg/h) is probably beneficial.

The use of tranexamic acid during NT and mild HT conditions, as well as desmopressin, during HT conditions, does not decrease rebleeding in cases of penetrating trauma.

6 CONCLUSIONS

- Hypothermia (32.5 °C) in pigs exposed to hemorrhagic shock induces a coagulopathy that is measurable by TEG as a retardation of clot formation. This retardation is visible < 35 °C and reversible upon rewarming. The final clot strength is unaffected by hypothermia.

- Hypothermia (32.5 °C) in pigs reduces metabolic rate during hemorrhagic shock even when shivering is not prevented. Upon rewarming, surviving pigs seem to have no short-term advantages or any obvious disadvantages from the hypothermic period.

- Tranexamic acid does not reduce the size or the number of rebleeding cases in penetrating trauma with uncontrolled hemorrhage in pigs.

- The number of episodes and size of rebleeding are important factors in the outcome after penetrating trauma and uncontrolled bleeding in pigs. An impaired coagulopathy, visible just before death or at the end of the study period, correlates with higher total bleeding volumes.

- Cooling and hypothermia increase the number of episodes and size of rebleeding in rats with penetrating trauma and uncontrolled bleeding. Hemodynamic changes, as with increased blood pressure provoked by cooling, seem to be important factors causing the higher bleeding volumes seen in the hypothermic group.

- After a short-term follow-up, a medium infusion regime (Lactated Ringers solution 25- 30 mL/kg/h) seemed to be the most beneficial resuscitation for rats exposed to a combination of hypothermia and penetrating trauma with uncontrolled hemorrhage.

- Desmopressin does not reduce the size or the number of bleeding in rats exposed to a combination of hypothermia and penetrating trauma with uncontrolled hemorrhage.

7 SAMMANFATTNING PÅ SVENSKA (SWEDISH SUMMARY)

Bakgrund

Hypotermi (en sänkning av kroppens temperatur till, vanligtvis, mellan 30 °C – 35 °C) har vid djurförsök visat sig öka överlevnaden vid blödningschock (ett livshotande tillstånd på grund av stora blodförluster). Teorin bakom denna skyddande effekt är att hypotermi sänker kroppens basala ämnesomsättning och skyddar organismen under det kritiska skede då inte tillräckligt med syre når fram till vävnaderna. Dessa djurförsök har oftast gjorts i modeller där man blöder djuren på en förutbestämd mängd blod.

Mot dessa kunskaper står erfarenheter från den kliniska verkligheten, där man från stora traumaregister, statistiskt har kunnat räkna ut att hypotermi är en oberoende faktor för ökad dödlighet hos traumapatienter med inre blödningar. Orsakerna till att ”teori och verklighet” inte stämmer överens är okända. En möjlig orsak skulle kunna vara en ökad blödningsbenägenhet hos traumapatienter som blivit nedkylda. Man vet nämligen att hypotermi påverkar blodets förmåga att levra sig, men man vet inte i vilken utsträckning denna försämring av koagulationsförmågan påverkar blödningsstorleken och hur viktig den är för överlevnaden. En försämring av koagulationsförmågan av andra orsaker än hypotermi är nämligen inte ovanlig vid svåra skador med inre blödningar.

Målet med denna avhandling var att studera hypotermins påverkan på risken att få återkommande blödningar vid en standardiserad kärlskada. Vi ville också försöka förstå hur hypotermi interagerar med andra orsaker till en försämrad koagulation och om det finns något samband mellan dessa orsaker och en fortgående blödning. Dessutom ville vi utreda, dels om dessa blödningar kunde påverkas av tillförsel av några av våra vanligaste blodstillande läkemedel, dels om blödningsmängden kunde påverkas av hur snabbt man tillför intravenös vätskeersättning. Slutligen ville vi undersöka om hypotermins skyddande effekt kvarstår även efter det att normal kroppstemperatur återställts.

Metoder

I arbete **I** och **II** användes grisar som försöksdjur. I arbete **I** kyldes en grupp grisar till en temperatur på 32,5 °C och återuppvärmdes sedan. En kontrollgrupp fick behålla normal kroppstemperatur under hela försöket. I början av försöket tappades alla djuren på 40 % av deras beräknade blodvolym för att skapa en blödningschock. Cirkulation, ämnesomsättning och blodprover för analys av viktiga kroppsfunktioner följdes regelbundet under dom 7 timmar försöken pågick. Tromboelastografi, vilket är ett speciellt instrument som exakt mäter blodets levringsförmåga över tiden, användes för analys av eventuella förändringar i koagulationsförmågan.

I arbete **II** fick en grupp grisar tranexamsyra (ett blodstillande läkemedel) och den andra gruppen fick placebo (ett icke verksamt preparat, i detta fall vanlig natriumklorid). Ingen av grupperna blev aktivt nedkylda. En blödningschock initierades genom att ett hål av standardiserad storlek skapades i stora kroppspulsådern. Den initiala blödningen var c:a 35 – 40 % av beräknad blodvolym, därefter avstannade

blödningen men kunde återigen börja, om koaglet lossnade från skadan på kärlet. Eventuella reblödningar kunde upptäckas och mätas genom att registrera flödet i kroppspulsådern ovanför och nedanför skadan med speciella flödesmätare. Observationstiden var drygt 2 timmar. Samma typ av kontroller som i försök **I**, inklusive tromboelastografi, utfördes.

I arbete **III** och **IV** användes råttor som försöksdjur. I arbete **III** kyldes en grupp råttor till 30 °C och återuppvärmdes sedan. En kontrollgrupp behöll normal kroppstemperatur hela försöket. En okontrollerad blödning skapades genom en punktion av ena ljumskartären. Den initiala blödningen uppgick till c:a 25 % av beräknad blodvolym. Denna blödning avstannade därefter, men kunde spontant börja igen. Alla reblödningar mättes avseende starttid, varaktighet och storlek. Basala cirkulationsdata som hjärtfrekvens och blodtryck mättes regelbundet. Blodprov som bl a avspeglade graden av chocktillstånd hos djuren, togs med regelbundna intervall. Observationstiden var 3 timmar.

I arbete **IV** indelades råttorna i 4 lika stora grupper. Tre grupper fick intravenös tillförsel av olika mängd av en saltlösning (Ringe Acetat), som också gavs med olika hastighet (”låg”, ”mellan”, ”hög”). En fjärde grupp fick ”mellan” infusionen plus desmopressin (ett blodstillande läkemedel). Alla råttor i detta arbete kyldes. Studieupplägget var i övrigt precis likadant som i arbete **III.**

Resultat

I arbete **I** sågs en försämring av blodets koagulationsförmåga när kroppstemperaturen föll under 35 °C. Denna förändring försvann vid återuppvärmning. Man såg också en sänkning av ämnesomsättningen under nedkylningsperioden, denna återgick till det normala vid återuppvärmningen. I slutet av försöket fanns däremot inga statistiskt säkerställda skillnader mellan de två grupperna avseende de antal djur som dött, eller i andra mätvärden som registrerats under försöket.

I arbete **II** fanns ingen skillnad i antalet reblödningar, eller reblödningsstorlek mellan den grupp som fått tranexamsyra och den grupp som fått placebo. När man jämförde döda och överlevande i båda grupperna, så visade det sig att de djur som dött, hade fler och större blödningar än de som överlevde. Det fanns ingen skillnad i kroppstemperatur mellan överlevande och döda, däremot fanns ett statistiskt säkerställt samband mellan den totala blödningsmängden och en försämring av koagulationsförmågan hos blodet.

I arbete **III** hade råttorna som nedkyldes, signifikant högre reblödningsstorlek, än kontrollgruppen (43 % jämfört med 3 % av totala blodvolymen). Man noterade ett mycket högre blodtryck bland de råttor som nedkyldes och det högre blodtrycket orsakades av själva nedkylningsprocessen.

I arbete **IV** blödde de råttor som fått ”hög” vätskeersättning mest, det fanns också en trend mot högre dödlighet bland de råttor som fått ”låg” vätskeersättning. Minst antal råttor dog i ”mellan” gruppen. Det fanns ingen skillnad i reblödningsstorlek mellan de råttor som fått desmopressin och kontrollgruppen.

Slutsatser

Hypotermi framkallar en försämring av blodets förmåga att levra sig, denna förändring försvinner dock helt när normal kroppstemperatur återställs. Vid pågående blödning med ökande blödningsmängder så bidrar också andra orsaker än

hypotermi till att koagulationsförmågan av blodet försämras. Nedkylning och hypotermi framkallar fysiologiska förändringar, som t ex en höjning av blodtrycket, som bidrar till återkommande blödningar och en ökad blödningsstorlek. Ifall en individ är utsatt för nedkylning och hypotermi och en penetrerande skada med okontrollerad blödning har uppstått, verkar en ”medelstor” infusionsmängd och infusionstakt av intravenös tillfört Ringer Acetat vara den typ av behandling som ger bäst resultat, åtminstone vid korttids uppföljning. Det finns ingenting som talar för att en behandling med tranexamsyra eller desmopressin minskar blödningsstorleken vid penetrerande skador där okontrollerad blödning uppstått.

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