Macro-hemodynamic targets and hemostatic effects of fluid resuscitation during experimental hemorrhagic shock

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PhD Thesis

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PhD Thesis

Szeged, 2017

“...So in everything, do to others

what you would have them do to you...”

Matthew 7.12

Publications related to the topic

I. **Tánczos K**, Németh M, Trásy D, László I, Palágyi P, Szabó Z, Varga G, Kaszaki J. Goal-Directed Resuscitation Aiming Cardiac Index Masks Residual Hypovolemia: An Animal Experiment. *Biomed Res Int*. 2015:160979. doi: 10.1155/2015/160979.
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II. **Tánczos K**, Németh M, Molnár Zs. What's new in hemorrhagic shock? *Intensive Care Med*. 2015;41:712.doi:10.1007/s00134-015-3658-8
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III. Németh M, **K. Tánczos**, G. Demeter, D. Érces, J. Kaszaki, A. Mikor, Z. Molnár. Central venous oxygen saturation and carbon dioxide gap as resuscitation targets in a hemorrhagic shock. *Acta Anaesthesiol Scand.* 2014;58:611-9.
IF:2.322

IV. **Dr. Tánczos Krisztián**, Dr. Fazakas János. Paradigmaváltás a trauma okozta koagulopátia ellátásában. *Aneszteziológia és Intenzív Terápia.* 2015. 45. évf. 1.ksz.
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2. Trásy D, Tánczos K, Németh M, Hankovszky P, Lovas A, Mikor A, László I, Hajdú E, Osztroluczki A, Fazakas J, Molnár Z; EProK study group. Early procalcitonin kinetics and appropriateness of empirical antimicrobial therapy in critically ill patients: A prospective observational study. *J Crit Care.* 2016;34:50-5.doi: 10.1016/j.jcrc.2016.04.007.
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3. **Tánczos K**, Németh M, Molnár Z. The multimodal concept of hemodynamic stabilization. *Front Public Health*. 2014; 30;2:34.
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4. **Tánczos K**, Molnár Z. Do no harm: use starches? *Minerva Anestesiol*. 2013;79(10):1101-2.

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5. **Tánczos K**, Molnár Z. The oxygen supply-demand balance: a monitoring challenge. *Best Pract Res Clin Anaesthesiol*. 2013;27(2):201-7. doi: 10.1016/j.bpa.2013.06.001.
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6. **Tánczos K**, Molnár Z Physiologic transfusion triggers and massive transfusion. *Sanamed* 2013; 8(1): 00-00, ISNN-1452-662X
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7. Suto B, Szitter I, Bagoly T, Pinter E, Szolcsányi J, Loibl C, Nemeth T, **Tanczos K**, Molnar T, Leiner T, Varnai B, Bardonicsek Z, Helyes Z. Plasma somatostatin-like immunoreactivity increases in the plasma of septic patients and rats with

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1. Introduction

Severe trauma is one of the major health care issues faced by modern society, resulting in the annual death of more than five million people worldwide, and this number is expected to increase to more than eight million by 2020 [1]. Uncontrolled post-traumatic bleeding is one of the leading causes of potentially preventable death among these patients [2]. The management of massively bleeding patients requires careful and ongoing considerations of a number of complex physiological relationships. Over the past decade the underlying pathological processes of trauma-related bleeding has been increasingly recognised and management strategies are evolving. Using pathophysiology-based clinical practice guidelines including early identification of bleeding sources, followed by prompt measures to minimise blood loss, restore tissue perfusion and achieve hemodynamic and hemostatic stability should improve outcomes of these patients. Therefore an awareness of the specific pathophysiological mechanisms associated with bleeding following traumatic injury by treating physicians is essential.

1.1 Hemodynamic issues in major bleeding

Hemorrhagic shock is a pathologic state in which intravascular volume and the blood hemoglobin content are depleted thus oxygen delivery to the cells is insufficient to sustain cellular activity and support organ function [3]. The primary goal of the cardiorespiratory system is to deliver adequate oxygen to the tissues to meet their metabolic requirements. The adequacy of tissue oxygenation is determined by the balance between the rate of oxygen transport to the tissues (driving pressure and oxygen delivery, DO_2) and the rate at which the oxygen is used by the tissues (oxygen consumption, VO_2) [4]. The standard formulae to determine oxygen delivery and oxygen consumption is shown in Figure 1 [5].

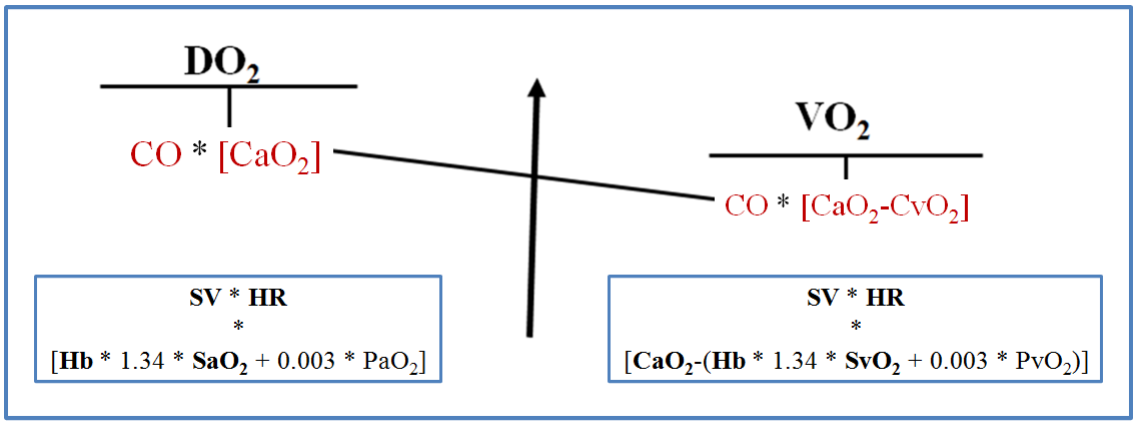

FIGURE 1. Oxygen supply and demand
DO_2: oxygen delivery; SV: stroke volume; HR: heart rate; Hb: haemoglobin; S_aO_2: haemoglobin arterial oxygen saturation; P_aO_2: arterial oxygen partial pressure; CO: cardiac output; C_aO_2: arterial oxygen content; VO_2: oxygen consumption; S_vO_2: haemoglobin mixed venous oxygen saturation; P_vO_2 : venous oxygen partial pressure; C_vO_2: venous oxygen content.

During massive bleeding various macrohemodynamic changes occur, which often lead to an imbalance between delivery and consumption. Oxygen delivery may be inadequate as a result of impaired arterial oxygen content (C_aO_2 – due to anaemia) and/or cardiac output (CO – as a result of hypovolaemia) [6]. However, oxygen consumption may also be raised in these patients due to stress, agitation and pain caused by the trauma. The circulation can adapt to this increased demand and compensation is possible to some extent by increasing CO with tachycardia and/or increasing the oxygen extraction ratio in order to maintain adequate tissue oxygenation. Under normal circumstances, VO_2 is usually independent during a wide range of DO_2. But after a critical threshold, these

compensatory resources become exhausted and VO_2 will be dependent on DO_2 hence without intervention (giving infusion, blood and/or catecholamines) oxygen debt can develop (Fig. 2) [7].

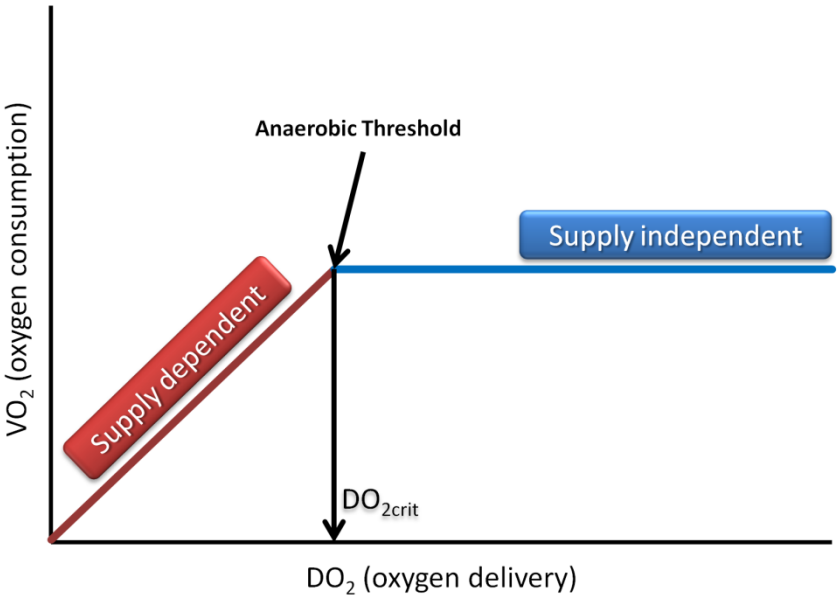

FIGURE 2. Relationship between O_2 delivery and consumption: curve showing a defined "knee", where consumption of oxygen by the tissues becomes dependent on delivery.

Once hypoperfusion takes place the microcirculation may also be damaged. At the cellular level, due to inadequate perfusion and oxygenation cells are deprived of essential substrates for normal aerobic metabolism and energy production. Anaerobic metabolism occurs, which results in the formation of lactic acid and metabolic acidosis occurs. If shock is prolonged the cellular energy-consuming enzyme systems become exhausted, and the cell membrane loses the ability to maintain its integrity. If the process is not reversed, progressive cellular damage and organ failure will develop ultimately leading to death. It is important to note, that severe hypoperfusion can also damage the cellular sensing mechanisms needed to regulate blood flow not only between organs but also within organs, between different cells, and even at the subcellular level where there is heterogeneity of oxygen consumption between the mitochondria [8]. It is obvious that the regulation of blood flow and oxygen transport in order to match oxygen supply to demand heterogeneity is a highly complex and regulated system that integrates cellular needs with vascular regulatory mechanisms. If this regulatory system remains intact the normalization of macrohemodynamic parameters will result in a parallel improvement in the perfusion of microcirculation. This condition is called "hemodynamic coherence". However, if the hemodynamic coherence between the marco-, and microcirculation is lost, simply restoring systemic hemodynamic abnormalities becomes ineffective in

restoring the microcirculation and in correcting tissue hypoperfusion. Four types of microcirculatory alterations underlying the loss of hemodynamic coherence can be identified (Fig. 3): type 1 - heterogeneity in microcirculatory perfusion with obstructed capillaries next to capillaries with flowing red blood cells (RBC); type 2 - hemodilution, in which dilution of blood causes a loss of RBC-filled capillaries and results in increased diffusion distances between the oxygen-carrying RBCs and tissue cells; type 3 - vasoconstriction/tamponade, where vasoconstriction of arterial vessels results in microcirculatory ischemia or raised venous pressures inducing microcirculatory tamponade, both resulting in compromised tissue oxygenation; and type 4 - tissue oedema caused by capillary leak, resulting in increased diffusion distances between the RBCs and tissue cells [9]. The pathogenic mechanisms underlying the loss of hemodynamic coherence include the generation of reactive nitrosative and oxidative species, which results in the loss of vascular regulation, in compromised endothelial cell function, and in compromised barrier function leading to reduced functional capillary density and thereby loss of the capacity of the microcirculation to transport oxygen to tissues [10].

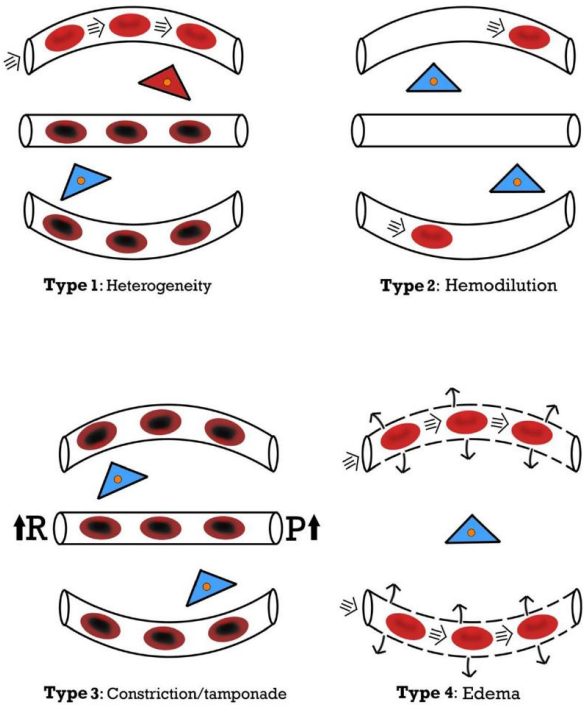

FIGURE 3. Microcirculatory alterations associated with loss of hemodynamic coherence. Adapted from Can Ince [9].
Red: well oxygenated RBC and tissue cells; purple: RBC with reduced oxygenation; blue: reduced tissue cell oxygenation

1.2 Hemostatic disorders after major trauma

About one-third of all bleeding major trauma patients present with a coagulopathy upon hospital admission [11]. The pathogenesis of the trauma-related coagulopathy was earlier thought to be a “triad” of loss and dilution of procoagulant clotting factors, hypothermia and acidemia [12]. However, there is emerging evidence that a combination of tissue injury and hypoperfusion also cause clotting disorders. Two recent observational studies on 1088 and 7638 patients have found that around 10 % of patients with acute coagulopathy on hospital admission presented with normal pH, normothermia and without prehospital fluid resuscitation. Hence, the hemostatic disorders of these patients cannot be explained solely on the basis of acidemia, hypothermia or dilutional coagulopathy [13, 14].

The coagulopathy in trauma is now recognised as an early, endogenous process that results from a combination of tissue trauma and systemic hypoperfusion which will activate the neurohumoral system and release catecholamines, resulting in endothelium damage, glycocalyx shedding and autoheparinization Endothelial injury will immediately and concurrently lead to an influence of many inflammatory and coagulation pathways including thrombin-thrombomodulin-complex generation, activation of anticoagulant and fibrinolytic pathways as well as platelet dysfunction (Fig. 4) [15, 16].

The central role of hypoperfusion-related endothelial dysfunction in trauma-induced coagulopathy has been established in a recently published study. High circulating levels of syndecan-1 (a marker of endothelial glycocalyx degradation due to hypoperfusion) was associated with high sympatho-adrenal activity and higher mortality in trauma patients. Furthermore, patients whose blood contains high level of syndecan-1 are associated with increased tissue damage, more severe inflammatory response and also with lower protein C level, hyperfibrinolysis and prolonged activated partial thromboplastin time. [17].

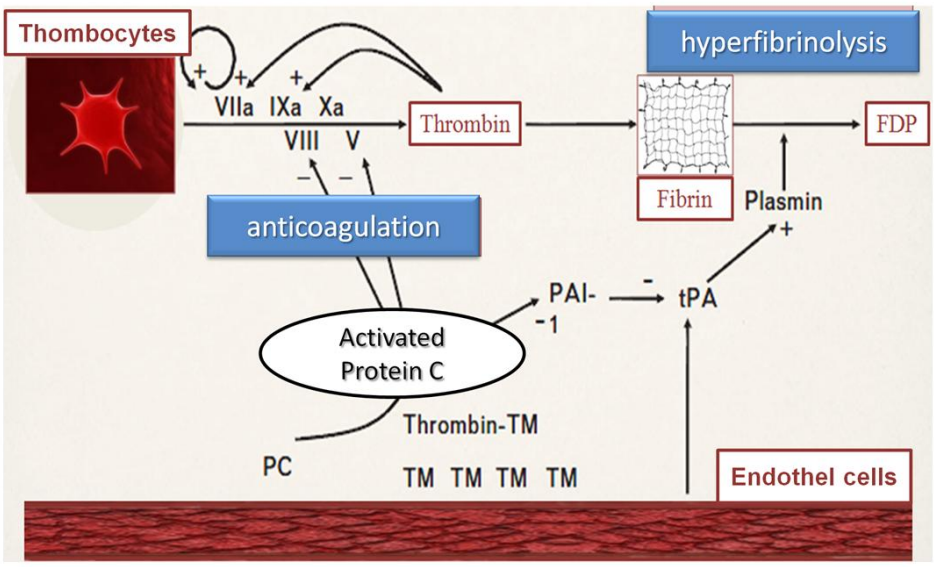

FIGURE 4. Hypoperfusion induced anticoagulation and hyperfibrinolysis.
In the presence of hypoperfusion the endothelium express thrombomodulin which complexes with thrombin, hence less thrombin will be available to catalyze the conversion of fibrinogen to fibrin. The thrombin-thrombomodulin complex activates protein C (APC) which potentiates anticoagulant state. Interestingly, APC in excess can effectively consume plasminogen activator inhibitor (PAI) and thus lead to a "de-repression" of fibrinolytic activity and systemic hyperfibrinolysis. Similar activation of fibrinolysis may occur as tissue plasminogen activator is released from the endothelium following injury or ischemia in the setting of hypoperfusion. [15]
PC: protein C; TM: thrombomodulin; V: factor V; VIIa: activated factor VII; IXa: activated factor IX; Xa: activated factor X; VIII: factor VIII; PAI: plasminogen activator inhibitor; tPa: tissue plasminogen activator; - inhibition; + activation.

Hypoperfusion or tissue injury related coagulopathy is further escalated or influenced by different environmental and therapeutic factors such as acidosis, hypothermia, dilution, and coagulation factor consumption [18, 19, 20]. Furthermore, this condition is modified by individual patient-related factors, including genetic background, co-morbidities, inflammation and medications, especially oral anticoagulants [21].

In the light of the new "cell-based" concept of hemostasis thrombocytes play a pivotal role in different part of physiological hemostasis such as vasoconstriction, clot formation and thrombin generation [22]. Recent evidence indicates that admission platelet counts are inversely correlated with early mortality and transfusion in critically injured trauma patients, even for platelet counts well into the normal range [23]. It is important to recognize that not all platelets accounted for in the platelet count are functional after major trauma thus a normal platelet count may provide the clinician with a false sense of security. Severe brain injury and acidosis as well as hypothermia and hemodilution were identified in the background of admission thrombocytes hypofunction [24]. Based on the endothelial dysfunction in severe bleeding we can hypothesize that hypoperfusion rather

than hypothermia or hemodilution may play a dominant role in trauma-related platelets dysfunction (Fig. 5).

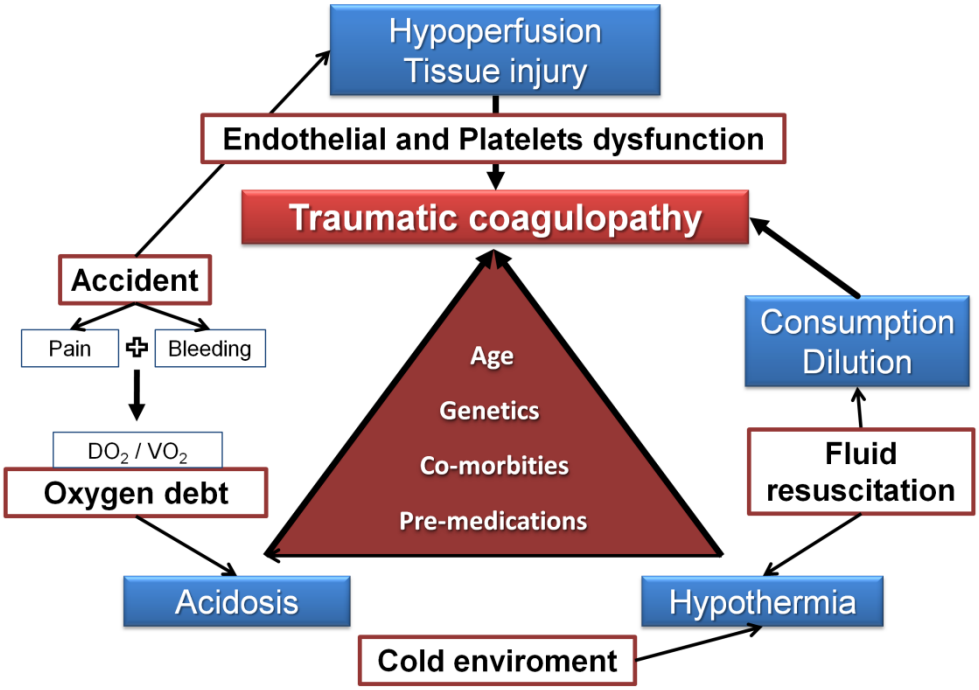

FIGURE 5. Trauma-related coagulopathy.
Hemostatic disorder occurred immediately after major trauma is resulted in the consumption of different components of coagulation due to hemorrhage as well as endothelial and platelets dysfunction caused by severe hypoperfusion and tissue injury. Later the coagulopathy may be modified by the elements of the "lethal triad" (acidosis, hypothermia and hemodilution) and also by different patient-related factors.
DO_2: oxygen delivery; VO_2: oxygen consumption.

Consequently, the phenotype of trauma induced coagulopathy (TIC) upon hospital admission is not uniform but varies according to the pattern of injury, severity of hypoperfusion, presence or absence acidosis or hypothermia and the amount of prehospital fluid administration. Therefore it is crucial to highlight the potential importance of point-of-care testing that can rapidly provide information on an actual individual patient's coagulation status.

1.3 Paradigm shift in the early management of trauma patients

It is well known that patients presented with trauma induced coagulopathy have a significantly increased incidence of multiple organ failure, longer intensive care unit stay and death compared to patients with similar injury patterns in the absence of a hemostatic disorder [11, 14, 25]. These recognition has brought a paradigm shift in clinical management of severely bleeding patients. The previous approach of maintaining an adequate circulating volume and oxygen carrying capacity before, and then dealing with coagulopathy as a secondary event has changed to hemodynamic and hemostatic resuscitation in parallel. In the acute phase of hemorrhage, the physician's therapeutic priority is to stop the bleeding by surgical or radiological interventions as quickly as possible [26]. As long as this bleeding is not controlled, the main goals of the treatment include the correction of the imbalance between oxygen delivery (DO_2) and consumption (VO_2), maintaining adequate perfusion pressure, and preventing or treating coagulopathy (Fig. 6) [27].

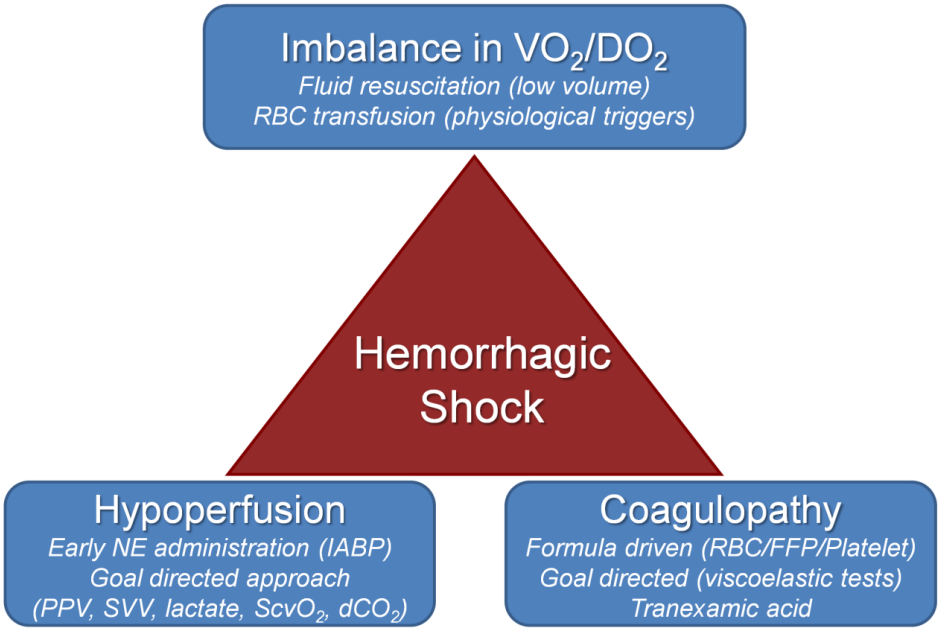

FIGURE 6. Cornerstones of pathophysiology-based interventions in hemorrhagic shock.
VO_2: oxygen consumption; DO_2: oxygen delivery; RBC: red blood cell; NE: norepinephrine; IABP: invasive arterial blood pressure; PPV: pulse pressure variation; SVV: stroke volume variation; $ScvO_2$: central venous oxygen saturation; dCO_2: venous-to-arterial CO_2 gap;

1.4 Fluid resuscitation in severely injured patient.

The cornerstone of the initial management of the bleeding patient is fluid resuscitation. Fluid therapy can increase stroke volume and subsequently enhance global perfusion and oxygen delivery thereby may improve tissue hypoxia by normalizing microcirculatory flow and so may limit progression to organ failure. However, too much fluid can induce excessive hemodilution and oedema formation and may cause type 2 and 4 alteration of microcirculation (Fig. 3), resulting in a reduction in the diffusion capacity of oxygen transport to the tissues especially at critical sites such as the lungs, kidneys and the brain [28]. Thus fluid management may be lifesaving at the time, however inadequate fluid resuscitation can lead to hypo-, or hyper-perfusion causing the development of multiorgan disorders at a later stage, which then severely affects the outcome of these patients [29, 30]. Therefore, the use of early and efficient therapeutic strategies able to detect and to treat the imbalance between oxygen delivery and consumption is of particular importance, which has been recognized for decades [31]. The recognition of hemorrhagic shock should be based on a combination of clinical, hemodynamic and biochemical signs. The simplest form of monitoring is the individual healthcare professional, inspecting the patient for consciousness, agitation or distress, assessing the breathing pattern (regular or laboured), the presence or absence of central and peripheral cyanosis; touching the patient's skin to note if it is cool and moist. Although well established and important as bedside diagnostic tools, these simple measures can be complemented by the use of the conventional parameters of hemodynamics, such as heart rate, blood pressure, and urine output. Table 1 summarises estimated blood loss and severity of shock state based on initial presentation of trauma patients according to the Advanced Trauma Life Support (ATLS) classification system [32]. The ATLS classification has been demonstrated to be a useful guide that allows the quantification of blood loss with acceptable accuracy in hemorrhagic shock [33].

TABLE 1. American College of Surgeons Advanced Trauma Life Support classification of blood loss. Adapted from [34]

	Class I	Class II	Class III	Class IV
Blood loss (ml)	Up to 750	750-1500	1500-2000	>2000
Blood loss (% blood volume)	Up to 15%	15%-30%	30%-40%	>40%
Pulse rate (bpm)	<100	100-120	120-140	>140
Systolic blood pressure	Normal	Normal	Decreased	Decreased
Pulse pressure (mmHg)	Normal or increased	Decreased	Decreased	Decreased
Respiratory rate	14-20	20-30	30-40	>35
Urine output (ml/h)	>30	20-30	5-15	Negligible
CNS / mental status	Slightly anxious	Mildly anxious	Anxious, confused	Confused, lethargic
Initial fluid replacement	Crystalloid	Crystalloid	Crystalloid and blood	Crystalloid and blood

Although these traditional parameters of resuscitation can be useful in the identification of inadequate perfusion but are limited in their ability to signal early or still ongoing, compensated shock [35]. The normal response of the body to hypovolemia is to attempt to maintain an adequate driving pressure (mean arterial pressure - MAP) by increasing sympathetic tone causing vasoconstriction, decreasing unstressed venous vascular volume, increased contractility, and tachycardia. These compensatory mechanisms may prevent a significant decrease in systolic blood pressure until the patient has lost more than 20-30 % of blood volume. Because of these reflex mechanisms, the sympathetic feedback aims to sustain MAP to maintain cerebral and coronary blood flow. Hypotension in bleeding patients not only occurs late but has already been associated with tissue hypoperfusion. Consequently, hypotension in the setting of circulatory shock reflects to exhausted intrinsic compensatory mechanisms to sustain effective blood flow and thus normal homeostasis [36]. In accordance with this, the new definition of circulatory shock emerging from a recent consensus conference does not require the presence of hypotension. Rather, the current definition of shock is “life-threatening, generalized form of acute circulatory failure associated with inadequate oxygen utilization by the cells”, which usually includes, but it is not limited to the presence of hypotension [30]. The latest European guideline on major bleeding and coagulopathy following trauma recommend a damage-control resuscitation concept in the early, rescue phase of hemorrhagic shock management in patients characterized by low arterial pressure, signs of hypoperfusion, or both. This strategy aims to achieve a lower than normal systolic blood pressure of 80-90 mmHg applying a concept of restricted fluid replacement, as well as early and transiently using of vasopressor in patients without TBI and/or spinal injury. Damage control resuscitation concept should be continued until the

bleeding source is identified and controlled. However, after the bleeding is under control the patient is no longer in immediate life-threatening danger but may be in a stage of compensated shock (but at high risk of decompensation) and any additional fluid therapy should be given more cautiously, and titrated with the aim of optimizing cardiac function to improve tissue perfusion with ultimate goal of mitigating organ dysfunction [37]. In order to notice and treat this compensated hypovolaemia before tissue hypoperfusion or evaluate the patient's response to the commenced fluid therapy, a more detailed assessment of global macrohemodynamic indices such as cardiac output and its derived variables, as well as measures of oxygen debt may be necessary [38, 39]. A recently published systemic review and meta-analysis showed that following severe trauma during the optimization phase of resuscitation early goal-directed therapy was associated with lower mortality and shorter durations of intensive care unit and hospital stays [40]. Although there is no consensus on the best or universally accepted parameter as resuscitation target, cardiac output calculated from thermodilution or pulse contour analysis is the most often used end-point during goal-directed therapy [41, 42].

Nevertheless, there are several issues, which have not yet been investigated and should be clarified in this bleeding-resuscitation context in order to help the clinician to choose the most reliable and adequate therapeutic targets during resuscitation of the acutely bleeding patient and to understand the underlying pathomechanism of hemostatic disorders.

2. Aims of the thesis

According to all the aboved detailed pathophysiological background and results of extensive clinical research cardiac output and its derived variables are the most often used target of resuscitation after the rescue phase of hemorrhagic shock management. However cardiac output-based fluid optimization – similar to MAP-guided therapy – may also lead to inadequate fluid therapy. Since CO is the product of heart rate and stroke volume, increased heart rate caused by compensatory sympathetic response may normalize cardiac output without the optimization of stroke volume resulting in residual, ongoing, compensated hypovolemia. Therefore we hypothesize that stroke volume-targeted fluid resuscitation may result in better hemodynamic optimization during the compensated shock phase of resuscitation.

In order to prove this hypothesis we decided:

I. *To produce a moderate bleeding-resuscitation animal model targeting stroke volume index (SVI) as therapeutically endpoint*

II. *To compare SVI as primary target of fluid resuscitation to cardiac index-based treatment*

Based on the aboved detailed hypoperfusion-related endothelial injury one can assume that hypoperfusion may be able to cause platelet dysfunction before severe acidosis developed and without severe tissue and/or brain injury. Therefore, we used our previously established bleeding-resuscitation animal model in order to:

III. *To assess the changes of platelet's function in moderate hemorrhage and fluid resuscitation animal model*

3. Experiments

The experiments were performed on the EU Directive 2010/63/EU on the protection of animals used for experimental and other scientific purposes and carried out in strict adherence to the NIH guidelines for the use of experimental animals. The study was approved by the National Scientific Ethical Committee on Animal Experimentation (National Competent Authority), with the license number V./142/2013.

I. Bleeding-resuscitation animal model

Materials and methods

Animals and Instrumentation.

Inbred Vietnamese mini pigs of both sexes (n = 12, weighing 23 ± 5 kg) were fasted for 6 hours preoperatively, but with free access to water. Anesthesia was induced with a mixture of ketamine (20 mg/kg) and xylazine (2 mg/kg) IM and maintained with a continuous infusion of propofol (6 mg/kg/hr IV). Nalbuphine (0.1 mg/kg IV) was used for pain control. The animals were placed in supine position on a heating pad for maintenance of the body temperature between 36°C and 37°C. The depth of anesthesia was monitored by assessing the jaw tone. After endotracheal intubation, the animals were mechanically ventilated with Harvard Apparatus Dual Phase Control Respirator (Harvard Apparatus, South Natick, MA). The tidal volume was set at 10 ml/kg, and the respiratory rate was adjusted to maintain the end-tidal carbon dioxide and partial pressure of arterial carbon dioxide in the range of 35-45 mmHg and the arterial pH between 7.35 and 7.45. Positive end-expiratory pressure was applied. After induction of the right femoral artery and jugular vein were cannulised for the measurement of mean arterial pressure (MAP) and cardiac output (CO) by thermodilution (PiCCO, PULSION Medical Systems SE, Munich, Germany). During the bleeding phase blood was drained from a sheat inserted in the left carotid artery. The central venous line (positioned by the guidance of intracavital ECG) was used for the injection of cold saline boluses for the thermodilution measurements, for fluid resuscitation and drug administration.

Hemodynamic measurements

Stroke volume (SV), heart rate (HR), mean arterial pressure (MAP), cardiac output (CO), global end-diastolic volume (GEDV), stroke volume variation (SVV), pulse pressure variation (PPV), left ventricular contractility (dPmax) and systemic vascular resistance (SVR) were measured by the PICCO Plus monitoring system at baseline and after equilibration of each interval. All hemodynamic parameters were indexed for body surface area. The average of three random measurements following 10 ml bolus injections of ice-cold 0.9% saline was recorded. Central venous pressure (CVP) was monitored continuously and registered with a computerized data acquisition system (SPELL Haemosys; Experimetria, Budapest, Hungary).

Experimental protocol

The flowchart of the experiment is summarized in Figure 7. After the preparation and 30 minutes rest, baseline (T_{bsl}) hemodynamic measurements were performed and then blood was drained from left carotid artery until the stroke volume index (SVI) dropped by 50% of its baseline value (T_0), then measurements were repeated. The difference of the SVI_{Tbsl}-SVI_{T_0} was divided into four equal target values, then the animals were resuscitated with boluses of balanced crystalloid Lactated Ringer (B. Braun AG., Melsungen, Germany) in 4 steps ($T_{1\text{-}4}$) in order to achieve the initial SVI by T_4. After reaching each step, 10 minutes were allowed for equilibrium, than hemodynamic parameters were measured. At the end of the experiment the animals were euthanized with sodium pentobarbital.

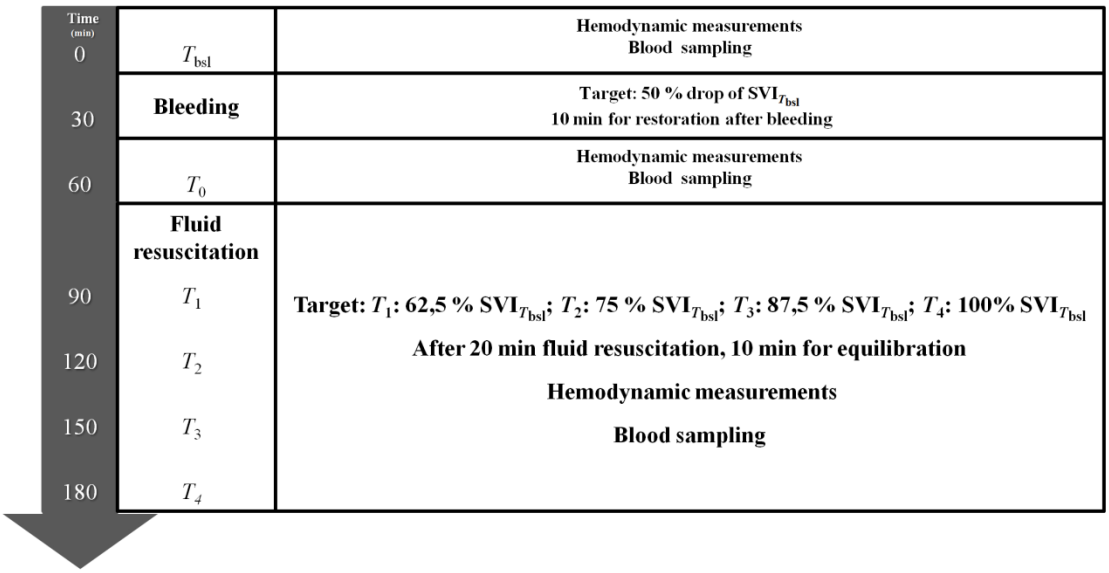

FIGURE 7. Schematic diagram illustrating the flow chart of the experiment.
After baseline measurement, animals were bled until the stroke volume index (SVI) decreased by 50% (T_0); then measurements were repeated. The difference of the $SVI_{T_{bsl}} - SVI_{T_0}$ was divided into four equal target values ($T_{1–4}$), and fluid resuscitation was performed in order to reach the initial SVI by T_4.

Data analysis and statistics

Data are presented as mean ± standard deviations unless indicated otherwise. For testing normal distribution the Kolmogorov-Smirnov test was used. Changes in all parameters throughout the experiment were tested by repeated measures analysis of variance (RM ANOVA). For pairwise comparisons Pearson's correlation was used. For statistical analysis SPSS version 18.0 for Windows (SPSS, Chicago, IL) was used and $p < 0.05$ was considered statistically significant.

Results

During the bleeding phase 314±65 ml blood had to be drained and in total 951±307 ml crystalloid infusion was administered for resuscitation. Hemodynamic changes during the experiment are summarized in Table 2. The goals of 50% reduction in SVI were achieved by T_0 and after resuscitation it returned to its initial value by T_4. The CI also decreased by T_0 and reached a higher value by T_4 as compared to T_{bsl}. There was an increase in heart rate from T_{bsl} to T_0, which remained elevated during the whole experiment while mean arterial pressure fell during the hemorrhage and remained lower until the end of the experiment as compared to T_{bsl}. Global end diastolic volume decreased at T_0 and increased during resuscitation, but remained lower as compared to T_{bsl}. The CVP also decreased from T_{bsl} to T_0 and returned to its baseline value at T_4. There was a tendency of gradually increasing myocardial contractility as indicated by dP_{max} but it did not achieve statistical significance. Pulse contour analysis driven stroke volume variation (SVV) and pulse pressure variation (PPV) increased from T_{bsl} to T_0 and normalized by T_4. Both the SVV and the PPV showed significant negative correlation with SVI determined by thermodilution (R=-0.53; p<0.001; R= -0.615; p<0.001). Lactate levels increased from T_{bsl} to T_0 and remained elevated throughout, with a non significant decrease from T_0 to T_4.

TABLE 2. Hemodynamic parameters during hemorrhage and fluid resuscitation

	T_{bsl}	T_0	T_1	T_2	T_3	T_4
Stroke volume index (ml/m^2)	26.8±4.7	13.4±2.3*	16.3±2.6$^{*\#}$	19.2±3.5$^\#$	22.3±4.1$^\#$	26.6±4.1$^\#$
Cardiac index (L/min/m^2)	2.6±0.4	1.8±0.3*	2.0±0.4$^{*\#}$	2.3±0.4$^\#$	2.6±0.4$^\#$	2.9±0.5$^{*\#}$
Mean arterial pressure (mmHg)	112±23	74±18*	73±20*	78±20*	84±19$^{*\#}$	91±19$^{*\#}$
Heart rate (beats/min)	95±12	131±27*	128±31*	121±22*	114±18$^{*\#}$	107±16$^{*\#}$
Central venous pressure (mmHg)	6.0±1.1	4.8±0.8*	5.5±2.1	5.6±1.5	6.0±1.3	6.1±1.4$^\#$
Global end-diastolic volume (ml/m^2)	309±57	231±61*	237±54*	245±45*	268±48$^{*\#}$	287±49$^{*\#}$
Stroke volume variation (%)	13.6±4.3	22.6±5.6*	21.8±5*	18.6±5.2$^\#$	16.6±5.4$^\#$	12.2±4.3$^\#$
Pulse pressure variation (%)	13.0±4.5	24.5±7.6*	23±7.3*	18.4±6.4$^\#$	16±5.6$^\#$	13±4.2$^\#$
Systemic vascular resistance index (dyn*s/cm^{-5}/m^2)	3425±816	3257±966	2711±733$^{*\#}$	2506±680$^{*\#}$	2460±561$^{*\#}$	2340±526$^{*\#}$
Dpmax (mmHg/s)	583±227	596±367	636±413	708±403	670±298	657±265
Lactate (mmol/l)	1.62±0.43	3.86±1.49*	4.75±1.88*	4.75±2.07*	4.17±2.06*	3.54±1.9*

Data are expressed as mean±standard deviation; *= (p<0.05) significantly different from tbsl ; #= (p<0.05) significantly different from t0

II. SVI- versus CI-based fluid resuscitation

Materials and Methods

Animals and Instrumentation.

Vietnamese mini-pigs (n = 27) underwent a 12-hour fasting preoperatively but with free access to water. Anesthesia was induced by intramuscular injection of a mixture of ketamine (20 mg/kg) and xylazine (2 mg/kg) and maintained with a continuous intravenous infusion of propofol (6 mg/kg/hr), while analgesia was performed with nalbuphine (0.1 mg/kg iv.). The animals' trachea was intubated and the lungs were ventilated mechanically with Dräger Evita XL (Dräger, Lübeck, Germany). The tidal volume was set at 10 mL/kg, and the respiratory rate was adjusted to maintain the end-tidal carbon dioxide and partial pressure of arterial carbon dioxide in the range of 35 – 45 mmHg. The adequacy of the depth of anaesthesia was assessed by monitoring the jaw tone. After induction of anaesthesia, the right jugular vein, the left carotid artery, and the right femoral artery were dissected and catheterized using aseptic technique. For invasive hemodynamic monitoring, a transpulmonary thermodilution catheter (PiCCO®, PULSION Medical Systems SE, Munich, Germany) was placed in the right femoral artery. Central venous catheter was inserted via the right jugular vein and was positioned by the guidance of intracavital ECG. During the bleeding phase blood was drained from a sheat inserted in the left carotid artery. Animals were kept warm (37 ± 1°C) by an external warming device.

Hemodynamic Monitoring and Blood Gas Sampling.

Cardiac output (CO), global end-diastolic volume index (GEDI), stroke volume (SV), cardiac function index (CFI), index of left ventricular contractility (dPmax), SV variation (SVV), pulse pressure variation (PPV), heart rate (HR), and mean arterial pressure (MAP) were measured by transpulmonary thermodilution and pulse contour analysis at baseline and at the end of each interval. All hemodynamic parameters were indexed for body surface area or bodyweight. Central venous catheter was used for the injection of cold saline boluses for the thermodilution measurements. The average of three measurements following 10 mL bolus injections of ice-cold 0.9 % saline was recorded. Central venous pressure (CVP) was measured via central venous catheter at the same times as the other hemodynamic variables. For blood gas measurements the right femoral artery served as

the site for arterial blood gas sampling (analyzed by Cobas b 221, Roche Ltd., Basel, Switzerland) simultaneously at baseline and at the end of each step.

Experimental Protocol.

The flowchart of the experiment is summarized in Figure 8. After the instrumentation, animals were allowed to rest for 30 minutes after which baseline (T_{bsl}) hemodynamic, blood gas analyses, including lactate measurements, and hemostatic laboratory testing were performed. After these measurements, blood was drained until the stroke volume index dropped by 50% of its baseline value (T_0); then measurements were repeated. At this point the animals were randomized into two groups. In the SVI-group the difference of the $\mathrm{SVI}_{T_{\mathrm{bsl}}} - \mathrm{SVI}_{T_0}$ was divided into four equal target values, which was aimed to reach in 4 steps during fluid resuscitation ($T_{1–4}$) to reach the initial SVI by T_4. While in the CI-group the difference of the $\mathrm{CI}_{T_{\mathrm{bsl}}} - \mathrm{CI}_{T_0}$ was divided into 4 target values and then the animals were resuscitated in 4 steps in order to reach the $\mathrm{CI}_{T_{\mathrm{bsl}}}$ by T_4. Fluid replacement was carried out with boluses of 200 mL of balanced crystalloid Ringerfundin (B. Braun AG., Melsungen, Germany) over 10 minutes, till the target SVI or CI value was reached. After reaching each step, 10 minutes was allowed for equilibrium; then hemodynamic and laboratory parameters were measured. At the end of the experiment the animals were euthanized with sodium pentobarbital.

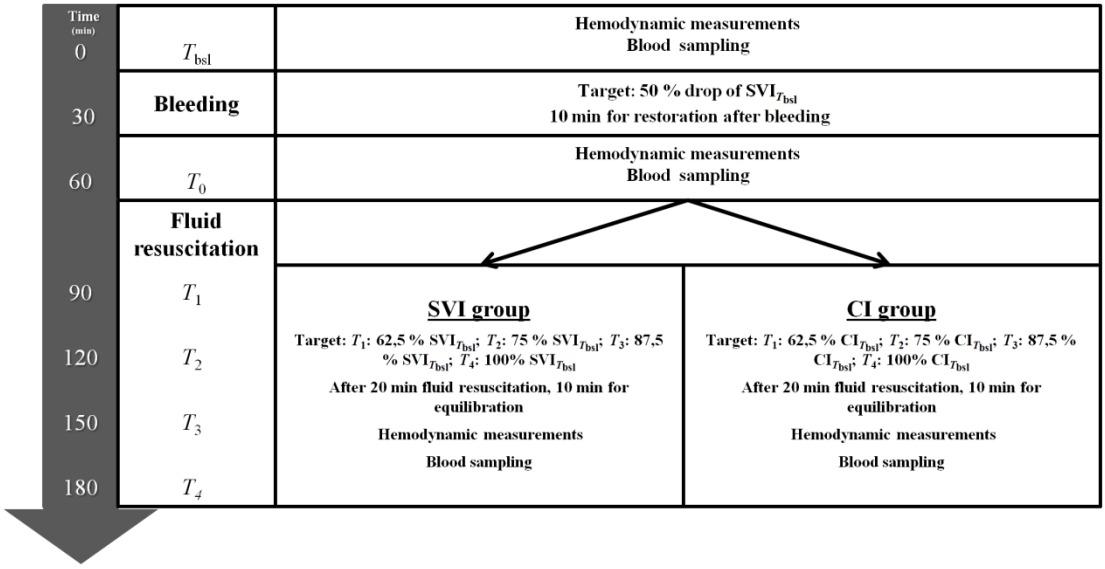

FIGURE 8. Schematic diagram illustrating the flowchart of the experimental protocol. After baseline measurement, animals were bled until the stroke volume index (SVI) decreased by 50% (T_0); then measurements were repeated and randomized into two group. In the SVI-group the difference of the $SVI_{T_{bsl}} - SVI_{T_0}$ was divided into four equal target values ($T_{1–4}$), and fluid resuscitated to reach the initial SVI by T_4. In the CI-group the difference of the $CI_{T_{bsl}} - CI_{T_0}$ was divided into 4 target values and then the animals were resuscitated in 4 steps in order to reach the $CI_{T_{bsl}}$ by T_4.

Data Analysis and Statistics.

Data are presented as mean ± standard deviations unless indicated otherwise. For testing normal distribution the Kolmogorov-Smirnov test was used. Changes in all parameters throughout the experiment were tested by two-way repeated measures analysis of variance (RM ANOVA) and for the post hoc test Bonferroni test was used. For pairwise comparisons Pearson's correlation was used. The primary end point of the study was the normalization of SVV, as the one of the best indicators of hypo-, normovolemia in mechanically ventilated subjects [10]. Based on the results of our previous animal experiment [9] SVV was found to be 12.2 ± 4.3% by the end of resuscitation. Considering that CI-based resuscitation remains inadequate, we regarded a clinically significant difference of 4% (i.e., 12% in the SVI group and 16% in the CI-group). In order the study to have 80% power to show a difference between the two groups if $\alpha < 0.05$, the required sample size is a minimum of 20 animals (10 in each group). For statistical analysis SPSS version 20.0 for Windows (SPSS, Chicago, IL) was used and $p < 0.05$ was considered statistically significant.

Results

All animals survived the experiment, apart from one (CI group), which had sudden cardiac arrest after induction of anaesthesia for unknown reasons. Therefore, the results of 14 animals in the SVI-group and 12 animals in the CI-group were analyzed. Demographics and fluid management data are summarized in Table 3. Animals were of similar weight in both groups. For a 50% decrease of SVI similar blood had to be drained in the two groups. During resuscitation animals in the SVI-group required more fluid in total, and taking into account the volume of crystalloid required to replace a unit of 10mL blood loss, animals in the SVI-group also received significantly more fluid (Table 3).

TABLE 3. Demographics and fluid therapy

	SVI-group (n=14)	CI-group (n=12)	p
Weight (kg)	29.00 ± 5,36	27.54 ± 5.46	0.606
BSA (m2)	0.98 ± 0.09	0.93 ± 0.91	0.390
Shed blood (ml)	485 ± 91	479 ±101	0.859
Shed blood (ml/ m2)	492 ± 59	508 ± 101	0.719
Total amount of the replaced fluid (ml)	1965 [1584-2165]	900 [850-1780]	0.020*
Required fluid (ml)/ unit blood loss (10 ml)	40 ± 12	25 ± 12	0.027*

Hemodynamic parameters were similar at T_{bsl} and goals of 50% reduction in SVI were reached by T_0 in both groups (Table 4). In the SVI-group SVI returned to its baseline value by T_4 and CI was significantly elevated as compared to T_{bsl}. In the CI-group SVI remained significantly lower as compared to Tbsl. Mean arterial pressure and heart rate showed a similar pattern in both groups, but in the CI-group heart rate remained significantly higher by T_4 as compared to T_{bsl}, while it normalized in the SVI-group. Mean arterial pressure changed significantly in each group with a similar pattern without significant differences between the groups. There was less change in the CVP throughout the experiment, with a significant increase at T_3 and T_4 only in the SVI-group. Global end-diastolic volume decreased and then increased in both groups, but while it normalized by T_4 in the SVI-group, it remained significantly lower in the CI-group as compared to the SVI-group and as compared to T_{bsl}. Stroke volume variation and PPV also followed a similar pattern, and SVV normalized in the SVI-group but it remained significantly elevated in the CI-group, both as compared to T_{bsl} and between the groups at T_4. There was an increased tendency in myocardial contractility throughout the experiment in both groups, as indicated by dPmax values, but it did not achieve statistical significance.

TABLE 4. Hemodynamic parameters during hemorrhage and fluid resuscitation

	Group	t_{bsl}	t_0	t_1	t_2	t_3	t_4
Stroke volume index (ml/m^2)	SVI	27.5 ± 5.4	13.8 ± 2.6 *	16.5 ± 2.8 *	19.5 ± 3.7 *$^{\#}$	23.6 ± 5.1 $^{\#}$	28.0 ± 5.0 $^{\#}$
	CI	31.4 ± 4.7	14.4 ± 9.0 *	18.1 ± 3.6 *	19.2 ± 3.6 *	23.2 ± 1.3 *$^{\#}$	23.8 ± 5.9 *$^{\#@}$
Cardiac index (l/min/m^2)	SVI	2.6 ± 0.3	1.8 ± 0.3 *	2.1 ± 0.4 *	2.4 ± 0.3 $^{\#}$	2.7 ± 0.4 $^{\#}$	2.9 ± 0.4 *$^{\#}$
	CI	2.8 ± 0.3	1.7 ± 0.5 *	2.1 ± 0.3 *	2.4 ± 0.2 $^{\#}$	2.6 ± 0.4 $^{\#}$	2.7 ± 0.3 $^{\#}$
Mean arterial pressure (mmHg)	SVI	116 ± 17	72 ± 17 *	75 ± 19 *	78 ± 18 *	86 ± 17 *	92 ± 16 *$^{\#}$
	CI	124 ± 12	75 ± 22 *	77 ± 18 *	80 ± 81 *	86 ± 22 *	96 ± 20 *$^{\#}$
Heart rate (beats/min)	SVI	95 ± 13	133 ± 22*	130 ± 29 *	121 ± 21 *	111 ± 18 $^{\#}$	101 ± 12 $^{\#}$
	CI	89 ± 11	139 ± 37 *	131 ± 13 *	127 ± 28 *	121 ± 24 *	117 ± 35 *
Central venous pressure (mmHg)	SVI	5.9 ± 1.0	4.8 ± 0.7	5.5 ± 1.9	5.6 ± 1.4	6.1 ± 1.2 $^{\#}$	6.2 ± 1.3 $^{\#}$
	CI	6.0 ± 0.6	4.7 ± 0.8	5.3 ± 0.6	5.6 ± 0.5	6.2 ± 1.5	6.5 ± 0.7
Global end-diastolic volume (ml/m^2)	SVI	308 ± 56	237 ± 61 *	243 ± 59 *	251 ± 46 *	282 ± 58 $^{\#}$	298 ± 53 $^{\#}$
	CI	312 ± 33	191 ± 56 *$^{@}$	204 ± 32 *	211 ± 27 *	243 ± 32 *$^{\#}$	247 ± 32 *$^{\#@}$
Stroke volume variation (%)	SVI	14.7 ± 4.7	22.1 ± 5.5 *	22.2 ± 4.9 *	18.5 ± 4.6	16.7 ± 5.2 $^{\#}$	12.1 ± 3.6 $^{\#}$
	CI	11.5 ± 5.3	18.6 ± 5.2 *	18.7 ± 3.7 *	21.3 ± 4.8	19.3 ± 4.1 *	17.4 ± 7.6 *$^{@}$
Pulse pressure variation (%)	SVI	14.2 ± 5.3	24.6 ± 6.9 *	23.3 ± 6.7 *	19.0 ± 5.8 $^{\#}$	16.7 ± 5.2 $^{\#}$	13.1 ± 4.1 $^{\#}$
	CI	12.2 ± 3.1	25.2 ± 6.7 *	22.8 ± 5.4 *	19.8 ± 4.5 $^{\#}$	17.4 ± 5.8 $^{\#}$	16.3 ± 6.7 $^{\#}$
Systemic vascular resistance index (dyn × s/cm^5/m^2)	SVI	3261 ± 942	3100 ± 873	2677 ± 734	2442 ± 698 *$^{\#}$	2410 ± 466 *$^{\#}$	2336 ± 475 *$^{\#}$
	CI	3507 ± 597	3191 ± 709	2767 ± 630 *	2652 ± 240 *	2508 ± 565 *	2481 ± 495 *$^{\#}$
dPmax (mmHg/s)	SVI	561 ± 226	560 ± 344	653 ± 404	682 ± 390	987 ± 269	674 ± 236
	CI	585 ± 87	595 ± 206	579 ± 95	597 ± 137	551 ± 105	639 ± 154
Lactate (mmol/l)	SVI	2.54 ± 1.01	3.97 ± 1.80 *	4.72 ± 2.29 *	4.37 ± 2.37 *	3.90 ± 2.25 *	3.26 ± 1.95
	CI	3.32 ± 1.26	4.49 ± 1.83	4.50 ± 2.40	4.32 ± 0.69	4.05 ± 2.52	3.77 ± 2.32

SVI (stroke volume index), SVI-group; CI (cardiac index), CI-group. Data are presented as mean ± standard deviation.
* $p < 0.05$ significantly different from T_{bsl}.
$^{\#}$ $p < 0.05$ significantly different from T_0.
$^{@}$ $p < 0.05$ significantly different between groups.

III. Platelet dysfunction in moderate bleeding – pilot study

Materials and methods

Animals and Instrumentation.

Inbred Vietnamese mini pigs of both sexes (n = 6, weighing 36, 16 ± 5,36 kg) were fasted for 12 hours preoperatively, but with free access to water. The induction and maintenance of analgesia/anaesthesia, the cannulation, the bloodletting, as well as the fluid resuscitation and the hemodynamic measurements were performed according to our previously set SVI-targeted bleeding-resuscitation animal model [9]. Pulmonary artery catheter was inserted via the right femoral vein and was positioned by the guidance of pressure waveform in pulmonary artery. Internal jugular catheter was placed in the right internal jugular vein and the tip of the catheter was positioned in the right jugular bulb. At the end of the experiment the animals were euthanized with sodium pentobarbital.

Hemostatic measurements.

Blood for testing standard hemostatic parameters and platelet function were collected from each animal via the indwelling venous catheters from 3 different sites: internal jugular catheter, positioned in the jugular bulb (Bulbus); pulmonary artery for mixed venous sample (Mix); and from the inferior vena cava (VCI), immediately after the cannulation, at the end of the bleeding phase and at the end of fluid resuscitation (Fig. 9). Standard laboratory vacuum-sealed tubes containing 3.8% (0.109M) sodium citrate were used for hematocrit, platelet number (Tct), prothrombin time (PT), international normalized ratio (INR), fibrinogen and anthithrombin III (AT III) measurements. Platelet function was assessed at point of care using multiple electrode aggregometer (Multiplate® analyzer, Roche Diagnostic International Ltd., Rotkreuz, Switzerland) immediately after sample collection in vacuum-sealed tubes containing hirudin (>15μg/ml) in order to prevent clotting. Briefly, 300 μL of whole blood was diluted in warmed normal saline and incubated for 3 minutes at 37°C with continuous stirring in a Multiplate® test cell. Each test cell contains two sets of 3mm silver-coated copper wires, across which electrical resistance is measured at 0.57 second intervals. Platelet activation was induced by adenosine diphosphate (ADP, final concentration 6.5μM; via P2 receptors), arachidonic acid (ASPI, final concentration 0.5mM; via the cyclooxygenase pathway), or collagen (COLL, final concentration 3.2μg/mL; via GpIa/IIa and GpVI receptors). Platelet

adhesion to the electrodes was detected as increasing electrical impedance, measured by duplicate sets of sensor wires in each test cell. Agonist responses are reported as area under the aggregation curve in units (U) over a 6-minute measurement period. Reference ranges for whole blood were provided by the manufacturer based on studies of healthy controls.

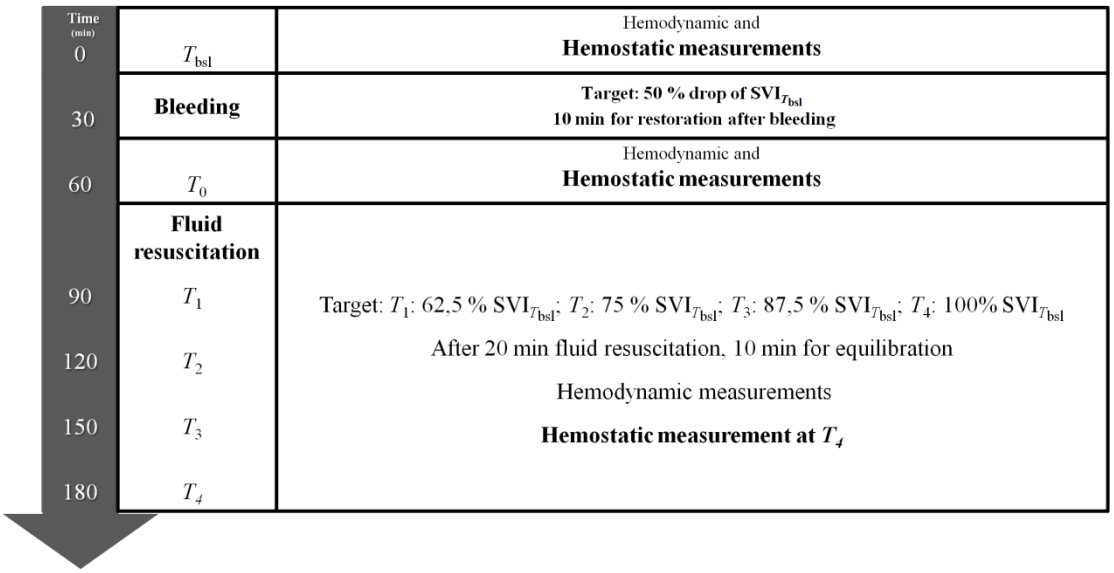

FIGURE 9. Schematic diagram illustrating the flow chart of the experiment.
Standard hemostatic parameters (Htc, PT, INR, fibrinogen, platelets number and function were measured at baseline, (T_{bsl}), at the end of bleeding phase (T_0) and at the end of fluid resuscitation (T_4).

Data analysis and statistics

Data are presented as mean ± standard deviations unless indicated otherwise. For testing normal distribution the Kolmogorov-Smirnov test was used. Changes in all parameters throughout the experiment were tested by repeated measures analysis of variance (RM ANOVA). For pairwise comparisons Pearson's correlation was used. For testing differences in platelet function between the 3 different sites (i.e.: Bulbus/Mix/VCI), a general linear model for ANOVA was applied. For statistical analysis SPSS version 18.0 for Windows (SPSS, Chicago, IL) was used and $p < 0.05$ was considered statistically significant.

Results

All animals apart from one survived the experiment. During bleeding phase 568±137 ml blood (which means 24,12 % of calculated blood volume) was drained and in total 1175ml±1036 ml crystalloid infusion was administered for resuscitation. Hemodynamic changes during the experiment are summarized in Table 5.

TABLE 5. Hemodynamic parameters during hemorrhage and fluid resuscitation

	T_{bsl}	T_0	T_1	T_2	T_3	T_4
Stroke volume index (ml/m^2)	36.4±1.3	15.5±2.5*	18±4.6*	21.5±3.5*	31.3±2.5$^{*\#}$	34.6±4.1$^\#$
Cardiac index (L/min/m^2)	2.86±0.25	1.88±0.51*	2.2±0.51	2.44±0.25$^\#$	3.0±0.43$^\#$	3.08±0.28$^{*\#}$
Mean arterial pressure (mmHg)	114±9	76±10*	82±7*	81±11*	92±8$^{*\#}$	98±7$^{*\#}$
Heart rate (beats/min)	80±8	127±25*	128±16*	110±14*	90±9$^\#$	97±16$^\#$
Central venous pressure (mmHg)	6.0±0.4	4.9±0.3*	5.3±0.5	5.4±1.2	5.7±1.3	5.5±1.4$^\#$
Arterial pH	7.47 ± 0.01	7.45 ± 0.02	7.46 ± 0.02	7.41 ± 0.03	7.48 ± 0.02	7.49 ± 0.01 *
Lactate (mmol/l)	2.20 ±0.1	2.75 ± 1.02	1.92 ± 0.92	1.50 ± 0.74$^\#$	1.40 ± 0.73$^\#$	1.20 ± 0.40 $^{\#*}$

Data are expressed as mean±standard deviation; *= ($p<0.05$) significantly different from tbsl ; #= ($p<0.05$) significantly different from t0

Among standard hemostatic parameters platelet number and fibrinogen level fell sharply during the bleeding phase (from T_{bsl} to T_0), while hematocrit, prothrombin time and INR changed significantly only after the fluid resuscitation (from T_0 to T_4). AT III level decreased during the whole experiment and reached a significantly lower level at the end of resuscitation as compared to baseline. These parameters were not affected by the sampling sites (Fig. 10).

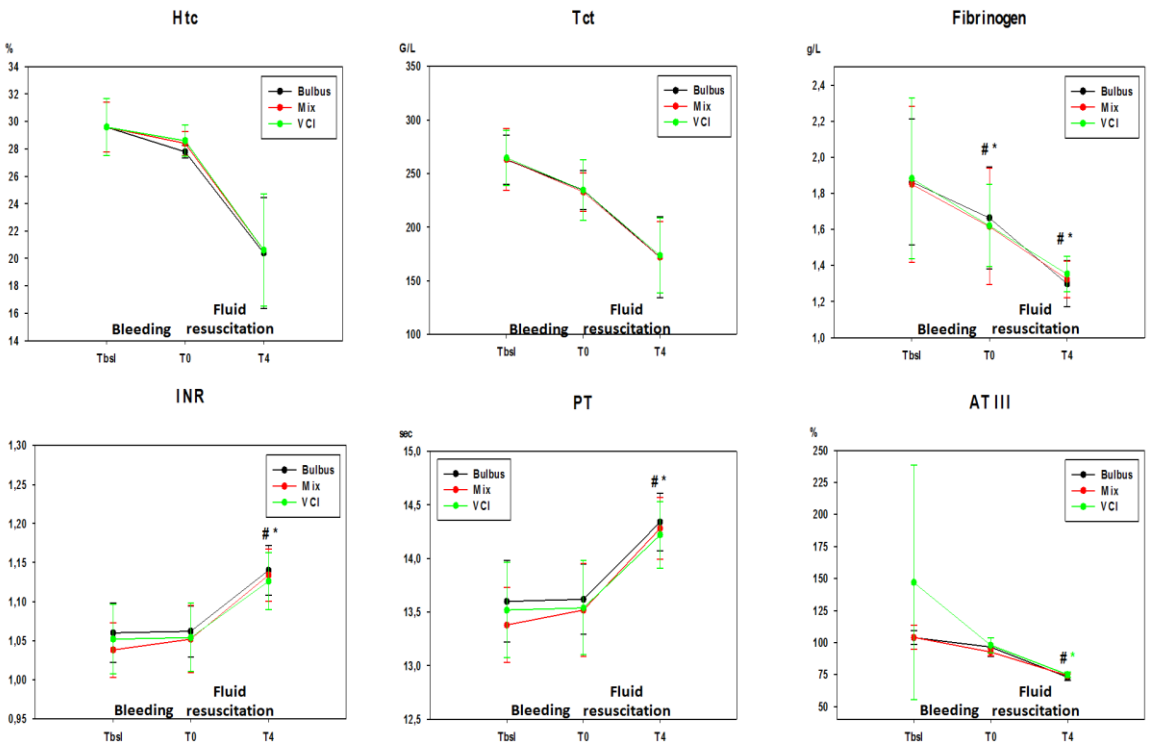

FIGURE 10.: Changes of standard hemostatic parameters
Htc: hematocrit; Tct: platelet number; INR: international normalized ratio; PT: prothrombi time; AT III: antithrombin III; Bulbus: blood taken from the jugular bulb; Mix: blood taken from the pulmonary artery; VCI: blood taken from the inferior vena cava. *= ($p<0.05$) significantly different from tbsl ; #= ($p<0.05$) significantly different from t0

Regarding the platelet function, mean platelet responsiveness to ADP, arachidonic acid, and collagen remained in the low-normal range according to manufacturer-provided reference values throughout the whole experiment. However, platelet responsiveness to ADP fell significantly by the end of bleeding and remained lower at the end of fluid resuscitation as compared to baseline in the Mix and IVC blood samples. In the Bulbus-samples there was a significant drop ADP responsiveness only between T_0-T_4. There was a tendency of gradually decreasing arachidonic acid response (ASPI), but it did not achieve statistical significance. Changes in platelet functions are summarized in Figure 11.

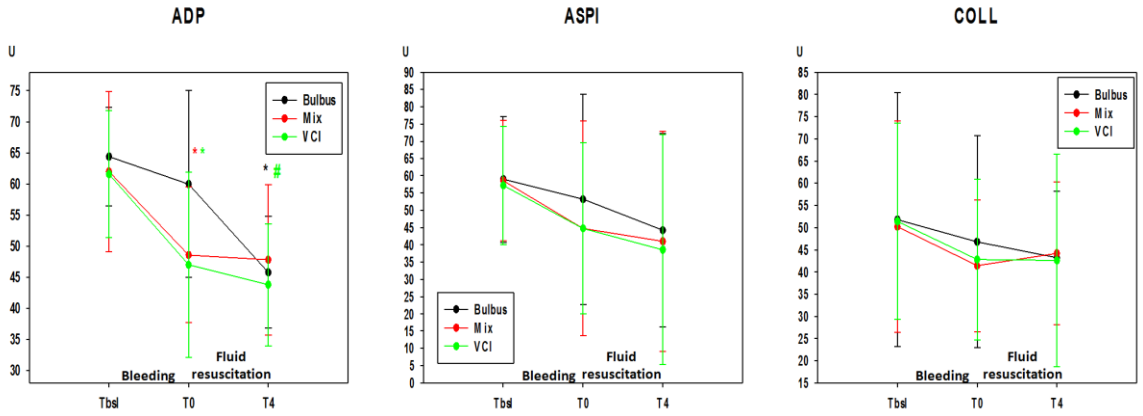

FIGURE 11. Mean platelet responsiveness during hemorrhage and resuscitation
ADP: adenosine diphosphate; ASPI: arachidonic acid; COL: collagen; Bulbus: blood taken from the jugular bulb; Mix: blood taken from the pulmonary artery; VCI: blood taken from the inferior vena cava. *= ($p<0.05$) significantly different from tbsl ; #= ($p<0.05$) significantly different from t0

4. Discussions

I. Bleeding-resuscitation animal model: Stroke volume, SVV and PPV as resuscitation end points

Our aim was to simulate a hypovolemic circulatory failure caused by moderate bleeding in a porcine model and to investigate the changes of different macrohemodynamic parameters during the optimization phase of fluid resuscitation. The physiological rationale of intravenous fluid administration to a hypovolemic patient is to increase stroke volume, therefore we chose the stroke volume index as a therapeutic end point in our experiment. The animals were exsanguinated until the SVI dropped by 50 % of its baseline value. This volume of bleeding proved enough to cause acute circulatory failure in animals, because in addition to the occurrence of significant hypotension by T_0, tissue hypoperfusion also developed with inadequate oxygen utilization by the cells indicated by elevated lactate levels, currently defined as "acute circulatory failure" by recent consensus criteria [30].

In acute circulatory failure the first questions physicians should ask themselves are: (1) whether the clinical problem can be resolved by increasing stroke volume and (2) whether fluid resuscitation will be effective to achieve this target? In patients, who are regarded hypovolemic (low stroke volume which can be improved by fluids) according to heart rate, mean arterial pressure and CVP only 50 % respond to fluid as defined by a 10–15% increase in stroke volume [43]. It is important to highlight that fluid therapy can only improve the stroke volume, hence be beneficial, if the patient is on the ascending limb of the Frank-Starling curve. In our experiment, during the optimization phase of fluid resuscitation (at T_3 and T_4 where lactate levels already decreased – compensated shock state) traditional end points such as HR, MAP and CVP failed to follow the changes in stroke volume. Although mean arterial pressure followed hemodynamic changes to some extent, it remained significantly lower at the end of the experiment as compared to T_{bsl}, which may indicate that for fine tuning of hemodynamics – after the rescue phase of resuscitation – MAP has limited value. This is due to the fact that MAP and CI do not correlate with each other [44]. The so called "static" markers of preload such as CVP and pulmonary artery occlusion pressure also poorly reflect intravascular volume and left ventricular preload as they are affected by altered venous tone, intrathoracic pressure, left and right ventricular compliance and geometry, which may all change in critically ill

bleeding patients. Nonetheless, recent large international surveys showed that more than 80% of physicians working in anaesthesiology or in critical care still rely mainly on these parameters [45, 46]. One of the most important messages of these large trials is that our everyday routine practice should be revised and may also be harmful. Our current results give further evidence that these measures should not be used routinely as resuscitation end points.

Dynamic variables of fluid responsiveness, such as stroke volume variation and pulse pressure variations allow to determine where the patient is on his individual Frank-Starling curve and thereby to differentiate between preload-dependent and preload-independent hemodynamic situations [47]. PPV and SVV are the result of the cyclic lung-heart interactions in mechanically ventilated patients when tidal volumes are kept over 8 ml/kg [48]. In the current model, all criteria were met for the correct interpretation of both SVV and PPV. Both parameters determined by pulse contour analysis became significantly elevated during hypovolemia and returned to their baseline values by the end of resuscitation and correlated well with changes of stroke volume. Our results are also in accord with the findings of several recent clinical studies, that PPV/SVV are better indicators of changes in stroke volume than conventional measures such as HR, MAP or CVP [48, 49]. However these dynamic variables cannot be used in patients with cardiac arrhythmia or with spontaneous breathing [50]. In such patients, the static variables such as cardiac output and its derivates remain the sole usable methods.

It is also important to note, that in our model normalising SVI resulted in higher cardiac output by the end of resuscitation as compared to baseline. It was possibly caused by the relatively fast bleeding induced sympathetic burst, such as tachycardia and a tendency of increased contractility, which was present until the end of the experiment. This situation is a reality in trauma and when major bleeding occurs on the wards, but in the operating room intravascular volume loss and bleeding caused hypovolemia usually occurs over a longer period of time.

II.1. SVI- versus CI-Guided Goal-Directed Resuscitation

During bleeding to restore homeostasis, the sympathetic nervous system becomes activated and releases epinephrine and norepinephrine. As a result, venous return will increase, while on the arterial side norepinephrine-caused vasoconstriction tries to maintain perfusion. Because of this sympathetic activation, heart rate and myocardial contractility will also increase. During resuscitation, our pivotal goal is to restore circulating blood volume by increasing SV to improve oxygen delivery. In several studies CI was applied as therapeutic goal [51–54], although CO is the product of heart rate and SV; thus compensatory mechanisms, such as tachycardia, may compensate residual hypovolemia.

In our next experiment we compared SVI-guided resuscitation to cardiac index-directed fluid therapy in a moderate bleeding animal model. Recent clinical investigations [55, 56] showed positive effects of SV optimization by SVV/PPV, and in our previously set bleeding-resuscitation animal model [57], SVV/PPV were well-established indicators of fluid responsiveness. Therefore in this study, SVV was the primary outcome variable as the closest to predict fluid responsiveness, hence hypovolemia. In both groups there was a significant increase in SVV and PPV after bleeding as expected but values returned to baseline only in the SVI-group. In the CI-group neither dynamic (SVV/PPV) nor static indicators of preload (GEDI) normalized to their baseline values, indicating that fluid resuscitation might had been inadequate and the normalization of CI was mainly due to the persistently elevated heart rate, rather than restoration of the circulating blood volume, leaving residual hypovolemia unnoticed. In accordance with this, the animals in the CI-group received significantly less fluid in total and also required less fluid to replace every unit of lost blood as compared to the animals in the SVI-group, showing that SVI-based goal-directed resuscitation of a bleeding subject seems superior to CI-guided resuscitation. It is interesting to note that CVP changed to a lesser degree than any other hemodynamic parameter and there was no difference between the groups; hence our results provide further evidence of the limitations to CVP as a goal during optimization phase of fluid resuscitation, also described by others [41].

Once the macrohemodynamic parameters look physiological, their effect on DO_2/VO_2 should also be assessed. Lactate, the product of anaerobic metabolism, is often referred to as one of the main biochemical targets to be treated during resuscitation [58]. In our experiment, levels were slightly elevated at baseline, possibly due to the relatively long

set-up time of the experiment, and there was an increase and then decrease during interventions, but these changes were not as dramatic as one may expect. However, it is important to note, that this experimental model is similar to a “moderate” bleeding event, and animals were resuscitated within a relatively short period of time. Due to the physiologic relationship between DO_2 and VO_2, namely, due to compensatory mechanisms when there is a drop in DO_2, up to a certain point VO_2 remains stable, in other words independent from DO_2. Therefore, although the VO_2/DO_2 ratio is increasing, but it does not cause and mean severe cellular hypoxia. To conclude, animals during this experiment were heading towards shock; they were in oxygen debt but remained close to the flat part of the VO_2/DO_2 curve, not reaching severe cellular hypoxia (Fig. 2). This is also supported by the arterial pH, which remained normal throughout in both groups. In general, this is the rationale and advantage of measuring lactate, and for similar reasons SVV or PPV, because we are “one step ahead” of cellular hypoxia or in other words our patients is in compensated circulatory shock.

One of the limitations of this experiment is that we could not provide data on microcirculation and regional blood flow, which would be interesting to see. Furthermore, these results can only partially be extrapolated for the real clinical settings. Reducing the SVI by 50% is a strictly controlled scenario, rarely happening in the everyday practice. The observation period at the end of the experiment was also short; therefore, long-term effects of SVI or CI-based fluid resuscitation could not be assessed.

II.2 The "Hemodynamic puzzle"

Over the last 20 years there were 21 clinical trials published on goal-directed fluid resuscitation [59]. In these studies hemodynamic goals showed a great variability. The most frequently used parameters to guide fluid management were CI, SV, SVV, PPV, CVP, MAP, echo-derived dynamic indices, pulmonary artery occlusion pressure, DO_2, and oxygen extraction ratio. This clearly shows that universally accepted hemodynamic target by which fluid therapy should be tailored is missing. According to our bleeding-resuscitation experiment, although the normalization of SVI is superior to CI guided-therapy due to elevated heart rate, SVI is also influenced by the activation of sympathoadrenerg system as indicated by the increased tendency of myocardial contractility. These results suggest that using SVI only may not be sufficient, and patients can still remain under resuscitated. Therefore, instead of using one single parameter to treat or follow as a target, it is necessary to put hemodynamic variables and measures of VO_2/DO_2 into context in a way that once macro-hemodynamic parameters are "normalized," adequacy of treatment has to be checked by measures of oxygen debt. Measuring SVI and SVV or PPV as well as simple blood gas driven variables such as $S_{cv}O_2$, dCO_2 and lactate are valuable tools to solve this puzzle as quickly as possible (Fig. 8) [5].

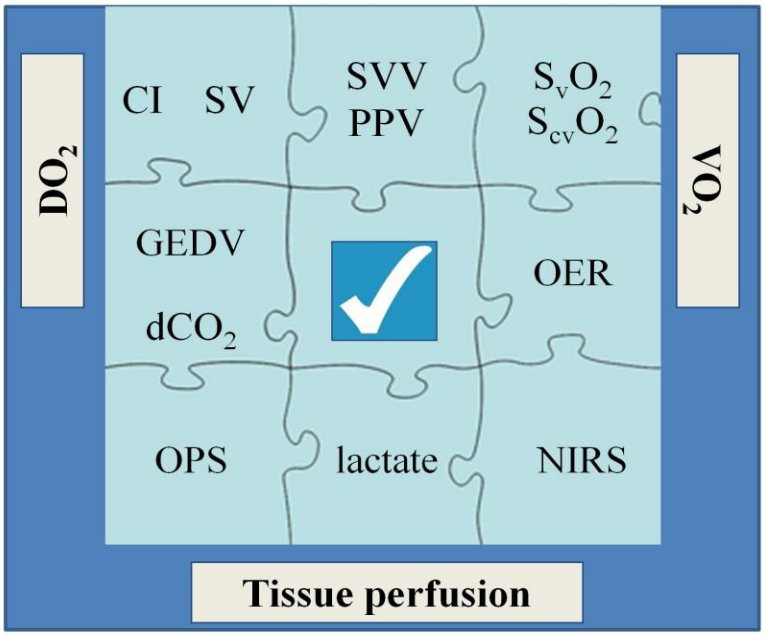

FIGURE 8: Hemodynamic puzzle.
CI cardiac index; SV stroke volume; GEDV global end-diastolic volume; dCO_2 central venous to arterial CO2-gap; OPS orthogonal polarization spectral imaging; SVV stroke volume variation; PPV pulse pressure variation; S_vO_2 mixed venous saturation; $S_{cv}O_2$ central venous saturation; OER oxygen extraction ratio; NIRS near-infrared spectroscopy;

However, “solving” the global hemodynamic puzzle is one thing, but normalizing the microcirculation is another. The physiological variables used to target resuscitation are based on correcting systemic hemodynamic variables of pressure, flow, and/or oxygen delivery and relies on the assumption that there is hemodynamic coherence between the macrocirculation and the microcirculation. In traumatic hemorrhagic shock Tachon et al. were successful in restoring systemic hemodynamic parameters (HR, MAP, CI and lactate) by applying fluids, blood, and vasoactive medication in the resuscitation phase [60]. However, despite the almost immediate correction of cardiac output and arterial pressure, the sublingual microcirculation took up to 4 days to recover, with the length of time to recover correlating with the occurrence of organ dysfunction [60]. With the introduction of a new generation of hand-held microscopy at the bedside, the nature of microcirculatory alterations has been elucidated and it opens the way to enabling titration of fluids to optimally recruit the microcirculation in such a way as to optimize its oxygen transport capacity [61].

Future clinical trials will be needed to determine whether such procedures will translate into improved outcomes in comparison with fluid therapy based on systemic hemodynamic variables in traumatic hemorrhagic shock [62].

III.1 Platelets dysfunction in moderate bleeding – pilot study

Despite uniformly normal standard coagulation parameters (PT, INR, APTT) and admission platelet counts, platelet dysfunction after major trauma is strikingly common, occurring in 45.5% of patients on admission and 91.1% at some time during their ICU stay [24]. It has been shown that injury severity score (severity of tissue injury) and severe base deficit (acidosis) as well as low GCS (severe brain injury) are multivariate predictors of admission platelet hypofunction in these patients. Therefore the latest European guideline on major bleeding and coagulopathy following trauma recommend platelet function testing after a major trauma complicated with brain injury [37]. However, based on hypoperfusion-related endothelial injury one can hypothesize that hypoperfusion may be able to cause platelet dysfunction before severe acidosis developed and without severe tissue and/or brain injury. Therefore, we used our previously established bleeding-resuscitation animal model in order to investigate the possible effect of moderate hypoperfusion on platelets function. Furthermore, as there is some evidence that the manifestation and degree of endothelial glycocalyx injury [17], which can have a profound effect of platelet function, may different from organ to organ, we decided to take samples from the jugular bulb (representing venous blood from the brain), from the inferior vena cava (lower body) and from the pulmonary artery (mixed venous blood representing the whole body).

Porcine models of coagulopathy in the setting of bleeding are popular and favored because they use a large mammalian species that shares gross cardiovascular physiology with humans. A review of experimental bleeding-associated coagulopathy models found that of 33 models deemed appropriate for review, 17 were porcine [63]. Swine are amenable to precise monitoring while providing also adequate sample volumes for platelet function testing. However, significant differences may exist in the type of coagulation changes produced in swine in response to hemorrhage and these differences are important to consider when interpreting our results.

Jacoby et al. used the PFA-100 platelet function analyzer and flow cytometric markers of platelet activation to prospectively assess platelet function in 100 trauma patients. In this study, significantly impaired platelet function were observed in non-survivors at later time point as compared to survivors, and similarly, platelet dysfunction occurred in patients with significant head injury compared to patients without head injury at 24h [64]. However, this study found no differences in non-survivors or brain-injured patients on

admission based on PFA-100 measurements, although platelet microparticle levels were significantly higher on admission in these populations. This may indicate that early alterations in platelet function are not reliably detected by PFA-100 aggregation. It was confirmed in another study using multiple electrode impedance aggregometry in order to assess platelet function disorders in 101 severely injured trauma patients. They found poor admission AA- and collagen-induced responsiveness in non-survivors and brain injured patients. Thus they identified that impedance aggregometry is more sensitive to these early differences, and appears superior to PFA-100 aggregation in identifying platelet dysfunction [24]. Therefore, we chose multiple electrode impedance aggregometry in our experiment in order to notice early changes of platelets function in moderate bleeding settings.

In our animal model moderate bleeding led to moderate hypoperfusion as indicated by significantly lower blood pressure but mildly elevated lactate level after bleeding phase, which did not cause metabolic acidosis. The whole experiment was performed within a relatively short period of time thus the animals developed a VO_2/DO_2 imbalance, but remained on or at least close to the flat part of the VO_2/DO_2 curve, not reaching severe cellular hypoxia.

Modest amount of bleeding was also confirmed by the changes of standard hemostatic tests. We found significant and clinically relevant deficiency in fibrinogen level only during the experiment, while platelet number and pro-and anticoagulation factors (PT, INR, ATIII) remained in the normal range. It is in accord with the results of a study performed on surgical patients with normal coagulation factors, in whom critical levels of fibrinogen (1.0 g/L) were reached, at least from the hemostatic point of view, at blood loss >150%, while shortage of platelets (50 × 103/mm3) and coagulation factors (II, V and VII) occurred after more than 200% of blood loss [65]. It can be due to a release of sequestered platelets from the spleen and lungs after bleeding and reduced thrombin activation is partially compensated by lower inhibitory activities of antithrombin and other protease inhibitors, whereas plasma fibrinogen is rapidly decreased proportional to the extent of bleeding and hemodilution [66].

Regarding platelets function Kutcher et al. identified injured activation (hyporesponsiveness to ADP in 30.7 %), adhesion (to collagen in 34.7 % in patients) and aggregation (to TRAP in 18.7 %) of platelets in severely injured trauma patients (median ISS: 25, mean pH:7.23 ± 0.20 and mortality rate of 22.7%). However our preliminary data suggest that damaged activation of thrombocytes (responsiveness to ADP) – “injured

platelets burst" during the primary hemostasis – may be present without significant acidosis and tissue injury and occur in the early phase of bleeding before fluid resuscitation. The mechanisms underlying trauma-associated platelet dysfunction are poorly understood. One of the potential mechanisms suggested that there is immediate platelet activation in response to hypoperfusion-related sympatoadrenal activation and cathecolamine release, which may induce a prolonged refractory state, in which a fraction of activated platelets remain in circulation but are dysfunctional [64]. Autonomic dysfunction measured by heart rate variability in the first day following trauma is associated with injury severity, coagulopathy and mortality [67]. Xu et al. demonstrated that ANS dysfunction, namely sympathetic–vagal disequilibrium, actually exists in TIC and may partly contribute to enhanced inflammation, endothelial and coagulation disturbances such as autoheparinization, hyperfibrinolysis and platelets dysfunction. Moreover, sympathetic denervation yielded anti-inflammatory, antifibrinolysis and endothelial protective effects in rats with TIC [68].

The finding that platelets taken from the jugular bulb responded differently during aggregometry is extremely interesting. It may indicate that indeed, there is organ specific response for bleeding, but it certainly requires further investigations.

Finally, it is important to highlight that hematocrit changed significantly only after fluid resuscitation (from T_0 to T_4), which gives further evidence that hemoglobin or hematocrit may not accurately reflect blood loss because patients bleed whole blood and compensatory mechanisms that move fluids from interstitial space or fluid resuscitation require time and may not be reflected in initial measurements. Therefore, the new European guideline on major bleeding and coagulopathy following trauma recommends the use of repeated hemoglobin measurements as a laboratory marker for bleeding, as an initial hemoglobin value in the normal range may mask bleeding [37].

Based on the preliminary results of our pilot study our plans for future research are:

1. to continue the investigation of changes of platelet function in this moderate bleeding animal model

2. to investigate the changes of platelet function and the endothelial glycocalyx in a severe hypoperfusion model but without severe tissue and brain injury

3. to assess the changes of platelet function and the endothelial glycocalyx in blood samples from different organs in a severe hypoperfusion model

III.2 Hemostatic resuscitation

Over the last 20 years, the management of coagulopathy in trauma patients has changed radically. The administration of red blood cells (RBCs) should be supplemented as soon as possible with fresh frozen plasma (FFP) to compensate for the deficit in coagulation factors. There are two strategies for correcting hemostasis.

So called 'formula-driven' resuscitation with a predetermined RBC:FFP ratio serves as a fast and easy-to-use approach to early correction of hemostasis. Recent retrospective studies suggested an RBC:FFP ratio of approximately 1:1:1 [69]. Although this concept is gradually expanding from trauma to non-trauma bleeding management, the results of these studies are conflicting and should be interpreted with caution because of the potential for survival bias and substantial differences between civilian and military trauma. In an international prospective cohort, only combined high-dose FFP, cryoprecipitate and platelet therapy with a high total fibrinogen load appeared to produce a consistent improvement in coagulation [70]. Undoubtedly the onset, course and severity of coagulopathy differ depending on the etiology. Therefore, it is likely that a given RBC:FFP ratio does not fit all patients. Furthermore, the timing of FFP administration may play a pivotal role in the outcome; hence, it may be just as important, or even more so, than the RBC:FFP ratio itself [71].

Unnecessary overuse of blood products can lead to several complications [72]. However, individualized 'goal-directed' hemostatic resuscitation may help to rationalize blood product utilization. Dynamic monitoring of whole-blood coagulation provides several advantages as compared to conventional plasma-based tests, such as the prothrombin time, activated partial thromboplastin time, international normalized ratio, fibrinogen and platelet count. The latter tests are slow and mainly reflect the initiation phase of thrombin generation. They cannot be used to evaluate the primary hemostasis, clot strength and fibrinolysis, which are also important pillars of hemostasis and can often be impaired in severe bleeding. Viscoelastic tests (such as the TEG or ROTEM), however, provide rapid and dynamic evaluation of clot formation, strength and stability, while impedance aggregometry reliably identifies the difference disorders of platelet-related primary hemostasis in severly injured patients [73]. There is emerging evidence that point-of-care tests based on "goal-directed" coagulation management can modify the transfusion strategy by providing better understanding of the underlying pathology and by the targeted use of not only FFP, but also fibrinogen and prothrombin complex concentrates

(PCC), hence reducing the need for blood products, which enables the clinician to tailor hemostasis management according to the patient's needs [74]. Current guidelines emphasize the importance of higher target fibrinogen levels (1.5–2.0 g/l) and platelets of 50 G/l in general, or 100 G/l in brain injury [37]. It is important to note that clinically significant platelet dysfunction after trauma exists in the presence of an otherwise reassuring platelet count and clotting studies, with profound implications for mortality. However there are debates on whether platelet transfusion should be done to the patients who have platelet dysfunction with normal count, because some studies showed that patient with TIC can receive benefits from platelet transfusion, but others showed that fibrinogen and prothrombin complex concentrate transfusion are enough and there is no need to transfuse platelet [75]. Regarding the adjunctive treatment of hemostasis, there is strong evidence that tranexamic acid reduces mortality; therefore, its early routine administration during hemostatic resuscitation is recommended by international guidelines [37, 76, 77]. Recombinant factor VII can be considered only if major bleeding persists after all efforts to support the fundamental pillars of hemostasis have failed (Fig. 4).

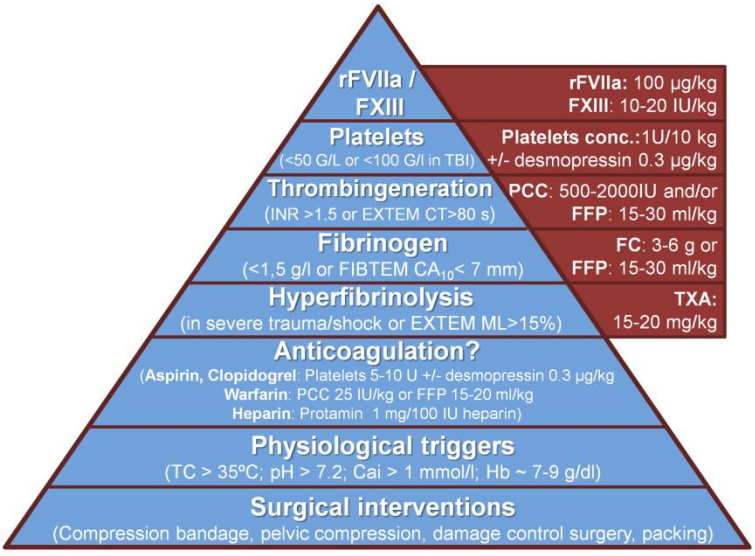

FIGURE 3: Cornerstones of coagulation management in hemorrhagic shock

Cai ionized calcium; Hb hemoglobin; PCC prothrombin complex concentrate; FFP fresh frozen plasma; INR international normalized ratio; TBI traumatic brain injury; TXA tranexamic acid; FC fibrinogen concentrate; rFVIIa recombinant activated factor VII; FXIII factor XIII

Considering the optimization phase of hemorrhagic shock management, in addition to restrictive transfusion protocols with a post-transfusion target of hemoglobin levels of 70–100 g/l, ‘physiological triggers’ such as the $S_{cv}O_2$ values have also been suggested to aid an individualized approach by tailoring transfusion according to the patient’s actual needs instead of a ‘numbers-driven’ protocolized care [27].

5. Main statements of the thesis

I/1. Moderate bleeding evoked by 50% reduction in SVI can induce acute circulatory failure indicated by significant hypotension and elevated lactate level in porcine model.

I/2. Dynamic hemodynamic parameters such as SVV/PPV, rather than conventional parameters (HR, MAP, and CVP) indicate macrocirculatory changes in a moderate bleeding animal model

II/1. CI-based resuscitation is significantly influenced by the compensatory sympathetic response for bleeding caused stress in this moderate bleeding model

II/2. CI-targeted resuscitation resulted in inadequate fluid resuscitation and thus residual hypovolemia in compensated shock states

II/3. SVI-targeted fluid management resulted in significantly better macro-hemodynamic indices as compared to CI-targeted resuscitation, and also normalized the values of most hemodynamic parameters

II/4. We do not recommend using cardiac output on its own as a resuscitation endpoint in the optimization phase of fluid resuscitation in acute bleeding events

III/1. Our preliminary data generates two hypotheses: a) that impaired platelet activation may be present in the systemic circulation without significant acidosis and tissue injury and occur in early phase of bleeding (T_{bsl}-T_0) before fluid resuscitation, which is a novel finding, never described before; b) the observation, that these changes did not take place to a similar manner in the jugular bulb may suggests that there might be an organ specific platelet function response for bleeding. (Based on these results we plan to continue to research of changes of platelet function in further animal experiments.)

6. Conclusions

In these experiments we have shown that SVI-based goal-directed resuscitation of a bleeding subject seems superior to CI-guided resuscitation as indicated by both hemodynamic parameters and measures of oxygen debt returning to baseline in the SVI-group, which was incomplete in the CI-group. However, we would like to emphasize that treating one single parameter during resuscitation is a grossly oversimplified approach, may also be harmful, therefore a multimodal concept, taking into account the full picture of the “hemodynamic puzzle” should be applied instead.

Finally, we strongly recommend a coordinated teamwork (including several different specialties), which is essential for the fast and appropriate management of patients requiring massive transfusions. In addition to educating specialists and trainees alike for the understanding of the underlying pathophysiology of hemostasis, the implementation of hospital-specific “massive transfusion protocols” is just as important, which may promote cooperation and accelerate the process, hence improving outcomes in patients with severe bleeding [18].

In summary, the current paradigm shift in the management of hemorrhagic shock evolving over the last decade changed our understanding and provides several new alternatives for improving outcomes in these patients; therefore, this knowledge should be spread and our current practice reviewed in order to implement the necessary changes in our institutes as soon as possible.

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APPENDIX

I.

II.

III.

IV.