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

- Summary of PhD Thesis -

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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.
IF:2,134

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
IF:10,125

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.
IF:-

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. Uncontrolled post-traumatic bleeding is one of the leading causes of potentially preventable death among these patients. 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. 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 ("Damage control resuscitation"). 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.

2. Aims of the thesis

Although there is no consensus on the best or universally accepted hemodynamic parameter as resuscitation target in bleeding patients, cardiac output calculated from thermodilution or pulse

contour analysis is the most often used end-point during goal-directed therapy. However cardiac output-based optimization may also lead to inadequate fluid resuscitation. 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 central role of hypoperfusion-related endothelial injury in trauma-induced coagulopathy one can assume that moderate hypoperfusion may also be able to cause platelet dysfunction before severe acidosis developed and without severe tissue 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 (dP_{max}) 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

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 in 4 steps (T_{1-4}) 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.

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. 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}. 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.

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. The induction and maintenance of analgesia/anaesthesia, the cannulation, the bloodletting, as well as the hemodynamic measurements were performed according to our previously set SVI-targeted bleeding-resuscitation animal model. For blood gas measurements the right femoral artery served as the site for arterial blood gas sampling simultaneously at baseline and at the end of each step.

Experimental Protocol.

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 $SVI_{T_{bsl}} - SVI_{T_0}$ 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 $CI_{T_{bsl}} - CI_{T_0}$ 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. Fluid replacement was carried out with boluses of 200 mL of balanced crystalloid 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.

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. 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. Hemodynamic parameters were similar at T_{bsl} and goals of 50% reduction in SVI were reached by T_0 in both groups. 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 *T*bsl. 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 dP_{max} values, but it did not achieve statistical significance.

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. 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 (hematocrit, platelet number, prothrombin time, INR, fibrinogen, anthithrombin III) and platelet function (responsivness to ADP, arachnoid acid and collagen) 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 (T_{bsl}), at the end of the bleeding phase (T_0) and at the end of fluid resuscitation (T_4). Platelet function was assessed at point of care using multiple electrode aggregometer (Multiplate® analyzer, Roche Diagnostic International Ltd., Rotkreuz, Switzerland).

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. This moderate amount of bleeding led to modest hypoperfusion as indicated by significantly decreased blood pressure during the bleeding phase (from 114±9 mmHg to 76±10 mmHg) but mildly elevated lactate level at T_0 (2.75 ± 1.02 mmol/l) which did not cause metabolic acidosis (pH=7.45±0.02). 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. 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 (from T_{bsl} to T_0), and remained lower at the end of fluid resuscitation as compared to baseline in the Mix and IVC blood samples. In the Bulbus-samples platelet responsiveness to ADP also decreased by the end of bleeding (from T_{bsl} to T_0), but a significant drop in ADP responsiveness occurred only at the end of fluid resuscitation as compared to baseline (from T_{bsl} -T_4). There was a tendency of gradually decreasing arachidonic acid response, but it did not achieve statistical significance.

4. 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.)

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