Effects of goal-directed fluid therapy on microcirculation: experimental and clinical studies

PhD thesis

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1. INTRODUCTION

Early fluid resuscitation remains the cornerstone of the treatment of severe hypovolemia, bleeding and septic shock. Although during these circumstances' fluid administration is a lifesaving intervention, it can also exert a number of adverse and potentially life-threatening effects, hence fluid therapy by-and-large is regarded a "double-edged sword". Unfortunately, for the three fundamental questions of: "when", "what" and "how much", we don't have universally accepted answers. Nevertheless, not giving enough volume may result inadequate cardiac output (CO) and oxygen delivery (DO_2), hence severe oxygen debt; while fluid overload can cause edema formation both in vital organs and on the periphery, hence impair tissue perfusion. In addition to using appropriate hemodynamic parameters to guide fluid resuscitation, the type of the infusion fluid should also be chosen carefully.

According to Starling's "3-compartment model" crystalloids (Cryst), with their sodium content similar to that of the serum, are distributed in the extracellular space, while colloids (Coll) should remain intravascularly due to their large molecular weight. Therefore, theoretically one unit of blood loss can be replaced by 3-4 units of Cryst and one unit of Coll solution. Understanding physiology especially the role of the recently discovered multiple function of the endothelial glycocalyx (GX) layer may cast a different light on these controversies. Fundamentally there are 3 infusion solutions that can be administer intravenously: 1) water, in the form of 5% dextrose, 2) Cryst's, containing sodium ions in similar concentration to that of the plasma, and 3) Coll's, which are macromolecules of either albumin or synthetic Coll molecules, such as hydroxyaethyl starches (HES). According to the classical Starling concept, the main determinants of the fluid transport between the 3 main fluid compartments of the intracellular, interstitial and intravascular spaces, are determined mainly by the 2 semipermeable membranes: the endothelium and the cell membrane. A web of membrane-bound glycoproteins and proteoglycans on the luminal side of endothelium has been identified to form the GX layer. This compartment consists of many highly sulfated glycosaminoglycan chains providing negative charge for the endothelium. Due to these electrostatic properties the subglycocalyx space produces a colloid oncotic pressure that might be an important determinant of vascular permeability and thus fluid balance. Nevertheless, under normal circumstances, when the GX is intact the Starling concept is still valid fluid transport is determined by the "Starling forces", and the volume replacement ratio should be several times higher for Coll's as compared to

Cryst's. There is mounting evidence that the GX becomes impaired or destroyed in several critically ill conditions. These include inflammation (both infectious or non-infectious), trauma, sepsis, ischemia-reperfusion injuries, but also persistent hypo-, and hypervolemia are known to affect the GX.

Although most recent large clinical trials were studies with end-points of 28-day mortality or organ dysfunction, it is worthwhile to analyze these results from a different perspective. Results showed strong association between acute kidney injury, increased use of renal replacement therapy and the use of HES, which was also accompanied with unfavorable patient outcome. There are several common features in these studies. First of all, the ratio of the administered volume of Cryst and Coll were completely different as what should have been expected according to the Starling principle. In general, only 30-50% more Cryst seemed to have the same volume expanding effect as Coll. Based on these results, a common view was formed that HES doesn't have higher potency for volume expansion than Cryst, but carries a greater risk of renal dysfunction and mortality. However, it is important to note that none of these trials applied detailed hemodynamic monitoring, which is the second common feature of these studies. The administration of iv. fluids was mainly based on the clinicians' subjective decision, or on parameters such as heart rate, blood pressure, central venous pressure, urine output, lactate levels or central venous oxygen saturation. CO, stroke volume (SV) was not measured in most of the trials, which is essential to prove volume responsiveness.

Inappropriate haemodynamic management during major surgery may lead to hypoperfusion or fluid overload, both of which are accompanied by a significant risk of impaired postoperative outcome. The same holds true for unnecessary use of vasoactive medications and blood transfusions. In reducing such adverse effects, advanced haemodynamic monitoring-based management has a strong pathophysiological rationale and, indeed, advanced haemodynamic monitoring-based peri-operative management has been shown to improve outcomes in high-risk surgery in several studies. To avoid the lethal consequences, intravenous fluid resuscitation is the first line of treatment, which has to be fast and efficient. Furthermore, as reported in recent prospective studies, non-survivor trauma patients also had significantly higher circulating syndecan-1 concentrations than survivors, indicating an impairment in the endothelial GX. These results suggest that critical illness in general predisposes the patient to GX damage; hence, the VR (volume replacement) ratio of Cryst and Coll may be different from what would have been expected.

2. AIMS AND SCOPES

I. The main aim of the current study was to compare the volume replacement effects of Cryst and Coll solutions during bleeding-resuscitation with moderate hemorrhage in an experimental animal model. We applied the model, which has been tested and reported in our previous experiments.

II. Second, we aimed to perform a randomised clinical trial to examine the effects of intra-operative Cryst and Coll fluid replacement guided by detailed haemodynamic monitoring on microcirculatory perfusion in patients undergoing free flap surgery for maxillofacial malignancy.

3. METHODS

3.1.Volume-replacement ratio

3.1.1. Ethical permission

The experiments were performed on the EU Directive 2010/63/EU for 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 experimental project was approved by the National Scientific Ethical Committee on Animal Experimentation (National Competent Authority), Hungary, with license number: V./142/2013. The study was conducted in the research laboratory of the Institute of Surgical Research in a manner that did not inflict unnecessary pain or discomfort upon the animals.

3.1.2Animals and instrumentation

Vietnamese pot-bellied pigs (n=30) underwent a 12-h preoperative fasting period with free access to water. The pigs were randomised into two groups: balanced crystalloid Ringerfundin, RF group (B. Braun AG) and a colloid (Voluven®, hydroxyethyl starch (HES)) group. Anesthesia was induced by intramuscular injection of a mixture of ketamine (20 $mgkg^{-1}$) and xylazine (2 $mgkg^{-1}$), maintained by a continuous intravenous propofol infusion (6 $mgkg^{-1}h^{-1}$ i.v.), and analgesia was performed with nalbuphine (0.1 $mgkg^{-1}$). Tracheal tubes were inserted in all animals, and the lungs were mechanically ventilated by Dräger Evita XL (Dräger, Lübeck, Germany). Tidal volume was adjusted to 10 $mlkg^{-1}$, and the respiratory rate was initialized to keep the end-tidal carbon dioxide and partial pressure of arterial carbon dioxide within 35–45 mmHg. After induction of anesthesia, catheters were inserted into the right jugular vein, the left carotid artery, and the right femoral artery. For invasive hemodynamic monitoring, a transpulmonary thermodilution catheter (PiCCO, PULSION Medical Systems SE, Munich, Germany) was placed in the right femoral artery. Throughout bleeding, blood was drained through a catheter from the left carotid artery to a cylinder.

3.1.3Experimental protocol

After instrumentation, 30 min was allowed for stabilization before baseline (Tbsl) measurements were taken. At each assessment point, hemodynamic measurements, blood gas analyses, and laboratory tests were performed. After Tbsl, the pigs were bled until the

stroke volume index (SVI) dropped to 50% of its baseline value (T0); then, measurements were repeated. The difference of SVI at Tbsl and T0 was divided into four equal target values, which was planned to be reached in four steps during fluid resuscitation (T1–4) to reach the initial SVI by T4. Fluid replacement was executed with boluses of balanced RF or HES solutions until the target SVI value was reached. After reaching each step, 20 min was allowed for equilibrium; then, blood gas, laboratory tests and hemodynamic parameters were measured. All of the pigs were euthanized with sodium pentobarbital at the end of the experiment.

3.1.4Hemodynamic monitoring and blood gas sampling

Cardiac function index (CFI), cardiac index (CI), left ventricular contractility (dPmax), global end-diastolic volume (GEDI), heart rate (HR), mean arterial pressure (MAP), pulse pressure variation (PPV), stroke volume index (SVI), and stroke volume variation (SVV) were measured via PiCCO. For blood gas measurements, the right femoral artery served as the site for arterial blood gas sampling and the catheter in the right internal jugular vein was used for taking central venous blood gas samples and measured central venous pressure (CVP). These were analyzed in parallel by co-oximetry (Cobas b 221, Roche Ltd., Basel, Switzerland) at each step. From these parameters, the following variables were calculated: Oxygen consumption (VO_2), Oxygen delivery (DO_2), Oxygen extraction. VR ratios were calculated by the resuscitation fluid over the total blood loss.

3.1.5 Glycocalyx degradation

Blood concentrations of syndecan-1 and glypican were quantified by enzyme-linked immunosorbent assay (ELISA) (MybioSource, Inc., San Diego, USA). For this purpose, blood samples were taken at Tbsl, T0, and T4.

3.2 Effects of goal-directed crystalloid vs. colloid fluid therapy on microcirculation during free flap surgery

3.2.1 Patient selection

This randomised, controlled study (Ethical Committee No. 44/2014) was undertaken between April 2014 and February 2018 and was approved by the Regional and Institutional Human Medical Biological Research Ethics Committee, University of Szeged, Hungary on 28 April 2014. The investigation was performed at the University of Szeged. The study was

registered at ClinicalTrials.gov with the registration number: NCT03288051. Written informed consent was obtained from all participants. Adult patients of both sexes undergoing radical forearm free flap surgery were recruited. Exclusion criteria included vulnerable individuals as defined in ISO 14155:2011, pregnant or lactating women, and end-stage oral cancer. The study's primary end-point was the difference in the perfusion units as determined by laser-Doppler at R_{12}. Patients were randomised either to a Cryst group (Ringerfundin; B. Braun Melsungen, Germany) or a Coll group [hydroxyethyl starch (HES), Voluven 6%; Fresenius Kabi Deutschland, Germany], using envelope block-randomisation in blocks of fifteen. Patient enrolment, sequence generation and assignment to interventions were performed by a responsible investigator. Only the patients were blind to group allocation.

3.2.2 Intra-operative protocol

Patients received routine anaesthetic management. In addition to standard monitoring, a radial artery catheter was inserted under local anaesthesia for invasive blood pressure monitoring. This arterial line was also connected to a noncalibrated haemodynamic monitor (ProAQT; PULSION Medical Systems SE, Munich, Germany). Anaesthesia was induced with 1 to 3 $mgkg^{-1}$ propofol [Propofol (1%), Fresenius Kabi Deutschland, Germany], 0.6 $mgkg^{-1}$ rocuronium (Esmeron, MSD Pharma Hungary,Hungary),and for analgesia, morphine (Morfina Jacopo Monico, Italy) was used. Anaesthesia was maintained with sevoflurane with the minimum alveolar concentration maintained around 1.3 vol%. Patients were ventilated with an 8 $mlkg^{-1}$ tidal volume, in pressure control mode, in order to have a reasonable effect on PPV. During the operation, core temperature was measured by rectal thermometer. Maintenance fluid was Ringerfundin $1mlkg^{-1}h^{-1}$. After induction of anaesthesia, a central venous catheter was inserted into the right internal jugular or the right subclavian vein based on the requirements of the surgical approach. In brief, fluid responsiveness was defined as PPV at least 10%, but this did not mean that fluid was given immediately: fluid administration was determined by a complex, multimodal algorithm. In cases when fluid loading was indicated, a bolus of 250ml of Ringerfundin or HES, as determined by randomisation, was administered within 15min. The aim was to maintain CI above 2.5 $lmin^{-1}m^{-2}$. If CI was low, and our haemodynamic model indicated that contractility had to be improved, then dobutamine (Dobutamine Hexal, Sandoz Hungaria, Hungary) was administered starting at a rate of 5 $\mu gkg^{-1}min^{-1}$. In the case of a drop in blood pressure, as indicated by MAP of 65 mmHg or less or at least 20% drop as compared with

baseline data, after excluding hypovolemia, or myocardial depression, norepinephrine was started as a continuous infusion. Global haemodynamic assessment was complemented with measuring hourly urine output and arterial and central venous blood gas analysis. Parameters included in the haemodynamic decision algorithm were central venous oxygen saturation ($ScvO_2$), central venous-to arterial CO_2-gap (dCO_2), arterial lactate, HCO_3 and pH. Normal values for these parameters were considered as $ScvO_2$: 70 to 80%, dCO_2 of 6 mmHg or less, HCO_3: 20 to 24 mmol l^{-1}, pH: 7.35 to 7.45. Arterial and central venous samples were taken at the same time hourly, or anytime in between, when a decision had to be supported in order to commence therapy. This approach was aimed at helping to individualise treatment, rather than following a preset target value. Data were recorded after instrumentation at baseline (T_0), at incision (T_i) and then hourly until the end of the surgery (T_{es}) and 24h after T_0 (T_{24}). All patients were monitored in the ICU until they were discharged to the Maxillofacial Surgery ward. Patients received standard ICU care according to our institutional protocols.

3.2.3 Laser-Doppler flowmetry

All flaps were monitored with noninvasive laser-Doppler flowmetry (PeriFlux 5000 LDPM; Perimed, Järfälla, Sweden). A probe with a standard fibre separation of 0.25mm, and a 780nm wavelength laser was used. The depth of the measurements was 0.5 to 1mm. Results are expressed as perfusion units. The first measurements were taken after the flap was prepared (R_{bsl}), then 1h after reperfusion and continued hourly for up to 12h (R_1–R_{12}). The probe was placed and fixed in a position in the centre of the forearm flap skin island. The skin in the deltoid region provided the control site. At both places, measurements were taken after active warming of the skin, at 35℃ and 44℃.

3.3. Data analysis and statistics

For statistical analysis, Statistical Program for Social Sciences version 23.0 for Windows (SPSS, Chicago, Illinois, USA) was used, and *P* value less than 0.05 was considered as significant. Data are presented as mean ± SD or median [IQR]. For testing normal distribution, the Shapiro–Wilk test was used. Independent samples were tested by independent samples t-test or Mann–Whitney U test. Changes in repeated measures throughout the experiment were tested by two-way repeated measures analysis of variance (ANOVA) with Bonferroni post hoc comparisons. Categorical data were compared using χ^2 tests. The Type I error probability associated with this test of the null hypothesis is 0.05.

4. RESULTS

4.1. Volume-replacement ratio

Out of the 30 animals, 27 survived the full experiment. Two in the HES group and one in the RF group had a sudden cardiac arrest after induction of anesthesia for reasons unknown. Therefore, the results of 27 animals (HES $n = 13$; RF $n = 14$) were finally analyzed. Animals were of similar weight, height, and body surface area in both groups. For a 50% decrease in SVI, a similar amount of blood had to be drained in both groups. Invasive hemodynamic (PiCCO) measurements were taken at similar frequencies in both groups. Urine output was significantly higher in the RF group.

4.1.1. Macro-hemodynamic effects of fluid resuscitation

Hemodynamic results were similar at Tbsl, and goals of 50% reduction in SVI were reached by T0 in both groups. Hemodynamic changes during the experiment did not show clinically relevant differences between the groups. At Tbsl, the SVI values were similar, after bleeding SVI decreased by the planned 50% to T0 and returned to its initial value by T4. Kinetics of the CI, MAP, HR, and GEDI showed similar pattern in both groups with significantly higher values in the HES group at the end of the experiment (T4). SVV and PPV almost doubled after bleeding in both groups and then returned to baseline values, being significantly lower in the HES group. Extravascular lung water index showed some changes during the experiment in both groups, without any significant differences between the groups. Contractility (dPmax) values, also showed same changes in both groups.

4.1.2. Changes in VO_2/DO_2 during fluid resuscitation

Arterial pH was elevated in both groups due to unintentional hyperventilation which was then corrected towards the end of the experiment. Partial pressure of arterial oxygen tension and oxygen saturation remained stable and within the normal range throughout the study. Central venous oxygen saturation fell during the bleeding phase in both groups, but baseline values were achieved earlier in the HES group. Changes in oxygen extraction followed a similar pattern in both groups. Venous to arterial carbon dioxide gap increased significantly after the bleeding phase, with significantly higher values in the RF group, and then returned to physiological values by T3 in both groups.

4.1.3. Volume-replacement ratios

While the hemodynamic profile was very similar, there were significant differences between the groups in the total amount of fluid required and in the ratio of the resuscitation fluid over the total blood loss. Significantly more RF was used during resuscitation than HES. Calculating the VR ratio, it was significantly higher in the RF group, where almost three times more RF was required to achieve the same hemodynamic parameters.

4.1.4. Endothelial function

Plasma concentration of syndecan-1 was significantly lower in the RF group at T0 and T4 between Tbsl values. Values of glypican in the RF group were significantly lower at T4 compared to Tbsl and T0. However, the syndecan-1 hematocrit ratio and the glypican hematocrit ratio showed no significant differences throughout the whole experiment.

4.2. Effects of goal-directed crystalloid vs. colloid fluid therapy on microcirculation during free flap surgery

There was no difference in the demographics. Complete flap failure occurred on five occasions (one in the Ringerfundin, four in the HES group, $P = 0.142$).

4.2.1. Macrohaemodynamic effects of fluid resuscitation

Patients remained haemodynamically stable throughout the observation period in both groups. PPV was in general higher in the Ringerfundin group, which became significant at T_3. CI also showed the same pattern in both groups, with significantly higher values in HES group at T_1. At T_0, the systemic vascular resistance index values were significantly elevated in the Ringerfundin group. Several other parameters showed changes during the experiment, without any significant differences between the groups. Intra-operative total urine output reached similar values in the HES and Ringerfundin-treated groups [355.0 (166.4) ml and 477.3 (212.5) ml, respectively, $P = 0.090$]. Creatinine values as measured 24 h after surgery were not different between the groups either [HES, 76 (19) mmol l^{-1}; Ringerfundin, 71 (26) mmol l^{-1} , $P= 0.505$].

4.2.2. Respiratory parameters

Overall, all parameters showed similar values in both groups. Although end-tidal CO_2 was significantly elevated in the HES group at T_0 and T_i, it still stayed within the normal range.

4.2.3. Blood gas parameters

All parameters remained within the physiological normal range throughout the observation period and, although there were certain statistically significant differences observed, these can be regarded as clinically nonrelevant. Haemoglobin concentration in the HES group showed a significant decrease over time, without the need for blood transfusion. Oxygen consumption and oxygen delivery were more or less stable throughout the study and followed similar patterns in both groups. Oxygen extraction changed accordingly with no major difference between the groups.

4.2.3. Total amount of the intra-operative and ICU medications

The Ringerfundin group required significantly more boluses and greater total amounts of fluid. During surgery, the Ringerfundin group was given 1.5 times more boluses of fluid than the HES group. Blood loss did not differ between the groups. There were no significant differences in the postoperative period. Nearly half of the patients required vasopressors (Ringerfundin, $n = 8$; HES, $n = 7$) and inotropic support (Ringerfundin, $n = 6$; HES, $n = 7$), without significant difference in the required doses between the groups. Total amount of anaesthetic and analgesic agents was similar in both groups.

4.2.4. Microcirculation and corresponding macrohaemodynamics

As evidenced by laser-Doppler flowmetry, baseline perfusion values were similar at the flap areas (*in situ*, before harvesting) and at the control sites in both groups (at 35℃ and 44℃). During reperfusion, however, significantly higher tissue perfusion values were observed at the free flap sites in both groups than those observed at baseline or at the control areas (at corresponding timepoints) at 35℃. A significant difference in the perfusion of the free flaps areas was observed only in the ninth hour of reperfusion between the groups when perfusion values appeared to be higher in the Ringerfundin group. Heat provocation (to 44℃) induced increases in tissue perfusion only at the control areas, whereas this effect was missing in the flaps during reperfusion in both groups. In the macrohaemodynamic values, a significant difference was found in the DAP and PPV during the second hour of reperfusion (R_2), but these changes were not accompanied by significant changes in microcirculatory perfusion at any sites. Changes in macrohaemodynamic parameters at different reperfusion measurement points varied according to the surgical section.

5. DISCUSSION

The main findings of our animal experiment study are that stable hemodynamic parameters were achieved by significantly more RF than HES boluses and that the VR ratio was more than three times higher in the RF group compared to the HES group. In our randomised human study we found that patients in the Cryst group required more fluid than patients in the Coll group, without any significant difference in the macrocirculation and microcirculation. To detect hypovolemia, advanced haemodynamic monitoring was applied in order to minimise the possibility of diagnostic errors and administer fluid boluses only when hypovolemia was strongly supported by physiological parameters.

5.1. Crystalloids vs. colloids – macrocirculation

Most of the large, multicentre, randomised clinical trials compared crystalloids with HES and concluded that there is no difference, or they observed worse outcome in the HES group. Regarding the VR effects, in these trials, there was a similar VR ratio for Cryst and Coll. Based on these results, a common view was formed that starch solutions do not have a significantly higher potency for volume expansion as compared to Cryst, but carry a greater risk of renal dysfunction and mortality. However, it is important to note that none of these trials used detailed hemodynamic monitoring. The administration of i.v. fluids was mainly based on the clinicians' subjective decision, or on a single parameter.

5.1.1 Volume-replacement ratio

Our first aim was to perform a bleeding-resuscitation animal experiment with detailed hemodynamic monitoring, predefined end points, and a pragmatic protocol. During the experiment, hemodynamic changes did not show clinically relevant differences between the two groups. Kinetics of CI, SVI, MAP, HR, and GEVI showed similar patterns in both groups with significantly higher values in the HES group at the end of the experiment. The higher macro-hemodynamic values in the HES group may be due to the more rapid hemodynamic effects of Coll in general as compared to Cryst. SVV and PPV almost doubled after bleeding in both groups and then returned to baseline values, with both being significantly lower in the HES group. dPmax values, also showed similar changes in both groups. In other words, we observed a similar hemodynamic course for these animals during the experiment, but the volume required was more than three times higher in the RF group. We detected elevated lactate and extravascular lung water (EVLW) levels from the

start. Nevertheless, EVLW did not reach extremely high values and regarding lactate, pigs can have higher blood lactate levels than humans, ranging from 0.5 to 5.5 mmol l^{-1}

5.1.2 Effects of goal-directed crystalloid vs. colloid fluid therapy on microcirculation during free flap surgery

Although the investigation of renal function was not the goal of our randomised clinical trial, the finding that there was no difference in urine output, and serum creatinine levels remained in the normal range in both groups, with no patient requiring renal replacement therapy in the HES group provides support that there is no current evidence that HES causes renal insufficiency in the peri-operative period. Theoretically, Coll have better volume expansion effects, therefore they restore the circulating blood volume and hence DO_2 faster than Cryst do. A fluid challenge study shows that fluid responsiveness is time-dependent and that the issue of optimal timing needs to be addressed. Our data support the theory of Starling's three-compartment model and provided additional information that using Coll may have the benefit of reaching haemodynamic stability two to three times faster compared with Cryst. This difference could potentially be important during fluid resuscitation.

5.2. Crystalloids vs. colloids – microcirculation

One of the main causes why the vasculature may be have differently than that described by Starling is the recently discovered role of the GX in the function of the endothelium. This compartment consists of many Glycosaminoglycan (GAG) chains providing negative charge for the endothelium. Due to these electrostatic properties, the subglycocalyx space produces a Coll oncotic pressure that might be an important determinant of vascular permeability and thus fluid balance. Theoretically, with an intact GX, the VR ratio is markedly different for Cryst compared to Coll and may behave as suggested by Starling. This is also supported by other studies, including our current experiment. Among the proteoglycans, syndecans are the main structural elements of GX and thus play a key role in the functional changes of the endothelium. Studies have confirmed that the increase in plasma levels of syndecans (syndecan-1 and 3), which also serve as indicators of GX degradation, show a positive correlation with mortality in critically ill patients in various shock states.

5.2.1. Volume-replacement ratio

In our experiment in healthy pigs, we also found similar differences between the volume expanding effects of RF and HES solutions. This suggests that during the early phase of bleeding, when theoretically the endothelium and the GX are intact, Coll have volume sparing effects compared to Cryst.

5.2.2. Effects of goal-directed crystalloid vs. colloid fluid therapy on microcirculation during free flap surgery

In our trial, microcirculation showed no clinically important difference between the Cryst and Coll groups either when the probe was placed on the control area or on the flap. Because the free flaps lose their innervation during harvesting, significantly higher perfusion values can be observed at these sites during reperfusion. Heat induced vasodilation, which requires both intact innervation and endothelial function, is apparently lost in the free flaps during reperfusion in both treatment groups. This latter reaction, however, is present in the control areas, and reaches similar values in both groups. These results suggest that haemodynamic stability probably has a higher impact on regional microcirculation than the type of solution used to achieve this state. Furthermore, intravenously administered vasopressors may affect denervated vessels via the endothelial nitric oxide system, but our results suggest that norepinephrine can be used safely and does not harm the flap when its administration is controlled by appropriate haemodynamic assessment. Although all flaps survived the study period, we observed that four flaps failed in the HES group and one in the Ringerfundin group within the first 10 days. However, in three cases, flap failure occurred most probably due to surgical complications, but our sample size is too small to draw any firm conclusions.

6. CONCLUSIONS

7.1. Volume-replacement ratio

- Our data provides experimental evidence that for the same hemodynamic effect, significantly more Cryst than Coll solution is required in healthy pigs.

- The VR ratio was very similar to that described by Starling. Our data also suggests that the Starling's "three-compartment model" requires an intact endothelial GX.

- Therefore, the clinical importance of our results is that Coll may have a place in the very early resuscitation phase, before the GX suffers impairment, to maintain haemodynamic stability faster that could be achieved with Cryst.

7.2. Effects of goal-directed crystalloid vs. colloid fluid therapy on microcirculation during free flap surgery

- In this randomised clinical trial performed during free flap surgery, we found that compared with Coll, larger volumes of Cryst were needed to achieve similar haemodynamic stability.

- There was no difference between the Cryst and Coll groups as far as haemodynamic parameters were concerned nor was there a difference in flap perfusion either.

- Our results indicate that in patients without relevant blood losses and relatively low infused fluid volumes, when haemodynamic stability is maintained with the aid of detailed haemodynamic assessment, there is no measurable benefit of Coll over Cryst.

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LIST OF PUBLICATIONS

LIST OF FULL PAPERS RELATED TO THE SUBJECT OF THE THESIS

I. **László I,** Demeter G, Öveges N, Érces D, Kaszaki J, Tánczos K, Molnár Z. Volume-replacement ratio for crystalloids and colloids during bleeding and resuscitation: an animal experiment. Intensive Care Med Exp. 2017 Dec 20;5(1):52. IF: 0

II. **László I**, Janovszky Á, Lovas A, Vargán V, Öveges N, Tánczos T, Mikor A, Trásy D, Lóderer Z, Piffkó J, Szabó A, Molnár Z. Effects of goal-directed crystalloid vs. colloid fluid therapy on microcirculation during free flap surgery: A randomised clinical trial. Eur J Anaesthesiol. 2019 Aug; 36(8):592-604. IF: 4.500

III. **László I**, Janovszky Á, Szabó A, Molnár Z. "Reply to: Crystalloids Should Be Second Choice for Goal-Directed Fluid Therapy." Eur J Anaesthesiol. 2020 May;37(5):415-416.

IV. **László I,** Janovszky Á, Szabó A, Molnár Z. Reply to: effects of goal-directed crystalloid vs. colloid fluid therapy on microcirculation during free flap surgery. Eur J Anaesthesiol. 2020 May; 37(5):413-414.

LIST OF BOOK CHAPTERS RELATING TO THE SUBJECT OF THE THESIS

V. **Ildikó, László** ; Nándor, Öveges ; Zsolt M. Distribution of Crystalloids and Colloids During Fluid Resuscitation: All Fluids Can be Good and Bad? In: Annual Update in Intensive Care and Emergency Medicine, Springer International Publishing. 2017, 91–103.

LIST OF ABSTRACTS RELATING TO THE SUBJECT OF THE THESIS

VI. **László** I, Demeter G, Öveges N, Tánczos K, Németh M, Trásy D, Kertmegi I, et al. 2016. "Physiological Volume Replacement Ratio Can Be Reached in Experimental Hemorrhage Model." INTENSIVE CARE MEDICINE 4 (Suppl.1.): 89–90.

VII. **László I**., Janovszky Á, Öveges N, Lóderer Z, József P, Szabó A, Vargán V, et al. 2018. "Effects of Crystalloid vs. Colloid Volume Replacement on Microcirculatory Perfusion in Free Flap Surgery." CRITICAL CARE 22 (Suppl. 1.): 126–127.