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Aspects of fluid therapy in the critically ill

Anaesthesiology and Intensive Care
Faculty of Medicine, Lund University, Sweden

Aspects of fluid therapy in the critically ill

Experimental and clinical studies on fluid therapy
in inflammatory conditions

Svajunas Statkevicius



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DOCTORAL DISSERTATION

by due permission of the Faculty of Medicine, Lund University, Sweden.
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Aspects of fluid therapy in the critically ill – experimental and clinical studies on fluid therapy in inflammatory conditions		
<p>Patients suffering from inflammatory conditions often present with severe hypovolemia due to vasodilatation and increased vascular permeability. Early administration of fluids is, therefore, a cornerstone and lifesaving therapy. However, a vigorous and aggressive fluid therapy increases tissue edema, worsen tissue perfusion and organ function. Based on this, the presented studies investigated different aspects of administration and choice of resuscitation fluids with the overall objective to obtain a long-lasting plasma volume expansion with minimal extravasation.</p> <p>Plasma volume expanding efficacy of albumin is suggested to be dependent on microvascular permeability whereas the efficacy of Ringers acetate is independent of permeability. In the first study, plasma volume expansion by 5% albumin was compared to that by Ringers acetate in a condition of normal (after mild hemorrhage) and increased microvascular permeability (in rat sepsis model). The results revealed that, while the efficacy of both albumin and Ringers acetate as plasma volume expanders decreased in sepsis, the ratio between the two as plasma volume expanders remained unchanged.</p> <p>In the second study, the objective was to investigate dose-response of a crystalloid in hypovolemia induced by two different etiologies- sepsis and severe haemorrhage. Rats were randomized to resuscitation with Ringers acetate at a dose of 10, 30, 50, 75 and 100 ml/kg in sepsis or after a severe (30 ml/kg) hemorrhage. The results showed that plasma volume expansion was lower than previously realized across those a wide range of doses and that normovolemia was not attained even at the highest doses in any of the conditions. In sepsis, crystalloid resuscitation induced a dose-depended decrease in plasma oncotic pressure which could not be explained only by dilution.</p> <p>The third study was a single-center, assessor-blinded, parallel-group, randomised prospective clinical study. Previous experimental studies showed that plasma volume expansion was greater after slow infusion compared to rapid infusion of a colloid of the same volume. Based on this experimental data the study aimed to test the hypothesis that plasma volume expansion is greater after slow infusion of colloid than after a rapid infusion of a given volume of colloid. A total of 70 patients with signs of hypovolemia after major abdominal surgery were included and a total of 34 and 31 patients completed the protocol in the slow and rapid infusion groups, respectively. The results have shown that a slow infusion of 5% albumin did not give a better plasma volume expansion than a rapid infusion in postoperative patients with suspected hypovolemia.</p>		
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*“As to diseases, make a habit of two things- to help,
or at least, to do no harm.”*

- Hippocrates

To Jurgita, Simon Elías and Mattias Aron

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Original studies

This thesis is based on the studies reported in the following papers, referred to in the text by respective Roman numerals (I-IV):

- I. Bansch P, **Statkevicius S**, Bentzer P. Plasma volume expansion with 5% albumin compared to Ringer's acetate during normal and increased microvascular permeability in the rat. *Anesthesiology*. 2014; 121: 817-24.
- II. **Statkevicius S**, Frigyesi A, Bentzer P. Effect of Ringer's acetate in different doses on plasma volume in rat models of hypovolemia. *Intensive Care Med Exp*. 2017; 5: 50.
- III. **Statkevicius S**, Bonnevier J, Bark BP, Larsson E, Öberg CM, Kannisto P, Tingstedt B, Bentzer P. The importance of albumin infusion rate for plasma volume expansion following major abdominal surgery - AIR: study protocol for a randomised controlled trial. *Trials*. 2016; 17: 578.
- IV. **Statkevicius S**, Bonnevier J, Fisher J, Bark BP, Larsson E, Öberg CM, Kannisto P, Tingstedt B, Bentzer P. The effect of albumin infusion rate on plasma volume expansion - a randomized clinical trial. Submitted.

Abbreviations

ASA	American Society of Anaesthesiology
AIR	albumin infusion rate
ANCOVA	analysis of covariance
ANOVA	analysis of variance
CI	confidence interval
CLI	cecal ligation and incision
HSA	human serum albumin
ICU	intensive care unit
IQR	interquartile range
MAP	mean arterial pressure
MOF	multiple organ failure
PACU	post anaesthesia care unit
P-POSSUM	Portsmouth-Physiological and Operative Severity Score for the enumeration of Mortality and morbidity
PP	pulse pressure
PPV	pulse pressure variation
PV	plasma volume
SD	standard deviation
SIRS	systemic inflammatory response syndrome
SOFA	Sequential [Sepsis-related] Organ Failure Assessment
SVR	systemic vascular resistance
TER	transcapillary escape rate

Introduction

Definitions

SIRS or Systemic Inflammatory Response Syndrome is defined by host's inflammatory reaction to infection or trauma when 2 or more of the following criteria are met: temperature $> 38\text{ }^{\circ}\text{C}$ or $< 36\text{ }^{\circ}\text{C}$, heart rate $> 90/\text{min}$, respiratory rate $> 20/\text{min}$ or $\text{PaCO}_2 < 32\text{ mm Hg}$ (4.3 kPa), white blood cell count $> 12\,000/\text{mm}^3$ or $< 4000/\text{mm}^3$ or $> 10\%$ immature bands (Bone et al., 1992).

Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection (Rhodes et al., 2017). In clinical practice, organ dysfunction is defined by an increase in the Sequential [Sepsis-related] Organ Failure Assessment (SOFA) score of 2 points or more, which is associated with in-hospital mortality greater than 10% (Singer et al., 2016).

Septic shock is an advanced septic condition, with profound circulatory, cellular, and metabolic abnormalities, which is associated with a greater risk of mortality than sepsis alone. This condition is defined by serum lactate above $> 2.0\text{ mmol/L}$ and the need for vasopressor administration to keep a mean arterial blood pressure above 65 mmHg despite adequate fluid resuscitation (Singer et al., 2016).

Hypovolemia is described by inadequate blood volume or suboptimal heart preload (Evers and Maze, 2004). In clinical practice, hypovolemia is defined by a beneficial response to fluid administration.

Hypoperfusion is described by inadequate oxygen delivery to the tissues usually by impaired microcirculation and results in tissue hypoxia and oxygen debt which, if not corrected early leads to cell damage, organ dysfunction, multiple organ failure (MOF) and death (Groeneveld et al., 1986, Shoemaker et al., 1992, Rivers et al., 2001).

Increased vascular permeability and glycocalyx

Starling's equation describes the transcapillary hydrostatic and osmotic pressures, which are responsible for the movement of fluid through the capillary membrane:

$$J_v = L_p A [(P_c - P_i) - \sigma(\pi_c - \pi_i)]$$

J_v = net fluid movement, *L* = fluid conductivity, *A* = surface area, *P_c* = capillary hydrostatic pressure, *P_i* = interstitial hydrostatic pressure, *π_c* = capillary oncotic pressure, *π_i* = Interstitial oncotic pressure, *σ* = reflection coefficient for macromolecules.

However, Starling's equation does not elucidate the mechanisms behind the transfer of proteins and other large molecules from the intravascular to extravascular space. Therefore the two-pore (small and large pores) model theory was developed to explain transport of water, solutes and macromolecules across the vascular wall. The two-pore theory postulate that flow of solutes and water occurs mainly through small pores with a diameter of 4-6 nm, whereas macromolecular transport only occurs through less abundant large pores with a diameter of 20-30 nm. Because of the large size of these pores, transport of macromolecules may occur both through diffusion and convection. The latter is dependent on a bulk flow of water through the pores which in turn is dependent on capillary hydrostatic pressure (Rippe and Haraldsson, 1994, Rippe et al., 2001).

The discovery of the endothelial glycocalyx demonstrated that fluid movement in the human vascular system is much more complex than Starling's original description of fluid dynamics across blood vessel walls (Pries et al., 2000). The glycocalyx consists of glycoproteins bound to the vascular luminal surface of the endothelium, providing a semi-permeable membrane between circulating blood and the cell surface (Woodcock et al., 2012). Glycocalyx is important in the initiation of tissue inflammation and has a key role in the regulation of vascular permeability. It is predicted that normally the glycocalyx might function as a barrier to large molecules, but when damaged by inflammatory states including sepsis, trauma or surgery, the passage of large molecules and fluid is not regulated, and fluid is lost from the microcirculation (Chelazzi et al., 2015).

Vasoplegia or vasoplegia syndrome is characterized by severe and persistent hypotension, decreased systemic vascular resistance (SVR), low intra-cardiac filling pressures, and normal or increased cardiac output (Byrne et al., 2003).

Fluid therapy

The first documented use of blood transfusion was in dogs in 1666 by Richard Lower. In 1667 by Jean Baptiste Denis was the physician to Louis XIV and transfused lambs' blood to a 15-year-old boy who was bleed with leeches. The recipient survived, most likely due to the small amount of blood that was transfused. The first successful transfusion of human blood was performed in 1818 at St. Thomas Hospital for a parturient. Thomas Latta used the first saline solution

for the treatment of shock secondary to cholera with remarkable results (Latta T., 1832). However, it was soon recognized that crystalloids are not universally effective in the treatment of hemodynamically unstable patients. In the early 20-ties Walter B. Cannon in his book on traumatic shock wrote “that all evidence, both clinical and experimental, indicates that the intravenous injection of warm normal salt or Ringers solution has only temporary effect, the injected fluid promptly passes from capillaries into the tissue spaces and within brief period the pressure is as low as before, if not lower” (Cannon, 1923). Based on this experience and experimental data from William M. Bayliss (Bayliss, 1918), Cannon suggested that infusion of a salt solution containing a colloid in sufficient amount to generate a normal colloid osmotic pressure may be superior to resuscitation with a crystalloid in some conditions. By this, he initiated a colloid vs. crystalloid debate, which is still unresolved.

Crystalloids are solutions containing water and small solutes, like sodium, chloride, potassium, glucose or bicarbonate. Crystalloids have traditionally been categorized as hypertonic, isotonic and hypotonic solutions relative to the tonicity of plasma. Resuscitation fluids as Ringers acetate, Ringers lactate, Plasmalyte® represent nearly isotonic solutions, while normal saline is slightly hypertonic (MacDonald and Pearse, 2017). Solutes of crystalloids are permeable to the most capillary membranes and easily distribute into the whole extracellular compartment, and traditionally only about 20% of the administered fluid is thought to remain intravascularly (Tonnesen et al., 1994, Jacob et al., 2012).

Colloids are solutions which, in addition to small solutes, also contain molecules with a molecular weight above 30 kDa. Large molecules in colloid solutions exert colloid-osmotic, or oncotic pressure and in comparison to crystalloids are more efficient plasma volume expanders (Mythen et al., 1993). Many different colloid solutions, with a diverse range of properties, have been developed. However, most of these have fallen from clinical use. Human albumin solution, dextran, hydroxyethyl starch and succinylated gelatins are the only types of colloid solutions still in widespread use, and their clinical value is hotly debated (MacDonald and Pearse, 2017). The findings of recent large randomized trials in critically ill patients suggest that starch solutions are associated with an excess rate of a kidney injury requiring renal replacement therapy, which may lead to a higher mortality rate (Myburg et al., 2012, Perner et al., 2012, Roberts et al., 2018). Succinylated gelatins are not available in all countries because of data suggesting a high incidence of anaphylaxis (Vervloet et al., 1983) and at present, the albumin is the only colloid which has not been associated with serious side effects. If albumin confers any beneficial effects in the critically ill is still unclear (Finfer et al., 2010, Finfer et al., 2011, Caironi et al., 2014).

Experimental and clinical background

During the past 100 years fluid therapy has become an integral part of perioperative and intensive care, and yet the question of the “ideal” fluid remains elusive (MacDonald and Pearse, 2017). Although fluid therapy is life-saving, it is also associated with side effects such as further oedema formation and compartment syndromes, which may impair organ function (Wiedemann et al., 2006; Holodinsky et al., 2013). Several studies indicate that such side effects may adversely affect outcome both in postoperative patients (Brandstrup et al., 2003, Rahbari et al., 2009) and in patients suffering from sepsis-induced SIRS in the ICU (Payen et al., 2008, Boyd et al., 2011). From a clinical point of view, it is therefore important that the administered fluid not just corrects hypovolemia, but also remains intravascularly as long as possible.

Distribution volumes for different solutions are suggested to be dependent on the vascular permeability of the solutes. Thus distribution volume for resuscitation fluids only containing small molecular weight solutes (crystalloids) is higher than that of resuscitation fluids containing high molecular weight solutes (colloids) it is low. This theory aligns with the suggested resuscitation ratio of albumin to crystalloid of 1:4 to 1:4.5 for a similar plasma volume expansion in postoperative patients and experimental haemorrhage models (Lamke and Liljedahl, 1976, Shoemaker, 1976, Persson and Grände 2005). In contrast, in recent randomized controlled trials in intensive care patients crystalloids and colloids were suggested to be almost equally efficacious as plasma volume expanders (Finfer et al., 2011, Myburgh et al., 2012, Perner et al., 2012). One explanation for these surprising findings could be that increases in vascular permeability in the critically ill decrease the efficacy of albumin as a plasma volume expander.

Crystalloids distribute mainly in the extracellular space and following equilibration showed volume effect of about 20 % of the administered dose in a prospective clinical study of controlled blood loss (Jacob et al., 2012). The similar distribution of crystalloids using only one volume (single dose) of crystalloid was shown both in experimental rat models with acute haemorrhage and in postoperative patients (Persson and Grände, 2005, Lamke and Liljedahl, 1976). Presumably, after acute blood loss, homeostatic mechanisms are active in this setting and strive to maintain normovolemia and contribute to the increase in plasma volume observed after resuscitation (Drobin and Hahn, 1999). This is in contrast to sepsis and other acute inflammatory conditions in which the dysfunction of homeostatic mechanisms striving to maintain normovolemia most likely aggravates hypovolemia (Radaelli et al., 2013, Terborg, 2001). Based on this it could be hypothesized that plasma volume expansion by crystalloid could be reduced in an inflammatory condition. Support for this hypothesis may be inferred

from the poor plasma volume expansion of 1-9% of the infused volume in experimental sepsis (Bark et al., 2013) and in post-operative cardiac surgery patients (Ernest et al., 2001). Surviving Sepsis Campaign and International Guidelines for Management of Sepsis and Septic Shock recommend initial resuscitation with crystalloids at least 30 ml/kg in sepsis-induced hypoperfusion (Rhodes et al., 2017), but very little is known about the efficacy of crystalloids as plasma volume expanders at different doses.

As mentioned above, the systemic inflammatory response syndrome (SIRS) disrupts the normal regulation of transcapillary fluid exchange with an increased vascular leak of macromolecules with tissue oedema and hypovolemia as a consequence (Gustot, 2011). As mentioned above, colloids are macromolecules for which the vessel wall has a low permeability and less volume is therefore required for an equal plasma volume expansion compared to crystalloids (Lamke and Liljedahl, 1976, Shoemaker, 1976, Ernest et al., 1999, Ernest et al., 2001, Persson and Grände 2005). However, extravasation of colloids is not only a function of the vessel wall permeability, but is also dependent on the volume of fluid that is filtered across the capillary wall, which in turn depends on the transcapillary hydrostatic pressure (Rippe and Haraldsson, 1994). This theory suggests that a slow rate of infusion may reduce extravasation of colloids by minimizing transient hypervolemia and transient increases in hydrostatic pressure. The potential importance of infusion rate of a colloid was illustrated by experimental studies in a rodent model of sepsis showing that plasma volume expansion is greater after a slow infusion compared to a rapid infusion of the same volume of colloid (Bark et al., 2013, Bark and Grände, 2014).

It is recommended that a fluid challenge technique should be applied where a fluid administration is continued as long as hemodynamic factors continue to improve (Rhodes et al., 2017, Hammond et al., 2017). However, recent surveys show that infusion rates of resuscitation fluids are highly variable (Cecconi et al., 2015) and no clinical study has addressed the importance of infusion rates on plasma volume expansion in a clinical setting. While rapid correction of suspected hypovolemia appears logical, the need for further knowledge in this aspect of fluid resuscitation was highlighted by the recent FEAST trial showing a surprising increase in mortality following resuscitation using fluid boluses compared to less aggressive fluid resuscitation (Maitland et al., 2011).

Aims of studies

- I. Test the hypothesis if the plasma volume expanding effect of 5% albumin relative to that of a crystalloid solution is reduced if microvascular permeability is increased.
- II. Investigate dose-response curves of a crystalloid resuscitation in hypovolemia induced by either sepsis or haemorrhagic shock in a rat model.
- III. Plan and describe a study investigating the importance of infusion rate for the plasma volume expansion of a colloid in a clinical setting.
- IV. Test the hypothesis if a slow infusion of a colloid results in better plasma volume expansion than a rapid infusion in postoperative patients after major abdominal surgery with suspected hypovolemia.
- V. Test the hypothesis that an infusion rate of a colloid influences markers of glycocalyx shedding and increased permeability.
- VI. Test the hypothesis that infusion rate influences plasma concentration of hormones involved in blood volume homeostasis.

Materials and Methods

Studies I and II

The experimental studies were approved by Ethical Committee for Animal Research of Lund University (M309-12), and animals were treated according to the guidelines of the National Institute of Health for Care and Use of Laboratory Animals.

Anaesthesia, preparation and plasma volume measurement

Anaesthesia was induced by inhalation of 5% isoflurane (Isoba Vet; Intervet AB, Sollentuna, Sweden). After a tracheostomy, the animals were connected to mechanical ventilation and ventilated with humidified air with a tidal volume of 10 ml/kg and a positive end-expiratory pressure of 3–4 cm H₂O. The core temperature was kept at 37.1–37.3°C using a heating pad. The left femoral artery was cannulated for the measurement of mean arterial blood pressure (MAP) and pulse pressure (PP), and to obtain blood samples for measurement of blood gases, sodium, lactate, and haematocrit (I-STAT, Abbot Park, Ill). Pulse pressure variation (PPV, %) during a ventilatory cycle was used as a measure of fluid responsiveness and calculated as: $[(PP_{max}-PP_{min})/(PP_{max}+PP_{min}/2)]*100$ and is expressed as the mean value for 5 consecutive ventilator cycles (Sennoun et al., 2007). The right jugular and the left femoral veins were cannulated for infusions and fluid administration. Following the start of a continuous 0.5 µg/kg/min fentanyl infusion, isoflurane concentration was lowered to 1.1–1.3%. Urine was collected in a vial placed at the external meatus of the urethra, and the bladder was emptied by external compression after completion of preparation and at the end of the experiment. After completion of the protocol, the animals were killed with an intravenous injection of potassium chloride.

Plasma volume (PV) was measured by determination of the initial distribution volume for I¹²⁵-labelled human serum albumin (HSA) (CSL Behring, King of Prussia, PA) at 5 minutes following an injection of a known dose (approximately 75 kBq/kg and 0.05 ml/kg of albumin) (Margaron and Soni, 2005, Bansch et al., 2011).

$$PV = C_{inj}/\Delta C$$

PV = plasma volume, Cinj = known injected amount of radioactivity, ΔC = radioactivity change per unit of plasma volume.

The administered dose was calculated by subtracting the radioactivity in the emptied vial, the syringe, and the needle. Samples were counted in a gamma counter (Wizard 1480; LKB-Wallac, Turku, Finland). Blood volumes were calculated dividing plasma volumes by 1-Hct.

The level of free ^{125}I was found to be $< 2.6\%$ in all administered doses measured after precipitation with 10% trichloroacetic acid and centrifugation.

Experimental protocols

Study I

The study consisted of two main groups of animals in which hypovolemia was induced by two different mechanisms: mild haemorrhage and sepsis.

A haemorrhage group in which rats were bled 8 ml/kg over 5 min and then resuscitated with 5% albumin (CSL Behring: 155 mmol/L Na^+ , 4 mmol/L caprylate, 4mmol/L N-acetyltryptophan, and Cl^- at approx. 150 mmol/L) in ratio 1:1 (8 ml/kg) or with Ringers acetate (Fresenius Kabi, Uppsala, Sweden: 131 mmol/L Na^+ , 4 mmol/l K^+ , 2 mmol/L Ca^{2+} , 1 mmol/L Mg^{2+} , 112 mmol/L Cl^- , 30 mmol/L acetate; osmolality 270 mosmol/kg) in ratio 1:4.5 (36 ml/kg).

A sepsis group in which rats were exposed to cecal ligation and incision (CLI) procedure and observed for 3 hours and then resuscitated with 5% albumin in ratio 1:1 or with Ringers acetate in ratio 1:4.5 of measured lost plasma volume.

Plasma volumes, haemodynamic and laboratory data were achieved at baseline, 5 minutes after haemorrhage or 3 hours after CLI, then 15 minutes, 2 hours and 4 hours after resuscitation (Figure 1).

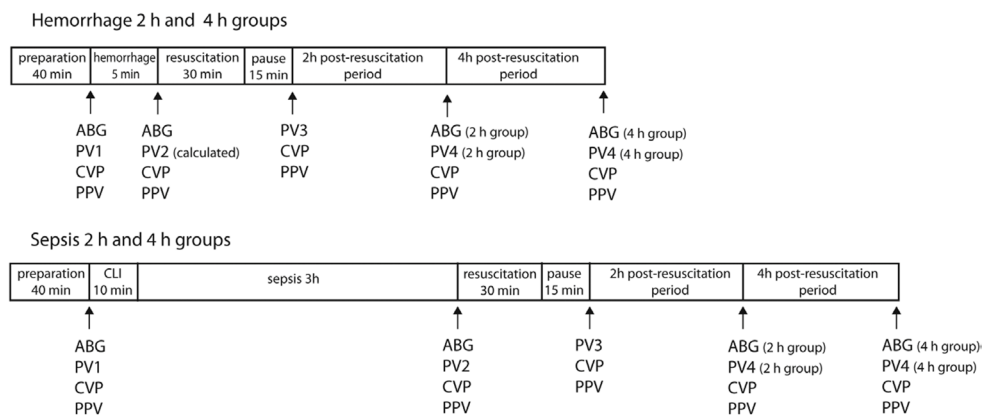


Figure 1. Schematic diagram of the experimental protocol in the haemorrhage and sepsis groups in the study I. ABG = arterial blood gas, PV = plasma volume, CVP = central venous pressure, PPV = pulse pressure variation.

Study II

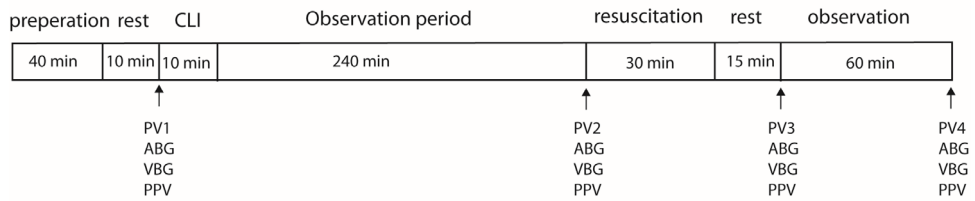
The study consisted of two main groups of animals in which inflammation and hypovolemia were induced by two different mechanisms: sepsis and severe haemorrhage.

A sepsis group in which rats were exposed to a cecal ligation and incision (CLI) procedure and observed for 4 hours and animals with a plasma volume loss ≥ 5 ml/kg were included in the study and were randomized to treatment with either 0 ml/kg, 10 ml/kg, 30 ml/kg, 50 ml/kg, 75 ml/kg or 100 ml/kg of isosmotic Ringers acetate solution over a 30 min period (Plasmalyte[®], Baxter: 140 mmol/L Na⁺, 5 mmol/l K⁺, 1.5 mmol/L Mg²⁺, 98 mmol/L Cl⁻, 27 mmol/L acetate, gluconate 23 mmol/L; osmolality 294 mosmol/kg).

A haemorrhage group in which rats were bled 30 ml/kg over 30 min and then after 2.5 hours resuscitated with either 0 ml/kg, 10 ml/kg, 30 ml/kg, 50 ml/kg, 75 ml/kg or 100 ml/kg of isosmotic Ringers acetate solution over a 30 min period (Plasmalyte[®], Baxter).

Plasma volumes, haemodynamic and laboratory data were measured at baseline, before resuscitation, at 15 minutes and again 60 minutes after the completion of the fluid resuscitation (Figure 2).

Sepsis model:



Hemorrhage model:

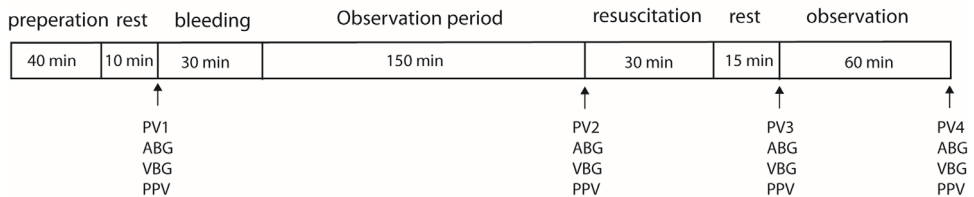


Figure 2.

Schematic diagram of the experimental protocol in the haemorrhage and sepsis groups in the study II. PV = plasma volume, ABG = arterial blood gas, VBG = central venous blood gas, PPV = pulse pressure variation.

The tissue water content was determined in the skin, subcutaneous tissue, muscle, lung, heart, liver, intestine, and kidneys. Tissues were extracted, precisely weighted and dried for 72 hours at 100 °C and then precisely weighted again. Water content was calculated as $(\text{wet tissue weight} - \text{dry tissue weight}) / \text{wet tissue weight} \times 100$ and presented in %.

Statistics

A sample size of least 8 animals in each group was chosen on the basis of previous experimental studies in rat sepsis and haemorrhage models (Jungner et al., 2010; Bansch et al., 2011; Bark et al., 2013). Physiological, laboratory parameters, and plasma volumes were presented as mean \pm SD if normally distributed and if not, as median with interquartile range. Results at baseline and after sepsis or haemorrhage were evaluated using paired Student's t-test. Differences in the plasma volume expansion, haemodynamic and laboratory data within the groups at different time points were analysed using one-way ANOVA, followed by an adjustment for multiple comparisons using Bonferroni method if normally distributed or one-way ANOVA on ranks using Dunn's method if not. Statistical analyses were performed using GraphPad Prism version 7.0a (GraphPad Software, San Diego, CA). P values <0.05 were considered as statistically significant.

Study III

The study was approved by regional ethical vetting board (Dnr. 2014/15) and the Swedish Medical Products Agency. The protocol was amended two times (the first, when patients after major gynaecological cancer surgery could be included, and the second, when vasopressor therapy was omitted as an exclusion criteria). Amendments were approved by the same ethical vetting board and the Swedish Medical Products Agency.

Inclusion and exclusion criteria

Postoperative patients following non-emergent operation ad modum Whipple or major gynaecological cancer surgery at the age of 40 years or more were screened for inclusion and exclusion criteria.

Inclusion criteria were:

1. Written consent by the patient to participate in the study obtained prior to operation.
2. Indication for fluid therapy as judged by the physician caring for the patient and at least one of the following criteria was fulfilled within 5 hours after admission to the post anaesthesia care unit (PACU):
 - a. a) positive "leg raising test" (pulse pressure increase > 9% or stroke volume increase by more than 10% as measured by cardiac ultrasound (Preau et al., 2016);
 - b. b) central venous oxygen saturation (ScvO₂) < 70%;
 - c. c) plasma lactate > 2.0 mmol/L;
 - d. d) urine output < 0.5 ml/kg the hour prior to inclusion;
 - e. e) respiratory variation of the inferior vena cava of more than 15% as measured by ultrasound (Feissel et al., 2004, Barbier et al., 2014);
 - f. f) systolic blood pressure < 100 mmHg or mean arterial blood pressure < 55 mmHg.

Exclusion criteria were:

1. Hypersensitivity to the study drug or the tracer;
2. Signs of postoperative bleeding;
3. History of heart failure;

4. Consideration of the caring physician that there are strong reasons to administer another fluid or the same fluid but in another way than stated in the study protocol;
5. Pregnancy;
6. The clinical judgment of caring physician that the patient should not participate in the study for the reasons other than described above.

Randomization and blinding

Eligible postoperative patients were observed for an indication for fluid administration during the first 5 hours after admission to the PACU. Patients who fulfilled inclusions criteria and met no exclusion criteria were randomized to either rapid or slow 5% albumin infusion by using sealed envelopes, which were prepared by an independent party (Clinical Research Unit, Skåne University Hospital, Lund). Randomization was performed using a computerized random number generator, and the research team was blinded to block size. A member of the research team who was blinded to the treatment allocation performed measurements of plasma volumes and transcapillary escape rate (TER) for albumin.

Study interventions and measurements

Patients were randomized to receive 5% albumin at a dose of 10 ml/kg either in 30 minutes or 180 minutes. The dose was calculated for ideal body weight (Wurtz et al., 1997).

Plasma volume was measured by calculating the distribution volume of an intravenous dose of I¹²⁵-HSA (SERALB-125[®], CIS Bio International, Gif-Sur-Yvette Cedex, France) before the start of the albumin infusion, at 30 minutes and 180 minutes after the start of the infusion (Figure 3). Blood samples were collected 5 minutes prior to injection of I¹²⁵-HSA and 10 minutes after injection of I¹²⁵-HSA for the plasma volume calculations. Plasma concentration is determined in a gamma counter (PerkinElmer 1480 Wizard; PerkinElmer, Waltham, MA, USA), and plasma volume was calculated by dividing the injected dose of I¹²⁵-HSA by the change in concentration of I¹²⁵-HSA in plasma at 10 minutes postinjection. Injected doses were corrected for remaining activity in the syringes.

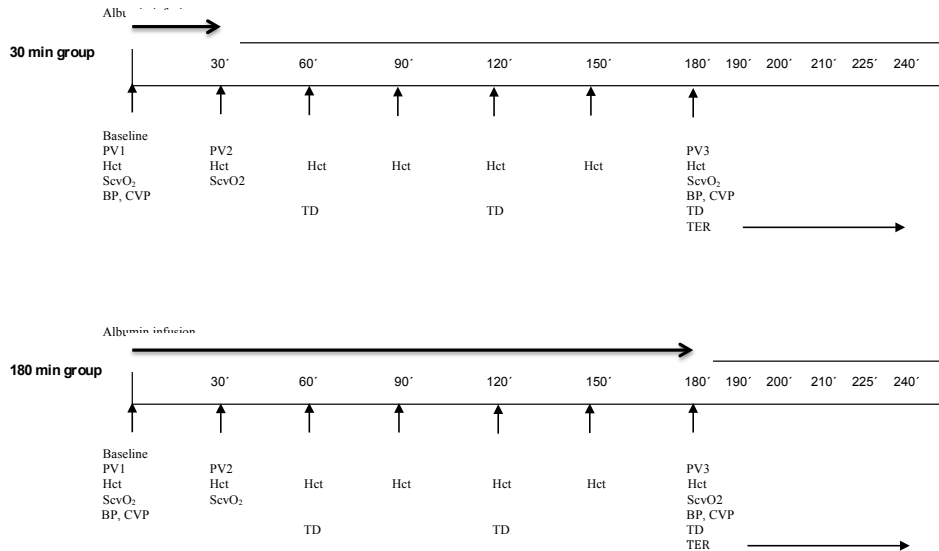


Figure 3.

Detailed study protocol. PV1 = baseline plasma volume, PV2 = plasma volume after 30 minutes, PV3 = plasma volume after 180 minutes, Hct = haematocrit, ScvO₂ = central venous oxygen saturation, BP = blood pressure, CVP = central venous pressure, TD = hourly diuresis, TER = transcapillary escape rate for albumin.

The primary outcome of the study was a change in plasma volume 180 minutes after the start of albumin infusion.

The secondary outcomes were differences in plasma volume over time (integral of plasma volume over time from the start of albumin infusion to 180 minutes) and the incidence of postoperative complications up to 30 days after surgery. Transcapillary escape rate (TER) for albumin from 180-240 minutes after the start of albumin infusion, change in heart rate, central venous oxygen saturation, haemoglobin concentration in blood, blood pressure, central venous pressure, lactate, diuresis, plasma concentration of hormones involved in fluid balance and plasma concentration of circulating components of the glycocalyx were other outcomes of interest.

The change in area under the plasma volume curve was calculated (plasma volume over time) using the trapezoid rule. TER for albumin is a measure of leakage of albumin from microvessels into the interstitium and was measured by measuring plasma concentration of I¹²⁵-HSA at five-time points after the last injection of the tracer. The decrease in plasma concentration of I¹²⁵-HSA as a linear function of time was then calculated and is expressed as % decrease in plasma concentration of I¹²⁵-HSA per hour.

Blood gases, haematocrit, and lactate were measured using a blood gas analyser (Radiometer 850, Radiometer, Copenhagen, Denmark). Plasma concentrations of glypican-4 (Cloud-Clone Corp), hyaluronan (Echelon Biosciences), Syndecan-1 (Diacclone), renin (IDS), copeptin (Brahms GmbH) and Mid Regional-pro Atrial Natriuretic Peptide (MR-proANP) (Brahms GmbH) were measured by immunologic assays according to manufacturer's instructions.

The participants received routine postoperative care after the study protocol was completed.

Statistics

Previously published standard deviation value for plasma volume measured with I¹²⁵-HSA method is about 5 ml/kg (Bonfils et al., 2012), so to detect 4 ml/kg difference in volume expanding effect following administration of 5% albumin about 30 patients in each group was required to obtain power of 80% using Student's t-test.

The analysis was performed per protocol. The primary outcome was analysed using Student's t-test. Secondary outcomes were analysed using Student's t-test or Fisher's test as appropriate. No adjustments for multiple comparisons were made. A 2-way ANCOVA was used to assess the interaction between either type of operation or baseline blood volume and treatment effect. Statistical analysis was performed blinded to treatment allocation using R (3.4.0).

Results

Study I

Twenty-nine rats were exposed to mild haemorrhage, 17 rats (n=8 resuscitated with albumin and n=9 with Ringers acetate) were observed for 2 hours after resuscitation and 12 (n=6 in each group) for 4 hours. No animals in haemorrhage group died during the experiment.

Twenty-eight rats were exposed to CLI, and 16 rats (n=8 resuscitated with albumin and n=8 with Ringers acetate) were observed for 2 hours after resuscitation and 12 rats (n=6 in each group) for 4 hours after resuscitation. 4 (20%) and 9 (36%) animals died in the 2-hours and 4-hours sepsis groups respectively, and their measurements were not included in analysis.

Plasma volume in CLI group decreased from 40.4 ± 2.1 ml/kg to 32.1 ± 3.4 ml/kg and from 39.6 ± 1.9 ml/kg to 32.7 ± 2.8 ml/kg in albumin and Ringers acetate groups, respectively ($P < 0.05$).

The study results showed that after haemorrhage resuscitation with albumin at a ratio of 1:1 relative the blood loss or Ringers acetate at a ratio of 1:4.5 relative measured plasma volume loss results in similar plasma expansion at 15 min, 2 and 4 hours after resuscitation. In sepsis model, the resuscitation with albumin and Ringers acetate at the same ratios as above resulted in a higher plasma volume in the albumin group than in the Ringers acetate group at 15 min, but no difference at 2 and 4 hours after resuscitation (Figure 4 and Figure 5).

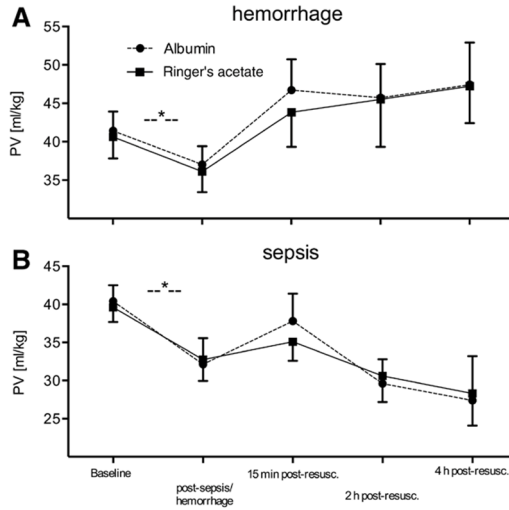


Figure 4. Absolute plasma volumes in haemorrhage and sepsis groups at baseline, after haemorrhage and sepsis, then 15 min, 2 and 4 hours after resuscitation.

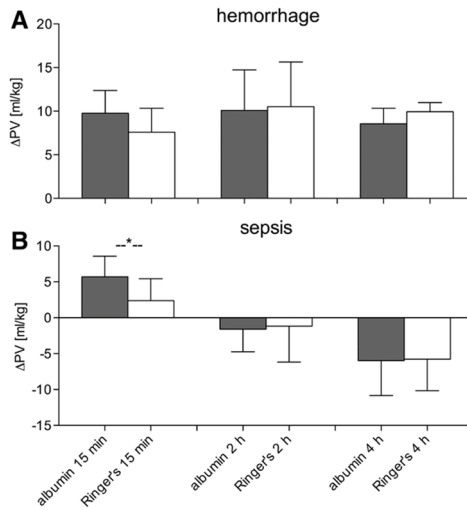


Figure 5. Change in plasma volume in haemorrhage and sepsis groups at 15 min, 2 and 4 hours after resuscitation.

In haemorrhage group, urine production was 2.3 ± 1.0 ml/kg/h in animals resuscitated with Ringers acetate and 1.8 ± 0.6 ml/ in those, resuscitated with albumin ($P < 0.05$). In CLI groups urine production was 0.9 ± 0.2 ml/kg/h and 0.8 ± 0.1 ml/kg/h in Ringers acetate and albumin groups, respectively.

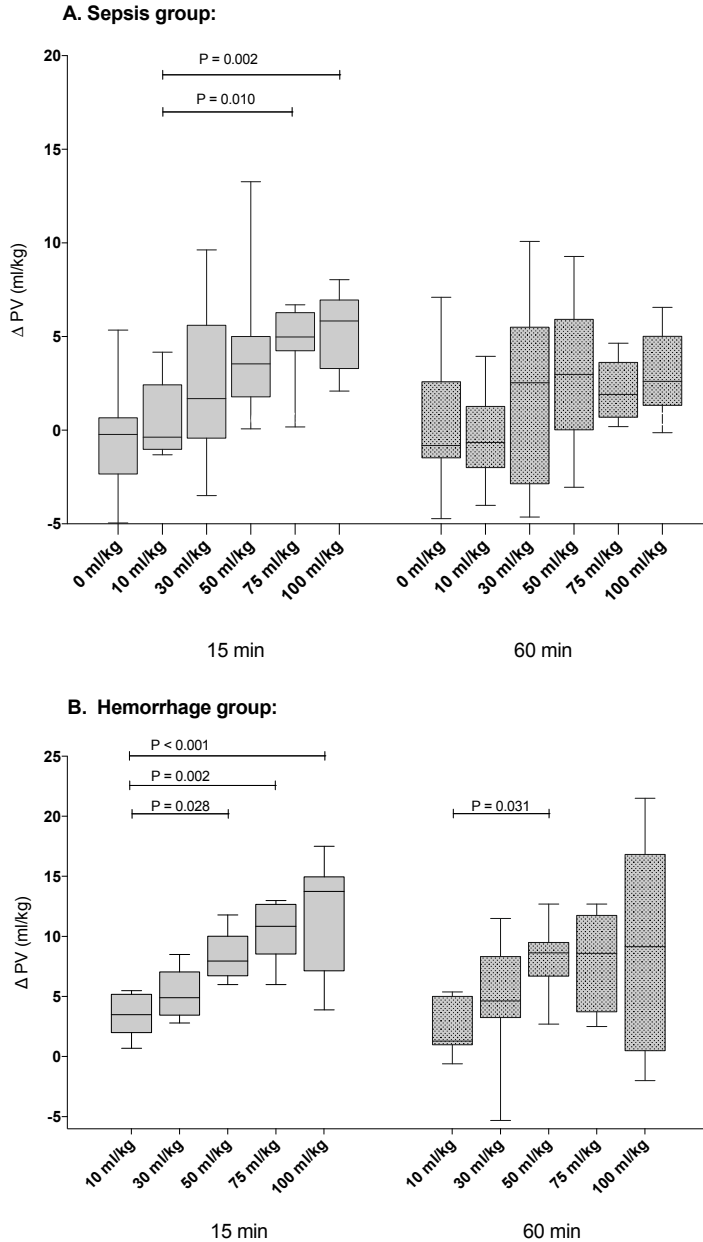
Study II

The baseline plasma volume in the sepsis group was 42.1 (39.5-46.4) ml/kg and decreased to 30.8 (28.7-32.5) ml/kg at 4 hours after CLI procedure ($P<0.001$). At 15 minutes after resuscitation a dose-dependent increase in plasma volume was observed (Figure 6A), but 60 minutes after resuscitation no differences in the plasma volume change could be detected ($P=0.17$, ANOVA).

The baseline plasma volume in haemorrhage group was 41.9 (39.8-43.5) ml/kg and decreased to 30.6 (28.7-32.5) ml/kg at 2.5 hours after bleeding ($P<0.001$). According to calculated blood volume, the bleeding corresponded to a 40% haemorrhage. 15 minutes after administered resuscitation the plasma volume change was dose-dependent (Figure 6B). At 60 minutes after resuscitation, only 50 ml/kg group showed increased plasma volume change compared to 10 ml/kg group.

The mortality in sepsis and haemorrhage groups was 14% and 33%, respectively.

Average plasma volume expansion as percentage of the administered dose was lower in the sepsis than in the haemorrhage (15 min; 5.9 (2.5-8.8) % vs. 14.5 (12.1-20.0) %, $P<0.001$, 60 min; 2.9 (-2.9-8.3) % vs. 13.3 (8.3-19.0) %, $P<0.001$, Mann-Whitney test). In sepsis, average efficacy decreased from 15 minutes to 60 minutes ($P=0.006$, Wilcoxon matched-pairs signed ranks test), whereas it did not differ between the different time points in haemorrhage ($P=0.108$).



We observed a dose-dependent decrease in colloid osmotic pressure in both conditions, which was more marked in sepsis than in haemorrhage ($P < 0.001$). In sepsis, the plasma oncotic pressure was 10.1 (9.8-10.3) mmHg in the group resuscitated with a dose of 100 ml/kg, which corresponds to a 32% lower plasma oncotic pressure than in the 10 ml/kg group. At this time point, the plasma volume in animals resuscitated with 100 ml/kg had increased by about 10% relative to the plasma volume change observed in animals resuscitated with 10 ml/kg. In haemorrhage, the plasma oncotic pressure was 9.6 (8.5-10.4) mmHg in the group resuscitated with a dose of 100ml/kg. This corresponds to a 20% lower plasma oncotic pressure than in the 10ml/kg group. At this time point, the plasma volume in animals resuscitated with 100 ml/kg had increased by about 23% relative to the plasma volume change observed in animals resuscitated with 10 ml/kg (Figure 7).

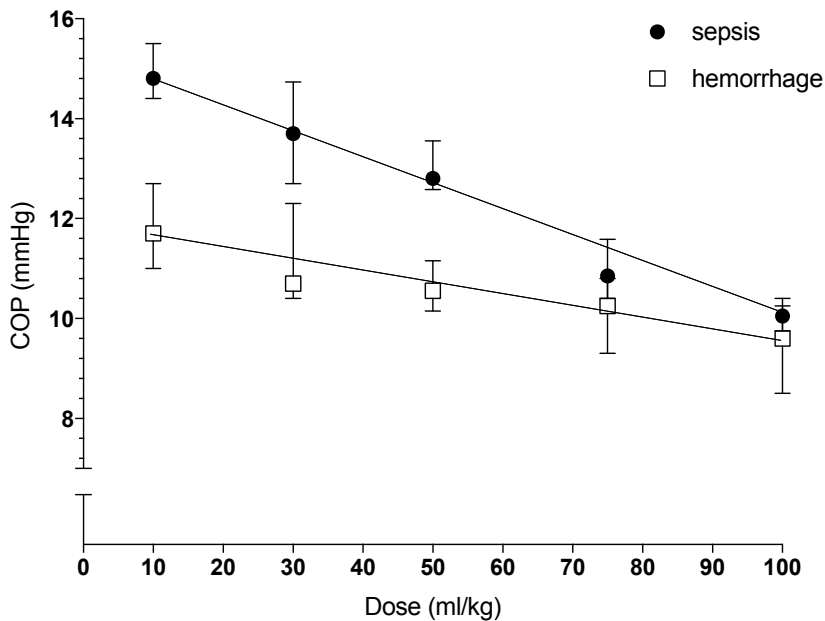


Figure 7. Plasma oncotic pressure at 60 minutes after completion of resuscitation. ($P < 0.001$ for the difference in slope using linear regression with an intersection term)

The tissue water content in the skin, intestine, muscle, and kidney was higher in the resuscitated group than in the control group in sepsis. The tissue water content in the skin, intestine, heart, and kidney was higher in the resuscitated group than in the control group in haemorrhage. In both conditions, water content increased the most in skin and intestines.

Study III

A total of 70 patients were enrolled in the study between the 18th of June 2014 and the 22nd of November 2016 and 35 patients were assigned to each treatment. One patient withdrew consent during the study protocol in the slow infusion group; then two patients received a mild allergic reaction to the tracer and plasma volume measurement failed in two patients in the rapid infusion group; therefore 34 patients receiving the slow infusion and 31 patients receiving the rapid infusion were included in the analysis.

Pre-treatment characteristics such as demographical, clinical and laboratory data, anaesthesia and surgery time, lost blood volumes and received intraoperative fluid volumes were similar in both groups. The two most common criteria for fluid administration were a positive passive leg raising test and elevated arterial lactate.

Plasma volume before the start of albumin infusion was 47.9 ± 10.3 ml/kg and 47.5 ± 6.3 ml/kg in the slow and rapid groups, respectively. The increase in plasma volume from start to 180 minutes after start of infusion did not differ between the different infusion rates and was 6.7 ± 5.0 ml/kg and 6.5 ± 4.1 ml/kg in the slow and rapid infusion groups, respectively (absolute difference, 0.16 ± 1.1 [95%CI, -2.4 - 2.1], $P=0.89$) (Figure 8).

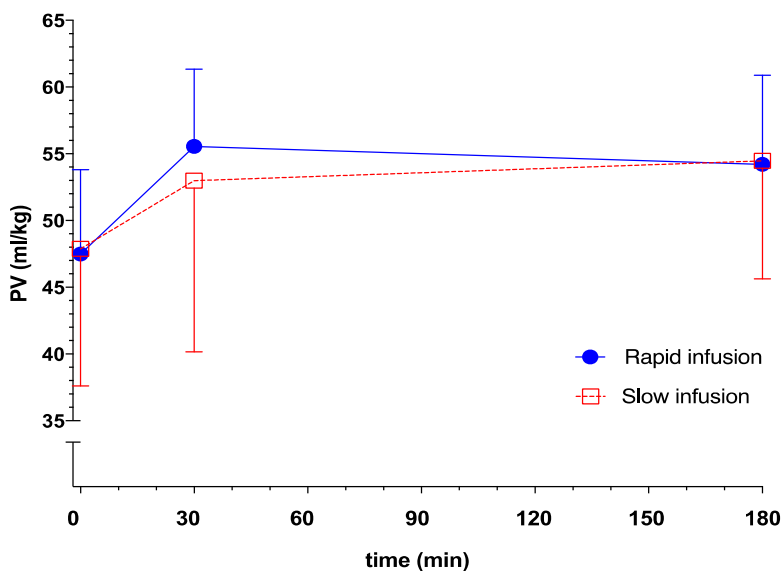


Figure 8. Plasma volumes in the slow and the rapid infusion groups. Data are shown as mean and SD.

Change in the area under the plasma volume curve over time did not differ between the different infusion rates and was 970 ± 119 ml*min/kg and 1226 ± 75 ml*min/kg, respectively in the slow and rapid groups, respectively (absolute difference, 256 ± 141 ml*min/kg [95%CI, -32 - 543], $P=0.08$). The number of patients with postoperative complications in the slow and rapid infusion groups were 8 and 6, respectively, and did not differ between the groups ($P=0.77$). Urine production was lower in the slow than in the rapid group. Treatment effect on TER, lactate, Hct or any of the haemodynamic parameters could be detected.

The pre-planned sensitivity analysis showed that low baseline blood volume was associated with a higher increase in plasma volume from the start to the 180 minutes point (2-way ANCOVA, $P=0.003$) (Figure 9).

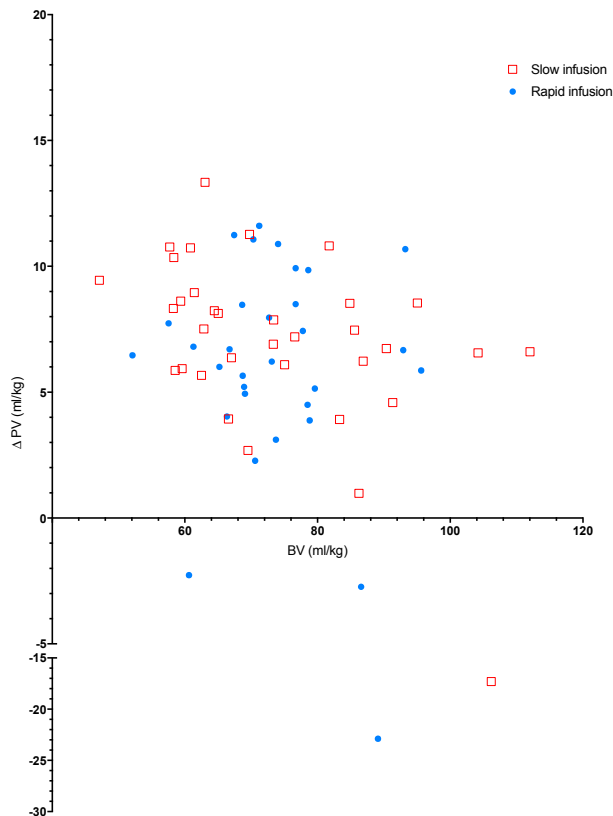


Figure 9. A plot of the change in plasma volume from start to 180 minutes after the start of the infusion of albumin in respective treatment groups.

The sensitivity analysis did not demonstrate an interaction between baseline blood volume and treatment effect ($P=0.38$, 2-way ANCOVA) or between the type of surgery and treatment effect ($P=0.89$, 2-way ANCOVA). Change in plasma volume from the start to 180 minutes after the start of the infusion of albumin in patients were analysed in concern if the baseline blood volume was above or below the median. The cohort could not be divided into two equally large groups; therefore results for both possible divisions were analysed (Figure 10).

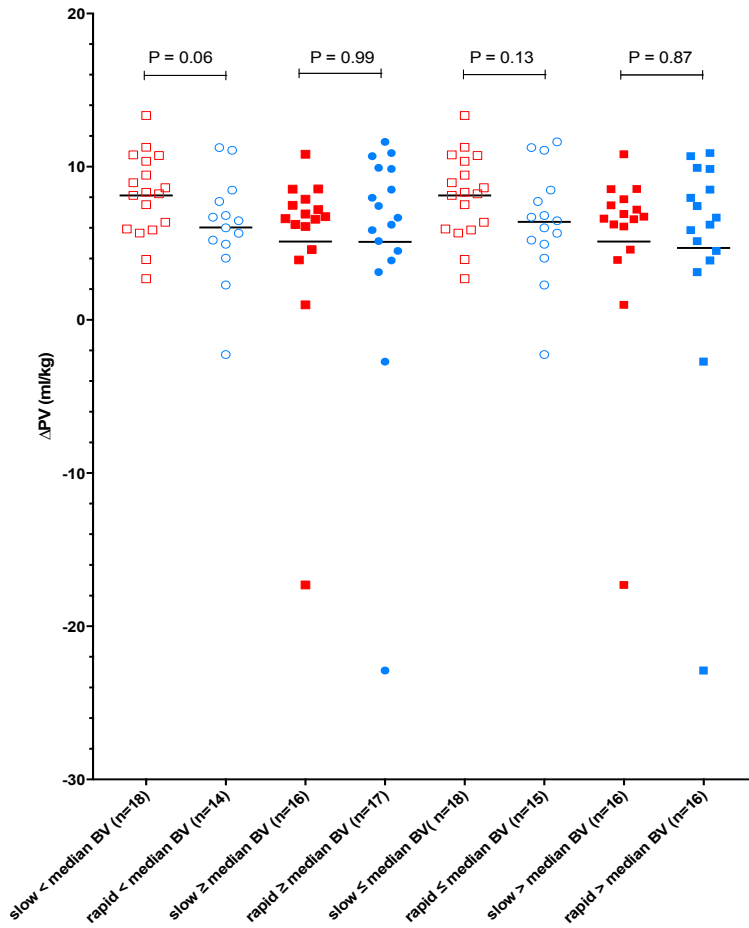


Figure 10. Change in plasma volume from the start to 180 minutes after the start of the infusion of albumin in patients with baseline blood volume above or below the median.

On a post hoc basis, the correlation between changes in the area under the plasma volume curve and urine production was analysed to further evaluation if the higher urine production reflected differences in change in preload. No interaction could be demonstrated (Pearson r : 0.16, P = 0.20) (Figure 11).

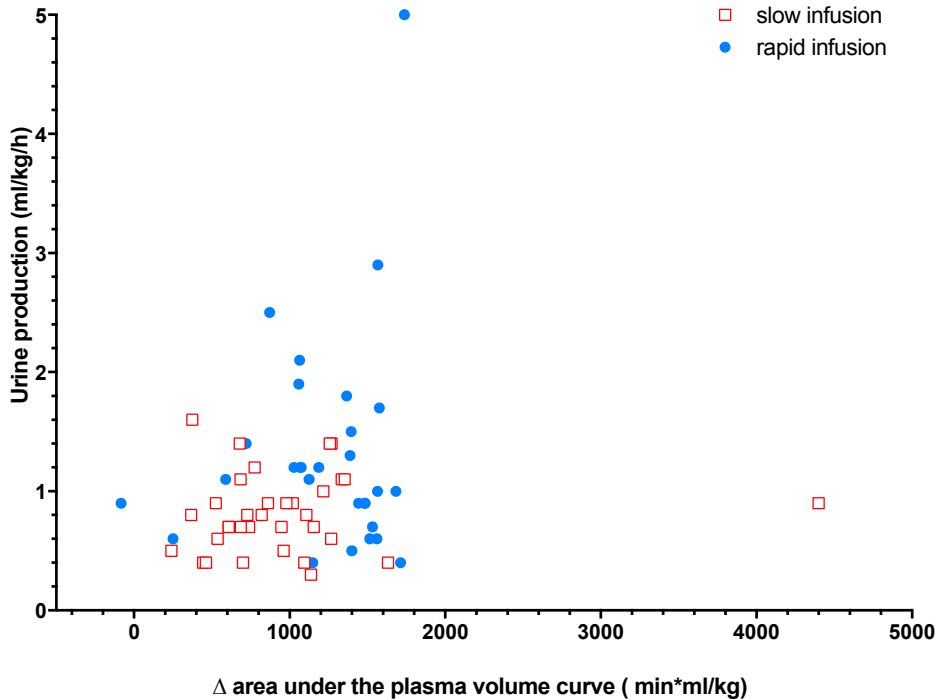


Figure 11.
The correlation between changes in the area under the plasma volume curve and urine production.

Plasma concentration of the stable precursor fragment of ANP, mid-regional pro-Atrial Natriuretic peptide (MR-proANP), increased more from the start to 180 min after start of infusion in the rapid infusion group than in the slow infusion group. The concentration of renin and copeptin, reflecting vasopressin release, does not differ between the groups (Table 1).

The change in concentration of circulating components of endothelial glycocalyx: hyaluronic acid and syndecan-1 did not differ between the slow and rapid infusion groups, whereas glypican-4 increased more in the slow infusion group (Table 1).

Change in glyocalyx components and hormones	Slow infusion (n = 34)	Rapid infusion (n = 31)	Absolute difference	P Value
Δ Hyaluronic acid, ng/ml	-4.6 ± 9.7	15.3 ± 14.9	19.9 (-14.8 – 54.6)	P = 0.26
Δ Syndekan 1, ng/ml	15.3 ± 13.3	20.3 ± 17.5	5.1 (-38.4 – 48.5)	P = 0.82
Δ Glypican-4, ng/ml	1.1 ± 0.9	-2.4 ± 1.5	3.5 (-7.3 – 0)	P = 0.048
Δ Copeptin, pmol/L	-73.9 (-148.6 - [-27.4])	-59.3 (-129.8 - 40.6])	14.6 (-29.9 - 33.8)	P = 0.88
Δ Renin, mU/L	-14.3 (-55.1- [-5.3])	-22.4 (-78.9- [-7.4])	8.1 (-24.1 - 6)	P = 0.36
Δ MR-proANP, pmol/L	20.6 (9.2 - 34.6)	45.1 (30.5 - 71.5)	24.5 (12.6 - 34.8)	P < 0.001

Table 1. Change in endothelial glyocalyx components in the slow and rapid infusion groups. Data are presented as mean with standard deviation or median with interquartile range.

General discussion

Plasma volume measurements were performed using radiolabeled I¹²⁵-HSA human serum albumin. The method is well established in experimental and clinical settings (Margaron and Soni, 2005, Dubniks et al., 2007, Bansch et al., 2011, Bonfils et al., 2012, Bark et al., 2013) and is often referred to as a gold standard. It is known, that free fraction of I¹²⁵ distributes into interstitial space and may cause an overestimation of true plasma volume (Valeri et al., 1973). Given that a free fraction of iodine after precipitation with 10% trichloroacetic acid was regularly measured in experimental studies and found to be low (< 2.6%) in all samples, this error is small and similar in all groups.

According to previous experimental studies a 5 minutes period was chosen for sufficient distribution of radiolabeled I¹²⁵-HSA human serum albumin from injection to collection of blood samples (Persson and Grände 2005, Bark et al., 2013). It could be argued that the I¹²⁵-HSA method overestimate plasma volume in inflammatory states with increased capillary permeability. However, assuming an increase in TER to at 20% per hour in septic rats (Bansch et al., 2011), it can be calculated that increased extravasation of I¹²⁵-HSA human serum albumin overestimated plasma volume by at most 1.7%.

The CLI method in rat sepsis model was used to induce increased capillary permeability and subsequent hypovolemia (Ottero-Anton et al., 2001, Dubniks et al., 2007, Scheiermann et al., 2009). Our results showing a decrease in plasma volume of approximately 7-8 ml/kg, haemoconcentration and elevated lactate levels suggest that the model resembles important aspects of early human sepsis. It should be noted, that anaesthesia and surgical animal preparation could induce even mild systemic inflammatory response with increased vascular permeability and contribute to vasodilatation (Christensen et al., 1967, Soehnlein et al., 2010). Although we cannot exclude that also the mild haemorrhage of 8 ml/kg in the first experimental study could induce increased capillary permeability (Nelson et al., 2016), the changes in measured I¹²⁵-HSA human serum albumin concentration revealed difference in capillary permeability between haemorrhage and sepsis groups. This in turn support that we compared groups with different permeability as intended. Interestingly, the study does not support the hypothesis that potency of albumin as a plasma volume expander is decreased in conditions characterized by increased capillary permeability. Providing that our results might be applicable

to human sepsis, it follows that the finding that equal volumes of albumin and crystalloid were administered as resuscitation fluids in the critically ill patients included in the SAFE trial cannot be explained by a relatively lower potency of albumin in the critically ill (Finfer et al., 2011). Interestingly, several studies have demonstrated that clinical signs used as triggers of fluid resuscitation cannot recognise fluid responders (Bentzer et al., 2016). This means that in a clinical setting colloid and crystalloid will be perceived as equally ineffective in a large fraction of patients regardless of their true efficacy as plasma volume expanders.

The second experimental study, investigating the dose-response relationship of Ringers acetate as a volume expander, shows that crystalloids expand the plasma volume by about 7% of the infused volume early after resuscitation in sepsis and correlates with the findings in our first study and other studies in experimental and clinical inflammation (Ernest et al., 2001, Bark et al., 2013 Bansch et al., 2014). Moreover, 1 hour after completed resuscitation with different doses no difference in plasma volume could be detected. After a haemorrhagic shock, crystalloids were more potent plasma volume expanders, but normovolemia was not achieved even with the highest dose. Observational studies have shown that it is not uncommon in clinical praxis when patients with septic shock receive more than 125 ml/kg during the first hours of resuscitation (Boyd et al., 2011), suggesting that the investigated doses are similar to those used in clinical practice. The study results also align with the recent clinical studies showing a short duration of crystalloid bolus (Nunes et al., 2014, Skytte Larsson et al., 2015). The question may be raised why crystalloids, which are permeable to the most capillary membranes and easily distribute into the whole extracellular compartment, have decreased plasma volume expanding effect under inflammatory conditions. It has been suggested that inflammatory conditions might be associated with structural changes in the interstitial matrix and interstitial pressure (Nedrebo et al., 1999), which raises the possibility that interstitial distribution volume of crystalloids could be increased in inflammatory conditions. Moreover, homeostatic mechanisms that strive to normalize intravascular volumes by mobilizing fluids from extravascular compartment are most likely dysfunctional in sepsis and in other acute inflammatory conditions, which will decrease plasma volume expansion of a given dose of crystalloid relative conditions with intact homeostatic mechanisms (Radaelli et al., 2013, Terborg, 2001).

After resuscitation, the plasma oncotic pressure decreased dose-dependently both after haemorrhage and sepsis, but in sepsis, the decrease in plasma oncotic pressure was more profound and could not be explained by dilution alone. There are several potential explanations for this intriguing finding. Thus it is possible that increased permeability in sepsis might be associated with an increased number of large pores (Bradley et al., 1988) and/or degradation of glycocalyx (Bansch et al., 2011), that increased water content in interstitium increases distribution

volume of albumin or that higher doses of crystalloid increases transcapillary hydrostatic pressure and induce dose-dependent extravasation of albumin and/or decrease its lymphatic return.

The results of the clinical study do not support the hypothesis that infusion rate of 5% albumin influences plasma volume expansion in patients with signs of hypovolemia after major abdominal surgery. Plasma volume expansion 30 minutes after infusion start was higher in the rapid infusion group, but after 180 minutes there was a similar plasma volume expansion in both groups. The results do not align with previous data from experimental studies in rodent sepsis (Bark et al., 2013, Bark and Grände, 2014). The reasons for the differences in results from previous experimental studies might include species differences, but could also be related to etiology and/or severity of the inflammatory condition and hypovolemia. The change in plasma volume from start to 180 min after start of infusion and a trend towards a bigger area under the plasma volume curve in the rapid infusion group suggest that in this clinical setting, a bolus approach may result in a better plasma volume expansion during the first hours after infusion.

Plasma volume expansion of 5% albumin as a fraction of an infused volume is shown to be in the range of 50-110 % immediately after infusion in previous studies using a similar methodology in postoperative or in septic patients (Lamke and Liljedahl 1976, Ernest et al., 1999, Ernest et al., 2001). Our results extend these previous findings and show that the volume expanding effect persists for at least 2.5 hours after termination of rapid infusion. In addition, the results from the sensitivity analysis suggest that the wide range of potencies for 5% albumin as a plasma volume expander reported previously, at least partly, may be explained by differences in baseline blood volumes. Patients with baseline blood volumes under median blood volume value showed a trend towards better plasma volume expansion in the slow infusion group, suggesting that slow infusion rate might be more efficient in more advanced hypovolemia. Future studies should be directed at investigating effects of infusion rate on plasma volume expansion in this subgroup of patients.

In an attempt to mimic clinical practice, hypovolemic patients were identified by clinical signs commonly used as indications for fluid therapy (Cecconi et al., 2015). The most common reason for inclusion was a positive passive leg raising (PLR) test, a test which has been shown to be an accurate predictor for fluid responsiveness (Preau et al., 2010). However, as mentioned above several of the other inclusion criteria are poor predictors fluid responsiveness in critically ill patients, and it is likely that several of the included patients were not truly hypovolemic (i.e., fluid responsive). By using more strict inclusion criteria a truly hypovolemic population could be targeted in the future studies.

The volume of albumin used in the study is within the range of that used in previous studies investigating hemodynamic effects of fluid bolus therapy (Glassford et al., 2014, Vincent and Weil, 2006). The rate of infusion in the rapid infusion group matches with that commonly used in clinical practice, and the rate of infusion in the slow infusion group was based on previous experimental studies and institutional practice (Bark et al., 2013, Bark and Grände, 2014). Every patient had received an average 4300 ml of crystalloid and 500 ml of colloid prior to inclusion. This aligns with the consensus statement on perioperative fluid therapy suggesting the use of both crystalloids and colloids for major surgery (Navarro et al., 2015).

Transient hypervolemia and increase in transcapillary hydrostatic pressure induced by rapid volume loading of colloids may induce shedding of components of the endothelial glycocalyx, which is in turn associated with reduced endothelial barrier function (Chappell et al., 2014). We, therefore, hypothesized that rapid infusion could induce an increase in shedding of glycocalyx components and increased endothelial permeability. Surprisingly, a rapid infusion significantly decreased plasma concentrations of glypican-4, but the change of plasma concentrations of syndecan-1 and hyaluronan was similar in both groups. It might be hypothesized that rapid infusion was more beneficial for endothelial integrity. However, the results that transcapillary escape rate for albumin did not differ between the groups indicates that such an effect, if present, is small.

The trend towards a larger area under the plasma volume curve over time in the rapid infusion group could be taken to indicate that the increased diuresis in this group reflects a better preload during the observation period. However, the absence of correlation between change in area under the plasma volume curve and diuresis does not align with this notion and suggests that mechanisms other than differences in preload over time contribute to this result. A higher plasma concentration of the stable ANP precursor fragment MR-pro ANP, an endogenous diuretic hormone, is probably the result of a transient increase in wall stress of the heart in the rapid infusion group.

The clinical study observed changes in haemodynamic, laboratory data, plasma volume expansion and endothelial glycocalyx shedding components only during 180 minutes: therefore long-term effects of infusion rate are still unknown and could be investigated in future clinical studies, especially in patients with more advanced hypovolemia.

Main conclusions

1. Albumin is equally effective relative to crystalloids as a plasma volume expander also in conditions with increased vascular permeability.
2. The potency of crystalloids as plasma volume expanders is context dependent and lower in inflammatory conditions.
3. In inflammatory conditions normovolemia was not achieved even with the highest resuscitation doses of crystalloids.
4. Crystalloid resuscitation in sepsis is associated with a dose-dependent decrease in the plasma oncotic pressure and cannot be explained only by dilution.
5. A slow infusion of a colloid does not result in a better plasma volume expansion than a rapid infusion in postoperative patients with suspected hypovolemia.
6. Rapid infusion of a colloid increases diuresis, at least in part, by inducing a release of an atrial natriuretic peptide (ANP).

Sammanfattning på svenska

Under de senaste 100 åren har intravenös vätsketerapi i syfte att korrigera en allt för låg blodvolym blivit en integrerad del av intensiv- och perioperativ vård. Även om vätsketerapi är livräddande, kan vätska om den läcker ut ur blodbanan orsaka svullnad i olika vävnader och därigenom ytterligare försämra organfunktion. Ur klinisk synvinkel är det därför viktigt att den administrerade vätskan stannar i blodkärl så länge som möjligt. Två huvudgrupper av vätskor används för att öka blodvolymen. Den ena typen är s.k. kristalloider vilka endast består av vatten och små molekyler som lätt passerar ut ur blodkärlen. Cirka 20% av den givna volymen har tidigare antagits stanna kvar i blodbanan. Den andra typen av vätska är kolloider, vilka i tillägg till små molekyler också innehåller stora molekyler som endast med svårighet kan passera ut ur blodkärlen. Denna grupp av vätskor anses stanna kvar i blodbanan i större utsträckning än kristalloider. Det övergripande syftet med detta avhandlingsarbete var att undersöka olika aspekter på vätsketerapi för att bättre förstå hur man skall kunna minimera biverkningar av vätsketerapi.

I ett första arbete testades hypotesen att kolloiden albumin är en, relativt sett, sämre plasmavolymexpander än kristalloiden Ringer-Acetat vid sjukdomstillstånd som kännetecknas av låg blodvolym (hypovolemi) på grund av att kärlen har en ökad genomsläpplighet (permeabilitet) för kolloider. En råttmodell av sepsis användes för att simulera ett tillstånd med hypovolemi pga ökad permeabilitet. En modell med lindrig blödning användes för att inducera hypovolemi med normal permeabilitet. Plasmavolymen undersöktes 2 och 4 timmar efter administration av 5% albumin eller Ringer-Acetat. I motsats till hypotesen visade studien att albumin var en lika potent plasmavolymexpander, relativt till Ringer-Acetat, också vid en ökad permeabilitet.

I ett andra arbete undersöktes dos-respons förhållandet mellan Ringer-Acetat och plasmavolymexpansion vid systemisk inflammation av två olika ursprung: dels i en råttmodell med sepsis och dels i en modell med blödningschock. Resultaten visade att Ringer-Acetat är en bättre plasmavolymexpander vid blödningschock än vid sepsis. Normal blodvolym uppnås inte vid någon av de undersökta doserna. Den dosberoende minskningen av det kolloidosmotiska trycket i plasma är mer uttalad vid sepsis än vid blödning och detta fynd kan inte i sin helhet förklaras av en spädningseffekt.

I ett tredje arbete testades hypotesen att långsam administration av 5% albumin ger en bättre plasmavolymsexpansion än en snabb infusion vid ett inflammatoriskt tillstånd orsakat av stor bukkirurgi. Postoperativa patienter med misstänkt hypovolemi randomiserades till att behandlas med 5% albumin i dosen 10ml/kg på antingen 30 minuter (snabbt) eller på 180 minuter (långsamt). Totalt analyserades 65 patienter och vi kunde inte finna någon skillnad mellan grupperna beträffande plasmavolymexpansion vid 180 minuter. Däremot tenderade arean under plasmavolymkurvan över tid var större efter snabb än efter långsam infusion av 5% albumin. Utifrån våra data finns det ingen anledning att ge albumin långsamt om det finns misstanke om hypovolemi.

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To my dearest sons—Símon Elías and Mattías Aron—you who are the purpose and extension of my love. May my work be a good example for you both to achieve the most from your lives.

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Appendix

- I. Bansch P, Statkevicius S, Bentzer P. Plasma volume expansion with 5% albumin compared to Ringer's acetate during normal and increased microvascular permeability in the rat. *Anesthesiology*. 2014; 121: 817-24.
- II. Statkevicius S, Frigyesi A, Bentzer P. Effect of Ringer's acetate in different doses on plasma volume in rat models of hypovolemia. *Intensive Care Med Exp*. 2017; 5: 50.
- III. Statkevicius S, Bonnevier J, Bark BP, Larsson E, Öberg CM, Kannisto P, Tingstedt B, Bentzer P. The importance of albumin infusion rate for plasma volume expansion following major abdominal surgery - AIR: study protocol for a randomised controlled trial. *Trials*. 2016; 17: 578.
- IV. Statkevicius S, Bonnevier J, Fisher J, Bark BP, Larsson E, Öberg CM, Kannisto P, Tingstedt B, Bentzer P. The effect of albumin infusion rate on plasma volume expansion - a randomized clinical trial. Submitted.

Paper I



Plasma Volume Expansion with 5% Albumin Compared to Ringer's Acetate during Normal and Increased Microvascular Permeability in the Rat

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ABSTRACT

Background: It is believed that the effectiveness of colloids as plasma volume expanders is dependent on the endothelial permeability for macromolecules. The objective of this study was to test the hypothesis that the plasma volume expanding effect of 5% albumin relative to that of a crystalloid solution is reduced if microvascular permeability is increased.

Methods: A control group was resuscitated with either 5% albumin (8 ml/kg) or Ringer's acetate (36 ml/kg) immediately after a hemorrhage of 8 ml/kg (n = 29). In a second group, permeability was increased by inducing sepsis through cecal ligation and incision (n = 28). Three hours after cecal ligation and incision, the animals were resuscitated with either 5% albumin in a ratio of 1:1 relative to the volume of lost plasma, or Ringer's acetate in a ratio of 4.5:1.

Results: In the hemorrhage group, plasma volumes at 15 min after resuscitation with albumin or Ringer's acetate had increased by 9.8 ± 2.6 ml/kg (mean \pm SD) and 7.4 ± 2.9 ml/kg and were similar at 2 and 4 h. Plasma volume 3 h after cecal ligation and incision had decreased by approximately 7 ml/kg, and at 15 min after resuscitation with albumin or Ringer's acetate it had increased by 5.7 ± 2.9 and 2.4 ± 3.0 ml/kg, respectively ($P < 0.05$). At 2 and 4 h after resuscitation, plasma volumes did not differ between the groups.

Conclusion: This study does not support the hypothesis that the plasma-volume-expanding effect of albumin relative to that of crystalloids is decreased under conditions characterized by increased permeability. (*ANESTHESIOLOGY* 2014; 121:817-24)

MAINTENANCE of normal intravascular volume is universally considered to be a cornerstone in the treatment of hemodynamically compromised patients, but the optimal type of fluid used to reach this therapeutic goal has been debated for a long time.^{1,2} Proponents of colloids have argued that less volume is required for equal plasma volume expansion and that crystalloids may compromise organ function secondary to edema formation.

Based on studies in postoperative patients and trauma victims as well as in experimental models of hemorrhage, it has been suggested that 4 to 4.5 times the volume of crystalloid solution is required to obtain the same plasma volume expansion as with a given volume of albumin.³⁻⁵ The difference in the distribution volumes for the different fluids is often attributed to the fact that microvascular permeability to small solutes is high, whereas permeability to colloids is low. This means that under conditions of increased permeability such as sepsis, it is plausible that the distribution volume of a colloid approaches that of a crystalloid solution as also suggested previously.⁴ If so, this could explain the observation that in recent randomized controlled studies of intensive care patients, the ratio between the volumes of crystalloids and colloids given to achieve clinical resuscitation end points was found to be 1:1 to 1.3:1.⁶⁻⁸

What We Already Know about This Topic

- It is thought that the better volume-expanding effect of albumin relative to crystalloids is dependent on low endothelial permeability for albumin

What This Article Tells Us That Is New

- One group of animals were subjected to a 11% hemorrhage and then given either 5% albumin in a volume equal to the shed blood volume or Ringer's acetate at 4.5 times that volume
- Another group of animals were subjected to abdominal sepsis, and at 3h, measured plasma volume loss was replaced with either 5% albumin or with Ringer's acetate in 4.5 times the measured loss
- Plasma volume expansion with albumin relative to Ringer's acetate did not differ between the two groups despite different etiologies for the decrease in plasma volume

Based on these considerations, the current study was designed to test the hypothesis that the difference between the volume of a colloid and the volume of a crystalloid required for equal plasma volume expansion decreases under conditions that are associated with increased permeability. For this purpose, rats subjected to either a controlled hemorrhage or abdominal sepsis were randomized to receive resuscitation with either 5% albumin at a ratio of 1:1 relative to the lost

The first two authors contributed equally to the study.

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volume of blood or plasma, or Ringer's acetate at a ratio of 4.5:1 relative to the lost volume of blood or plasma. Plasma volume was measured for up to 4 h after resuscitation by measuring the initial distribution volume of radiolabeled albumin.

Materials and Methods

Materials and Anesthesia

The study was approved by the Lund University ethics committee for animal research (M87-09), and the animals were treated in accordance with the guidelines of the National Institutes of Health for Care and Use of Laboratory Animals. Seventy male Sprague–Dawley rats weighing 354 ± 13 g were used in the study. The animals had free access to water and food until anesthesia was induced by placing them in a covered glass container with a continuous supply of isoflurane (Isoba® Vet; Intervet AB, Sollentuna, Sweden). After a tracheostomy, the animals were connected to a ventilator (Ugo Basile; Biological Research Apparatus, Comerio, Italy) and ventilated with tidal volumes of 6 ml/kg with a positive end-expiratory pressure of 3 to 4 cm H₂O. Anesthesia was maintained by inhalation of 1.6 to 1.8% isoflurane in humidified air through the tracheal cannula. Body temperature, measured rectally, was kept at 37.1° to 37.3°C *via* a feedback-controlled heating pad. End-tidal partial pressure of carbon dioxide was monitored continuously and kept between 34 and 42 mmHg (Capstar-1000; CWE, Ardmore, PA). The left femoral artery was cannulated for measurement of mean arterial pressure (MAP) and pulse pressure variation (PPV) and to obtain blood samples. The right jugular vein and the left femoral vein were cannulated and used for injections and to measure central venous pressure (CVP) intermittently. Following administration of an intravenous bolus of fentanyl (25 µg/kg; Braun Melsungen AG, Melsungen, Germany) and the start of a continuous fentanyl infusion ($0.5 \mu\text{g kg}^{-1} \text{min}^{-1}$), isoflurane concentration was reduced to 1.1 to 1.3%. After a 10-min equilibration period, MAP, CVP, and PPV were recorded, and baseline values for arterial blood gases, electrolytes, hematocrit, and lactate were measured (I-stat; Hewlett Packard, Böblingen, Germany). PPV was calculated by measuring PPV over a single respiratory cycle: $\text{PPV (\%)} = (\text{PP}_{\text{max}} - \text{PP}_{\text{min}}) / ((\text{PP}_{\text{max}} + \text{PP}_{\text{min}}) / 2) \times 100$ and is presented as the mean of 3 to 4 calculations. A PPV above 13% has previously been shown to be highly predictive of preload responsiveness in the rat.¹⁰

Urine was collected in a glass vial placed at the external meatus of the urethra, from the end of the preparation until the end of the experiment, when the bladder was emptied by external compression. Urine production is presented in ml kg⁻¹ h⁻¹ (total production divided by the length of the collection period). After the experiment, the animals were killed with an intravenous injection of potassium chloride.

Measurement of Plasma Volume

Plasma volume was estimated by determination of the initial volume of distribution for human serum albumin (CSL

Behring, King of Prussia, PA) labeled with ¹²⁵I as described in detail previously.^{11,12} This was accomplished by measuring the increase in radioactivity following injection of a known amount ¹²⁵I-human serum albumin (approximately 75 kBq/kg and 0.05 mg of albumin per kilogram) by subtracting the activity in a 250-µl blood sample taken just before the injection from the activity 5 min after the injection. The administered dose was calculated by subtracting the radioactivity in the emptied vial, in the syringe, and in the needle from the total radioactivity in the prepared dose. The volume of distribution for the tracer was then calculated by dividing the administered dose by the resulting change in concentration. The amount of unbound radioactivity in the injected ¹²⁵I-albumin was measured regularly after precipitation with 10% trichloroacetic acid and was found to be less than 1% in all experiments. All samples were counted in a gamma counter (Wizard 1480; LKB-Wallac, Turku, Finland).

Experimental Protocol

Animals were randomized with regard to experimental conditions and resuscitation fluid after preparation, and an investigator blinded to the type of experiments performed the analysis of the data. The investigator performing the experiments was not blinded to group assignment or treatment.

Hemorrhage Group

In the hemorrhage group, animals were bled a total of 8 ml/kg in 5 min. They were then resuscitated with either 5% albumin (8 ml/kg) (CSL Behring: 155 mmol/l sodium, 4 mmol/l caprylate, 4 mmol/l *N*-acetyltryptophan, and chloride at approximately 150 mmol/l) or Ringer's acetate (36 ml/kg) (Fresenius Kabi, Uppsala, Sweden: 131 mmol/l sodium, 4 mmol/l potassium, 2 mmol/l calcium, 1 mmol/l magnesium, 112 mmol/l chloride, 30 mmol/l acetate; osmolality 270 mosmol/kg) during a 30-min resuscitation period. Plasma volumes were measured at baseline, 15 min after resuscitation was completed, and again 2 h later. Arterial blood gases, lactate, hematocrit, and electrolytes were measured at baseline, after hemorrhage, and 2 h after resuscitation. Plasma volumes directly after hemorrhage were calculated as follows: $[\text{Baseline value} - (8 \text{ ml} \times (1 - \text{hematocrit}))]$. MAP, CVP, and PPV were measured at baseline, after hemorrhage, 15 min and 2 h after resuscitation (fig. 1). On a *post hoc* basis, a second set of experiments were performed in animals subjected to hemorrhage, using an identical protocol except that the last plasma volume measurement was performed 4 h instead of 2 h after resuscitation (fig. 1).

Sepsis Groups

Following surgical preparation and baseline measurements as described above, animals were subjected to a cecal ligation and incision (CLI) procedure. This procedure has been

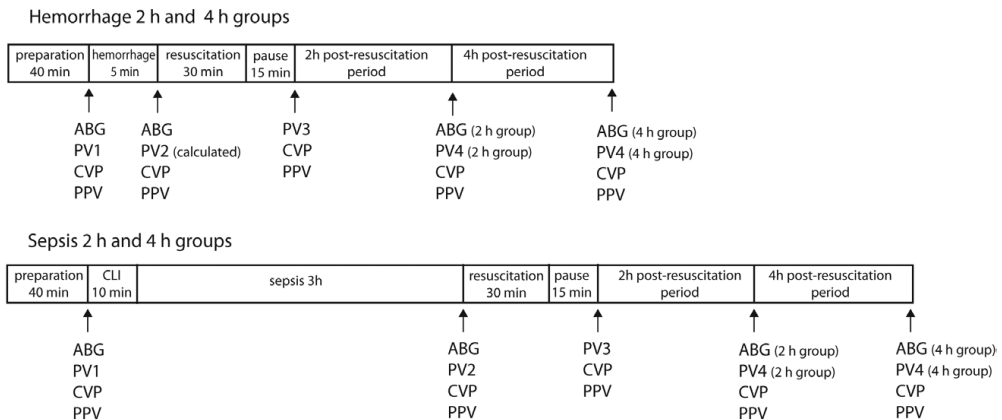


Fig. 1. Schematic diagram of the experimental protocol in the hemorrhage and sepsis groups. ABG = arterial blood gas; CLI = cecal ligation and incision; CVP = central venous pressure; PPV = pulse pressure variation; PV = plasma volume.

described in detail previously.^{11,13} Briefly, following a 3- to 4-cm midline abdominal incision, the cecum was mobilized while carefully avoiding hemorrhage. It then was ligated with a 3.5 silk ligature and a 1-cm incision was made with a scalpel blade. The abdomen was closed with metal clips. Three hours after the CLI procedure, MAP, CVP, and PPV were recorded and blood samples for analysis of plasma volume, arterial blood gases, electrolytes, hematocrit, and lactate were collected. Plasma volume loss was calculated, and the loss was replaced with the same volume of 5% albumin or with 4.5 times this volume of Ringer's acetate over the next 30 min. Plasma volume was measured again at 15 min and at 2 h after completion of the infusion (fig. 1). MAP, CVP, and PPV were measured immediately before plasma volume measurements, and arterial blood gases were measured again at the end of the experiments. On a *post hoc* basis, a second set of experiments were performed in septic animals, using an identical protocol except that the last plasma volume measurement was performed 4 h instead of 2 h after resuscitation (fig. 1). The change in plasma concentration of ¹²⁵I-human serum albumin from 15 min after resuscitation to either 2 h or 4 h after resuscitation was calculated to estimate differences in transcapillary leakage of albumin between the hemorrhage and sepsis groups.

Statistics

A sample size of a least eight in each of the 2-h groups was chosen based on previous studies using the same methodology demonstrating differences in plasma volume between different treatment groups.^{11,14} All data were analyzed by the Kolmogorov–Smirnov test and found to be normally distributed. Changes in plasma volumes and physiological data from baseline to 5 min after hemorrhage or 3 h after sepsis were analyzed with a paired Student *t* test. Effects of albumin and Ringer's acetate on plasma volume in the hemorrhage

and sepsis groups were evaluated by comparing the changes in plasma volume from baseline at the different time points with an unpaired Student *t* test. To test for differences in plasma volume between the hemorrhage and the sepsis animals immediately after resuscitation, an unpaired Student *t* test was used. Bonferroni correction was calculated by dividing the desired α -level with the number of comparisons within the sepsis and hemorrhage groups, respectively. All tests are two tailed. Data are presented as mean \pm SD. Prism 5.0c software was used for the analysis (GraphPad Software, La Jolla, CA)

Results

Hemorrhage Groups

Physiological and Laboratory Data. A total of 29 animals were included in the hemorrhage groups. In the 2-h group, eight and nine animals were resuscitated with albumin or Ringer's acetate, respectively. In the 4-h group, six animals were included in each group. No animals died during the experiment. MAP decreased and PPV increased after bleeding in both groups. After resuscitation, MAP and PPV returned to baseline values. There was a slight decrease in hematocrit already 5 min posthemorrhage. No difference between the resuscitation fluids on any of the physiological or laboratory data shown in tables 1 and 2 could be detected. Urine production was 2.3 ± 1.0 ml kg^{-1} h^{-1} in the Ringer's acetate groups and 1.8 ± 0.6 ml kg^{-1} h^{-1} in the albumin groups ($P < 0.05$).

Plasma Volumes. Plasma volume was 41.4 ± 2.5 ml/kg at baseline and decreased to 37.0 ± 2.4 ml/kg after hemorrhage in the albumin groups, and corresponding values were 40.5 ± 2.8 ml/kg and 36.1 ± 2.7 ml/kg in the Ringer's acetate groups. In the albumin groups, plasma volume was 46.7 ± 4.0 ml/kg 15 min after resuscitation, 45.7 ± 4.4 ml/kg at the end of the experiment in the 2-h group, and

Table 1. Hemodynamics for Animals Resuscitated with either 5% Albumin or Ringer's Acetate

Hemodynamic Values	Hemorrhage		Sepsis	
	Albumin	Ringer's	Albumin	Ringer's
Baseline				
MAP (mmHg)	109 ± 27	111 ± 23	110 ± 15	106 ± 14
PPV (%)	11 ± 3	10 ± 5	8 ± 2	8 ± 3
CVP (mmHg)	1.5 ± 0.7	2.0 ± 0.8	1.6 ± 0.5	1.5 ± 0.3
5 min after hemorrhage/3 h after CLI				
MAP (mmHg)	77 ± 23*	80 ± 24*	104 ± 15	102 ± 14
PPV (%)	17 ± 7*	17 ± 5*	15 ± 4*	17 ± 7*
CVP (mmHg)	1.1 ± 0.8	1.7 ± 0.7	1.5 ± 0.6	1.4 ± 0.4
15 min postresuscitation				
MAP (mmHg)	110 ± 11	103 ± 16	109 ± 14	100 ± 11
PPV (%)	9 ± 6	9 ± 4	12 ± 3	13 ± 5
CVP (mmHg)	1.8 ± 0.9	2.2 ± 0.9	1.5 ± 0.4	1.5 ± 0.4
2 h postresuscitation				
MAP (mmHg)	106 ± 17	100 ± 17	90 ± 13	88 ± 17
PPV (%)	9 ± 2	13 ± 5	17 ± 6	21 ± 9
CVP (mmHg)	1.8 ± 0.7	2.3 ± 0.9	1.4 ± 0.5	1.3 ± 0.6
4 h postresuscitation				
MAP (mmHg)	109 ± 23	103 ± 7	56 ± 7	65 ± 15
PPV (%)	9 ± 3	7 ± 2	30 ± 10	18 ± 8
CVP (mmHg)	2.1 ± 0.8	2.4 ± 0.5	1.0 ± 0.6	1.0 ± 1.0

Change in parameters from baseline to 5 min after hemorrhage or 3 h after CLI was analyzed with the paired Student *t* test.

* *P* < 0.05. Differences between albumin and Ringer's groups at the different time points in the hemorrhage or sepsis groups were evaluated using the unpaired Student *t* test with Bonferroni correction to adjust for multiple comparisons.

CLI = cecal ligation and incision; CVP = central venous pressure; MAP = mean arterial pressure; PPV = pulse pressure variation.

46.7 ± 4.0 ml/kg at the end of the experiment in the 4-h group (fig. 2). In the Ringer's acetate group, plasma volume was 43.8 ± 4.5 ml/kg 15 min after resuscitation, 45.5 ± 6.2 ml/kg at the end of the experiment in the 2-h group, and 47.2 ± 4.8 ml/kg at the end of the experiment in the 4-h group (fig. 2). At 15 min after resuscitation, the mean difference (±95% CI) between the mean of the albumin and Ringer's acetate groups was 3.0 (±3.3), and corresponding values at 2 and 4 h were 0.2 (±5.6) and 0.2 (±7.2), respectively (fig. 3).

Sepsis Groups

Physiological and Laboratory Data. A total of 28 animals completed the protocol and were included in the analysis. In the 2-h group, eight animals were included in each of the albumin or Ringer's acetate groups. In the 4-h group, six animals were included in each group. No difference in the effect of the resuscitation fluids on any of the physiological or laboratory data shown in tables 1 and 2 could be detected. Average MAP from the start of resuscitation until the end of the 2-h experiment was 107 ± 10 mmHg in the albumin group and 93 ± 12 in the Ringer's acetate group. The corresponding values for the 4-h experiments were 86 ± 12 mmHg and 87 ± 15 mmHg. Urine production was 0.8 ± 0.1 ml kg⁻¹ h⁻¹ in the albumin group and 0.9 ± 0.2 ml kg⁻¹ h⁻¹ in the Ringer's acetate group.

In the 2-h group, four animals (20%) died, and in the 4-h group nine animals (36%) died prior to completion of

all measurements. The animals randomized to receive treatment with either Ringer's acetate (*n* = 7) or albumin (*n* = 6) that died prior to completion of the experimental protocol had a plasma volume loss of 9.7 ± 2.6 ml/kg and 7.9 ± 2.9 ml/kg, respectively, at 3 h after CLI. Plasma volume increased by 4.8 ml/kg to 36.3 ± 1.7 at 15 min after resuscitation with Ringer's acetate and by 5.6 ml/kg to 38.0 ± 1.8 ml/kg after resuscitation with albumin. Lactate in the Ringer's acetate group was 2.3 ± 0.2 mmol/l at baseline and 4.0 ± 1.0 mmol/l at 3 h after CLI, and corresponding values for the albumin resuscitated animals were 2.1 ± 0.3 at baseline and 3.5 ± 0.5 mmol/l. Data from the animals that died prior to completion of all measurements are not included in the analysis below.

Plasma Volumes. Plasma volume in the albumin groups was 40.4 ± 2.1 ml/kg at baseline and decreased to 32.1 ± 3.4 ml/kg 3 h after the CLI procedure. Corresponding values in the Ringer's acetate groups were 39.6 ± 1.9 ml/kg and 32.7 ± 2.8 ml/kg. In animals resuscitated with albumin, plasma volume was 37.8 ± 3.6 ml/kg at 15 min, 29.6 ± 3.2 ml/kg at 2 h, and 27.4 ± 5.8 ml/kg at 4 h after resuscitation (fig. 2). In animals resuscitated with Ringer's acetate, plasma volume was 35.1 ± 2.5 ml/kg at 15 min, 30.6 ± 3.4 ml/kg at 2 h, and 28.3 ± 4.2 ml/kg at 4 h after resuscitation (fig. 2). The relative increase in plasma volume at 15 min postresuscitation by albumin was higher than after resuscitation with Ringer's acetate (adjusted *P* < 0.05). No differences between albumin and Ringer's acetate could be detected after 2 or 4 h (fig. 3). At 15 min after resuscitation, the mean difference

Table 2. Arterial Blood Gas Values, Hematocrit, Lactate, and Potassium for Animals Resuscitated with either 5% Albumin or Ringer's Acetate

	Hemorrhage		Sepsis	
	Albumin	Ringer's	Albumin	Ringer's
Baseline				
pO ₂ (mmHg)	80.3±9.0	79.5±9.0	83.3±9.0	83.3±8.3
pCO ₂ (mmHg)	39.8±3.0	39.8±3.8	40.5±3.8	39.8±3.8
pH	7.46±0.04	7.47±0.3	7.47±0.04	7.48±0.05
Base excess (mEq/l)	4±2	5±1	6±2	5±2
Hct (%)	44±2	45±2	43±3	43±2
Lactate (mmol/l)	2.2±0.5	2.3±0.5	2.0±0.5	2.2±0.3
Potassium (mmol/l)	4.7±0.5	4.8±0.3	4.5±0.4	4.6±0.4
5 min after hemorrhage/3h after CLI				
pO ₂ (mmHg)	78.8±7.5	78.8±12.0	81.8±10.5	81.0±6.0
pCO ₂ (mmHg)	39.0±3.0	38.3±3.8	4.8±12.8	39.8±3.0
pH	7.46±0.03	7.46±0.04	7.40±0.03	7.41±0.03
Base excess (mEq/l)	4±1	5±1	0±2*	1±2*
Hct (%)	41±2*	41±3*	48±3*	47±2*
Lactate (mmol/l)	2.3±0.6	2.6±0.9	3.5±0.9*	3.3±0.8*
Potassium (mmol/l)	5.2±0.3	5.2±0.3	4.8±0.3	4.8±0.3
15-min postresuscitation				
No blood gas analysis performed				
2-h postresuscitation				
pO ₂ (mmHg)	75.0±8.3	71.3±13.5	80.3±12.0	78.8±16.5
pCO ₂ (mmHg)	37.5±2.3	38.3±5.3	37.5±3.8	34.5±3.8
pH	7.45±0.02	7.42±0.04	7.38±0.05	7.37±0.05
Base excess (mEq/l)	2±2	0±2	-2±3	-4±2
Hct (%)	35±2	36±3	48±5	43±7
Lactate (mmol/l)	1.9±0.5	1.9±0.7	2.3±0.4	2.8±1.0
Potassium (mmol/l)	4.9±0.4	4.5±1.7	5.5±0.7	5.8±0.5
4-h postresuscitation				
pO ₂ (mmHg)	66.0±10.5	70.5±7.5	84.0±9.8	87.8±6.0
pCO ₂ (mmHg)	40.5±6.0	41.3±3.0	33.0±3.0	30.8±4.5
pH	7.42±0.03	7.42±0.03	7.28±0.11	7.36±0.06
Base excess (mEq/l)	1±1	2±1	-10±5	-7±3
Hct (%)	35±2	35±2	45±7	47±4
Lactate (mmol/l)	1.5±0.6	1.4±0.3	5.7±2.7	4.0±2.1
Potassium (mmol/l)	5.0±0.5	4.7±0.2	7.5±1.1	6.9±1.2

Change in parameters from baseline to 5 min after hemorrhage or 3h after CLI was analyzed with a paired Student *t* test.

* *P* < 0.05. Differences between albumin and Ringer's groups at the different time points in the hemorrhage or sepsis groups were evaluated using the unpaired Student *t* test with Bonferroni correction to adjust for multiple comparisons.

CLI = cecal ligation and incision; Hct = hematocrit; pCO₂ = partial pressure of carbon dioxide; pO₂ = partial pressure of oxygen.

(±95% CI) between the albumin and Ringer's acetate groups was 2.7 (±2.4), and corresponding values at 2 and 4 h were 1 (±3.5) and 0.9 (±6.5), respectively (fig. 3). Concentration of ¹²⁵I-human serum albumin decreased faster in the sepsis groups than in the hemorrhage groups (table 3).

Plasma volume changes 15 min following completion of resuscitation with albumin differed between the hemorrhage and sepsis groups and were 122±32% and 73±30% of the infused volume, respectively (unpaired Student *t* test, corrected *P* < 0.05). Also in the animals resuscitated with Ringer's acetate, plasma volume changes at 15 min postcompletion of resuscitation differed between the hemorrhage and sepsis groups and were 22±7% and 7±9% of the infused volume, respectively (unpaired Student *t* test, corrected *P* < 0.05).

Discussion

Our results show that resuscitation following hemorrhage with albumin or Ringer's acetate at 4.5 times the volume of albumin results in similar plasma volume expansion at 15 min, at 2 h, and at 4 h postresuscitation in rats. In sepsis animals, resuscitation with albumin or Ringer's acetate in the same ratio results in a higher plasma volume in the albumin group at 15 min, whereas plasma volumes at 2 and 4 h are similar. In sepsis, both albumin and Ringer's acetate are less efficient as plasma volume expanders at 15 min compared to the same time points after hemorrhage.

Plasma volume measurement using radiolabeled albumin is an established method, both experimentally and in clinical practice.^{11,12,15} As discussed previously, transcapillary escape

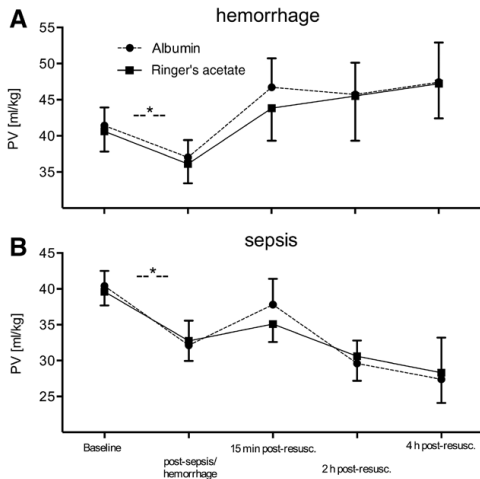


Fig. 2. (A) Absolute plasma volumes (PVs) at baseline, immediately following a controlled hemorrhage of 8 ml/kg, 15 min, 2 h, and 4 h after resuscitation with either 5% albumin or Ringer's acetate (n = 29 until 15 min after resuscitation, n = 17 for the 2-h group, and n = 12 for the 4-h group). (B) Plasma volumes at baseline, 3 h after cecal ligation and incision, at 15 min, 2 h, and 4 h after resuscitation with either 5% albumin or Ringer's acetate (n = 28 until 15 min after resuscitation, n = 16 for 2-h group, and n = 12 for 4-h group; *P < 0.05).

of albumin will cause an overestimation of true plasma volume during conditions of increased permeability.^{12,16} By measuring plasma concentrations of radiolabelled albumin at the earliest time point at which complete mixing of tracer probably has occurred, this source of error is minimized.¹² Based on our previous observation that transcapillary escape of albumin increases from approximately 15% per hour during basal conditions to approximately 20% per hour after the CLI procedure,¹¹ it can be calculated that the overestimation of plasma volume is small and in the range of 1.25 to 1.75% under basal or septic conditions, respectively. This in turn means that only 0.5% of the difference in plasma volume between the hemorrhage and sepsis groups can be attributed to a measurement error due to increased leakage of tracer. Free iodine in the injected tracer solution will rapidly distribute in the extracellular space, which may cause an overestimation of plasma volume. Given that free iodine was found to be less than 1% on all experiments, this error will also be small and similar in both hemorrhage and sepsis groups. Taken together, these sources of error are small and will not influence the conclusions made below.

The CLI method has been shown to result in a bacteremia within hours with a high mortality rate.¹³ The observed decrease in plasma volume of approximately 7 ml/kg prior to resuscitation in combination with hemoconcentration suggests that the model induces plasma leakage secondary to increased microvascular permeability. The ongoing plasma

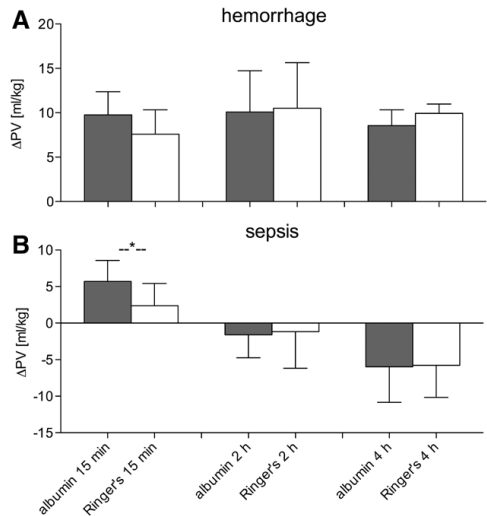


Fig. 3. (A) Change in plasma volume (PV) in animals subjected to a controlled hemorrhage of 8 ml/kg at 15 min, 2 h, and 4 h after resuscitation with either 5% albumin or Ringer's acetate (n = 29 until 15 min after resuscitation, n = 17 for the 2-h group, and n = 12 for the 4-h group). Unadjusted P values for comparison between Ringer's acetate and albumin are P = 0.04 at 15 min, P = 0.86 at 2 h, and P = 0.54 at 4 h. (B) Change in plasma volume in animals subjected to cecal ligation and incision at 15 min, 2 h, and 4 h after resuscitation with either 5% albumin or Ringer's acetate (n = 28 until 15 min after resuscitation, n = 16 for 2-h group, and n = 12 for 4-h group; *P < 0.05). Unadjusted P values for comparison between Ringer's acetate and albumin are P = 0.006 at 15 min, P = 0.84 at 2 h, and P = 0.94 at 4 h.

loss after resuscitation in the current study and the increase in the transcapillary escape rate of albumin after CLI in a previous study from our group further supports the hypothesis that microvascular permeability is increased.¹¹

It could be argued that hemorrhage may have induced a systemic inflammatory response syndrome, which could have increased microvascular permeability. However, a hemorrhage of 8 ml/kg is only 11% of the total blood volume in the rat and is unlikely to have increased permeability to a major degree. Our finding that plasma volumes remained

Table 3. Change in Concentration of ¹²⁵I-HSA from 15-min Postresuscitation to 2 or 4 h Postresuscitation, Respectively, in the Hemorrhage and Sepsis Groups

	Hemorrhage	Sepsis
2-h group	14.6 ± 2.6% (n = 17)	19.2 ± 4.5% (n = 16)*
4-h group	25.2 ± 4.3% (n = 12)	32.3 ± 5.1% (n = 12)*

Differences between the hemorrhage groups and sepsis groups at the different time points were analyzed using the unpaired Student t test.

*P < 0.05.

HSA = human serum albumin.

stable after resuscitation and that concentrations of radiolabeled albumin decreased at a higher rate in the sepsis groups supports this assumption and we conclude that the hemorrhage and sepsis groups differed with regard to permeability as intended when designing the protocol.

The ratio between volumes of Ringer's acetate and albumin of 4.5 to 1 resulted in a similar plasma volume expansion in the hemorrhage groups. The adequacy of this crystalloid to colloid ratio is supported by a previous study showing that if Ringer's acetate is administered in a lower-volume ratio to that of 5% albumin, plasma volume expansion will be significantly lower than in the group resuscitated with albumin.¹⁴ Similar ratios between 0.9% NaCl and 5% albumin have been reported in hemorrhage models, indicating that, with regard to plasma volume expansion, 0.9% NaCl and Ringer's acetate have similar efficacy.⁵ Also, clinical studies support that crystalloid solutions have poor plasma-expanding properties and that only approximately 20% of 0.9% NaCl and Ringer's solutions remain intravascularly immediately after resuscitation.^{3,4,9,17}

Our hypothesis that 5% albumin would be a relatively less potent plasma volume expander than a crystalloid in sepsis with increased plasma leakage compared to conditions with a normal microvascular permeability was not supported by our result that plasma volume was equal in the Ringer's acetate and albumin groups 2 h after resuscitation, and initially even higher in the albumin-treated animals. Based on the concern that 2 h was too short a time for an increased permeability to affect the plasma-volume-expanding properties of albumin, we added a second group of sepsis animals in which plasma volume was evaluated 4 h after resuscitation. Also the 4-h data failed to demonstrate a difference in plasma volume between the Ringer's acetate group and the albumin group. It could be argued that a difference in the plasma-volume-expanding effect might have been detected by measuring plasma volume at even later time points after resuscitation. However, given that more than one-third of the animals died before the end of the 4-h period, longer experiments were not considered feasible.

It could be argued that the lack of difference in plasma volume expansion between albumin and Ringer's acetate could be attributed to low statistical power. On average, sepsis animals were resuscitated with 8 ml/kg of albumin. If our hypothesis that the 1:1.4 ratio of albumin to Ringer's acetate observed in the "Saline *versus* Albumin Fluid Evaluation" study could be explained by a decreased plasma volume expansion by albumin this corresponds to approximately 6-ml/kg difference in plasma volume between animals resuscitated with albumin and animals resuscitated with Ringer's acetate. As can be seen from the 95% CI of the difference between the groups this is highly unlikely. However, we cannot exclude that a smaller change in plasma volume expansion by albumin could have been detected at 2 and 4 h after resuscitation if we had included a higher number of animals.

Our result of lower plasma volume expansion by both albumin and Ringer's acetate in the sepsis group than in the

hemorrhage group at 2 and 4 h was expected and probably reflects the ongoing loss of plasma in the septic animals. However, our finding of an almost 50% lower plasma-volume-expanding effect of both albumin and Ringer's acetate during sepsis compared to that observed after a hemorrhage already 15 min after resuscitation is unlikely to be fully explained by ongoing plasma leakage at the rate observed prerescuscitation. Sepsis animals lost approximately 7 ml/kg of plasma on average prior to resuscitation, and even if we assume that this loss occurred during the last hour preceding resuscitation, when animals were most strongly affected by the sepsis, this could at most account for 35% of the observed difference in the plasma volume expansion of approximately 5 ml/kg between the sepsis and the hemorrhage group, and other mechanisms most likely contributed to this result. Following hemorrhage, homeostatic mechanisms such as activation of the baroreceptor reflex will strive to normalize intravascular volumes by mobilizing fluid from the extravascular compartment. This hypothesis is supported by our result that the iso-oncotic 5% albumin solution increased plasma volume by more than the infused volume and that hematocrit had decreased already prior to resuscitation. The mobilized fluid is added to that given during the resuscitation and contributes to the measured plasma volume expansion. In contrast, sepsis disrupts homeostatic mechanisms such as autoregulation of capillary pressure. This means that the increased blood pressure seen during resuscitation will be transferred to the exchange vessels and may transiently increase plasma leakage, thereby contributing to the reduced volume-expanding properties of both colloids and crystalloids in sepsis.^{18,19}

Our rat model differs from human sepsis in several aspects. We chose to fluid-resuscitate the animals at one single occasion according to measured loss of plasma in order to make the groups comparable and to isolate effects of fluid resuscitation on plasma volume. This contrasts to clinical practice in which plasma volume is rarely measured and fluid resuscitation is a continuous process with end points such as MAP, lactate, and central venous oxygen saturation and is often combined with the use of inotropes and vasopressors. The latter may influence permeability and transcapillary hydrostatic pressures, which in turn may differentially influence the plasma-volume-expanding properties of colloids and crystalloids.²⁰ Normal transcapillary escape of albumin in humans is approximately 5% per hour in healthy subjects and is reported to increase to approximately 15% per hour in sepsis, and corresponding values in the rat are 15% per hour and 20% per hour, respectively.^{11,21} These differences in transcapillary escape of albumin suggest that also species differences may be of importance for the clinical relevance of this study.

Conclusion

The current study does not support the hypothesis that pathophysiological conditions associated with increased

microvascular permeability change the plasma-volume-expanding properties of 5% albumin relative to that of crystalloids and indicates that also in severe sepsis the ratio of albumin to crystalloid for equal plasma volume expansion is approximately 1 to 4.5.

The findings indicate that mechanisms other than changes in permeability may influence the plasma volume expansion of albumin relative to that of crystalloids and further research in this area is warranted.

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Competing Interests

The authors declare no competing interests.

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Paper II



RESEARCH

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Effect of Ringer's acetate in different doses on plasma volume in rat models of hypovolemia

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Abstract

Background: Even though crystalloids are the first choice for fluid resuscitation in hemodynamically unstable patients, their potency as plasma volume expanders in hypovolemia of different etiologies is largely unknown. The objective of the study was to investigate dose–response curves of a crystalloid in hypovolemia induced by either sepsis or hemorrhagic shock.

Results: Rats were randomized to resuscitation with Ringers acetate at a dose 10, 30, 50, 75, or 100 ml/kg at 4 h after induction of sepsis by cecal ligation and puncture (CLP) or 2.5 h after a 30 ml/kg hemorrhage. Plasma volume (¹²⁵I–albumin) was the primary outcome. Plasma volume decreased by about 11.8 (IQR 9.9–14.5) ml/kg relative baseline after CLP and increased dose-dependently by at most 5.8 (IQR 3.3–7.0) ml/kg in the 100 ml/kg group at 15 min after resuscitation. In the hemorrhage group, the plasma volume increased by at most 13.8 (IQR 7.1–15.0) ml/kg in 100 ml/kg group. Blood volumes at baseline, calculated using hematocrit and plasma volumes, were 72.4 (IQR 68.2–79.5) ml/kg in sepsis group and 71.1 (IQR 69.1–74.7) ml/kg in hemorrhage group. At 15 min after resuscitation with a dose of 100 ml/kg blood volumes increased to 54.8 (IQR 52.5–57.7) ml/kg and ; 49.6 (IQR 45.3–56.4) ml/kg, in the sepsis and hemorrhage groups, respectively. Plasma volume expansion as the percentage of dose at 15 min was 5.9 (IQR 2.5–8.8)% and 14.5 (IQR 12.1–20.0)% in the sepsis and hemorrhage groups, respectively. At 60 min, average plasma volume as the percentage of dose had decreased to 2.9 (IQR [–2.9] – 8.3)% ($P = 0.006$) in the sepsis group whereas no change was detected in the hemorrhage group. A dose-dependent decrease in the plasma oncotic pressure, which was more marked in sepsis, was detected at 60 min after resuscitation.

Conclusions: We conclude that the efficacy of Ringers acetate as a plasma volume expander is context dependent and that plasma volume expansion is lower than previously realized across a wide range of doses. Ringers acetate decreases plasma oncotic pressure in sepsis, in part, by mechanisms other than dilution.

Keywords: Plasma volume, Crystalloid, Dose–response, Inflammation, Sepsis, Hemorrhage

Background

Despite the fact that clinical guidelines recommend crystalloids for the initial fluid resuscitation in hemodynamically unstable patients with suspected hypovolemia, very little is known about the efficacy of crystalloids as plasma volume expanders at different doses [1, 2]. Crystalloids distribute primarily in the extracellular space and following equilibration only 20–25% is thought to remain in the circulation [3]. Data supporting this distribution is mainly based on experiments performed in models of an acute hemorrhage and on postoperative patients using only one volume (dose) of crystalloid [4–7]. In these settings, homeostatic mechanisms acting to replace the lost fluid are likely to contribute to the increase in plasma volume after resuscitation [5, 8]. This is in contrast to sepsis and other inflammatory conditions in which the disruption of homeostatic mechanisms striving to maintain normovolemia most likely contributes to hypovolemia [9–12]. Based on this, it could be hypothesized that crystalloids are less potent as plasma volume expanders during inflammatory conditions. Results showing that only 1–9% of a crystalloid remain intravascularly immediately after resuscitation in experimental sepsis [13, 14] or in postoperative cardiac surgery patients, align with this hypothesis [15]. These results raise the following questions: Can normovolemia be achieved with a crystalloid-based resuscitation strategy in severe inflammation? Is the efficacy of crystalloids as plasma volume expanders context dependent, i.e. different in inflammatory conditions of different origin?

The present study was designed to investigate the dose–response relationship of a crystalloid in inflammatory conditions of different etiology. For this purpose, we evaluated the effect of resuscitation with an isosmotic Ringers acetate solution in different doses on the plasma volume in rat models of abdominal sepsis or hemorrhagic shock.

Methods

Animals

The study was approved by the Lund University Ethical Committee for Animal Research (M309–12) and animals were treated under the guidelines of the National Institutes of Health for Care and Use of Laboratory Animals. A total of 127 adult male Sprague–Dawley rats weighing 351 ± 21 g were used in the study.

Anesthesia and preparation

Anesthesia was induced by inhalation of 5% isoflurane. After a tracheostomy, the animals were connected to a ventilator and ventilated with humidified air with a tidal volume of 10 ml/kg and a positive end-expiratory pressure of 3–4 cm H₂O. The core temperature was kept at 37.1–37.3 °C using a heating pad. The left femoral artery was cannulated for the measurement of mean arterial blood pressure (MAP) and pulse pressure (PP), and to obtain blood samples for measurement of blood gases, sodium, lactate and hematocrit (I-STAT, Abbot Park, Ill). PP variation (PPV)(%) during a ventilatory cycle was used as measure of fluid responsiveness and calculated as; $[(PP_{max} - PP_{min}) / (PP_{max} + PP_{min} / 2)] * 100$ and is expressed as the mean value for 5 consecutive ventilator cycles [16]. The right jugular and the left

femoral veins were cannulated for injections and fluid administration. Following the start of a continuous 0.5 µg/kg/min fentanyl infusion, isoflurane concentration was lowered to 1.1–1.3%. Urine was collected in a vial placed at the external meatus of the urethra and the bladder was emptied by external compression after completion of preparation and at the end of the experiment. After completion of the protocol, the animals were killed with an intravenous injection of potassium chloride.

Measurement of plasma volume

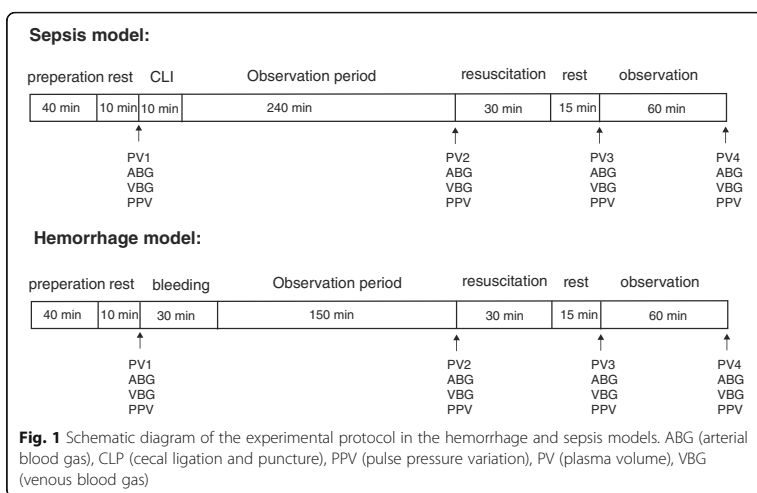
The plasma volume (PV) was estimated by a calculation of the volume of distribution for ¹²⁵I-labeled human serum albumin (HSA) at 5 min following an injection of a known dose as described previously [17]. The volume of ¹²⁵I-HSA (0.5 ml) was not included in the resuscitation volume. The amount of free ¹²⁵I in the administered dose was measured after precipitation with 10% trichloroacetic acid and centrifugation and was found to be < 2.6% in all experiments. Samples were counted in a gamma counter and blood volumes were estimated by dividing plasma volumes by 1-Hct.

Experimental protocol

The study consisted of 2 main groups: a sepsis group and a hemorrhage group. Following the preparation and the induction of sepsis or a hemorrhage as described in detail below, animals were randomized with regard to the dose of resuscitation fluid.

Sepsis model

Following baseline measurements (see Fig. 1), animals were subjected to a cecal ligation and puncture (CLP) procedure as described previously [14]. Four hours after the CLP procedure, plasma volume was measured again and the change in plasma volume was calculated. Animals with a plasma volume loss ≥ 5 ml/kg were included in the study



and were randomized to treatment with either 0 ml/kg, 10 ml/kg, 30 ml/kg, 50 ml/kg, 75 ml/kg or 100 ml/kg of isosmotic Ringers acetate solution over a 30-min period (Plasmalyte®, Baxter). The plasma volume was measured again at 15 min and at 60 min after the completion of the fluid resuscitation.

Hemorrhage model

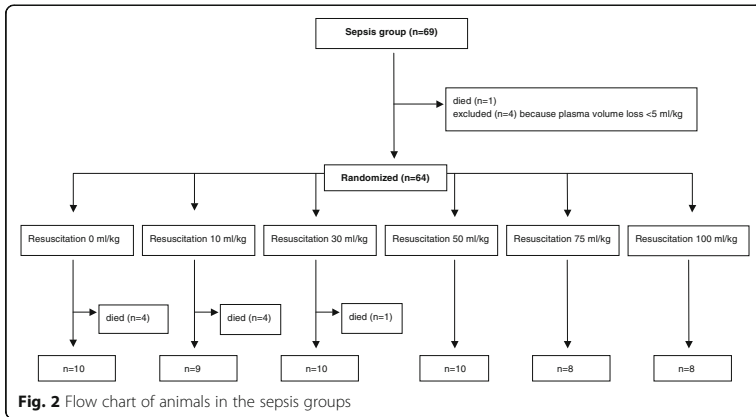
After baseline measurements (see Fig. 1), animals were bled 30 ml/kg over 30 min via the cannulated femoral artery. At 2.5 h after the hemorrhage, the plasma volume was measured again and the change in plasma volume was calculated. The volume of bleeding and the duration of unresuscitated shock was chosen on the basis of a previous study demonstrating a shock induced inflammatory response during these condition [18]. Animals were then randomized to receive resuscitation with isosmotic Ringers acetate (Plasmalyte®, Baxter) at a dose of 0, 10, 30, 50, 75, or 100 ml/kg over a 30 min period. The plasma volume was measured at 15 and 60 min after completion of the fluid resuscitation.

Plasma oncotic pressures and tissue water content

Oncotic pressure was measured using an osmometer with a 10-kDa cutoff membrane (Osmomat 050; Gonotec, Berlin, Germany) by an investigator blinded to the treatment status. To investigate the distribution of the resuscitation fluid, water content in the skin and subcutaneous tissue (from the abdominal wall), muscle (from the abdominal rectus muscle), lung, heart, liver, intestine and kidneys was measured. Tissue water content was also measured in two control groups of animals exposed to the CLP ($n = 4$) or the hemorrhage ($n = 4$) protocols and sacrificed before resuscitation. Tissues were extracted, weighted and dried for at least 72 h at 100 °C and then weighted again. Water content was calculated as (wet tissue weight – dry tissue weight)/wet tissue weight $\times 100$.

Statistics

Data was analyzed per protocol. Change in plasma volume at 15 min after resuscitation was the primary outcome. Because of the exploratory nature of these experiments, no formal power analysis was performed and the group size was based on previous studies in which differences in plasma volume expansion could be detected using the same methodology [13, 14]. Randomization was performed in blocks of four in each of the treatment groups with the objective to have eight to ten animals in each group. Animals that did not complete the protocol were replaced. Changes in plasma volume, hemodynamic parameters and laboratory data from the baseline to the end of the CLP or the hemorrhage procedure (before randomization) were evaluated using the paired Student's *t* test. Differences in the plasma volume expansion and in hemodynamic and laboratory data within the sepsis and hemorrhage groups at the different time points after resuscitation were analyzed using one-way-ANOVA on ranks followed by an adjustment for multiple comparisons using the Dunn's method. Differences in dose-dependent decrease in plasma oncotic pressure between the two groups were analyzed by comparing the slope of the dose–response curves as calculated by linear regression.



Differences in tissue water content were analyzed using Mann–Whitney test. Data are presented as the median and interquartile range (IQR) unless stated otherwise.

Results

Mortality

The mortality in sepsis and hemorrhage was 14% (95% confidence interval [CI], 8–25%) and 33% (95% CI; 22–45%), respectively. A flow chart of the animals in both study arms is presented in Figs. 2 and 3.

Hemodynamic and laboratory data

Sepsis

At the start of resuscitation, lactate, hematocrit and PPV had increased, and base excess, MAP and ScvO₂ had decreased compared to the baseline values ($P < 0.001$ for all, see Table 1 for all hemodynamic and laboratory data). Both at 15 and at 60 min after resuscitation lactate decreased and base excess increased in the group receiving

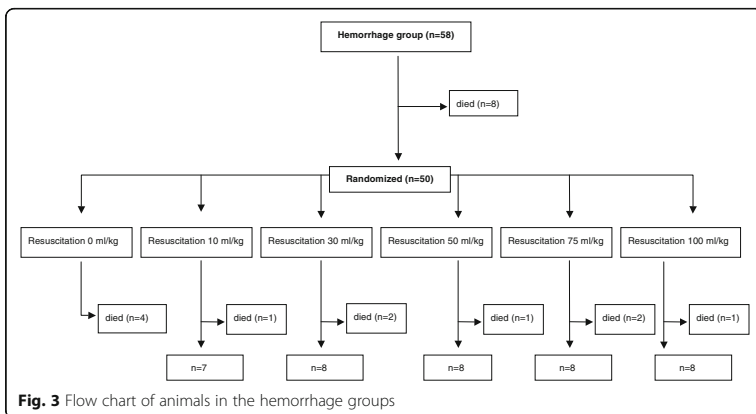


Table 1 Hemodynamic and laboratory data. Data are presented as median and IQR. The change in hemodynamic and laboratory data within the sepsis and hemorrhage groups at 15 and 60 min after resuscitation were analyzed using one-way ANOVA on ranks (* = $P < 0.05$ for comparisons with the 10 ml/kg group using the Dunn's method to correct for multiple comparisons). MAP = mean arterial pressure, PPV = pulse pressure variation, ScvO₂ = central venous saturation, Hct = hematocrit, BE = base excess, Δ = change in respective parameter, ns = no survivors

	Sepsis					Hemorrhage						
	0 (n = 10)	10 (n = 9)	30 (n = 10)	50 (n = 10)	75 (n = 8)	100 (n = 8)	0 (n = 4)	10 (n = 7)	30 (n = 8)	50 (n = 8)	75 (n = 8)	100 (n = 8)
Baseline												
Resus. volume ml/kg												
MAP, mmHg	99 (73-106)	98 (89-105)	95 (86-109)	102 (81-117)	116 (93-121)	93 (79-115)	88 (71-106)	99 (85-105)	95 (84-106)	89 (78-104)	83 (65-98)	85 (76-94)
PPV, %	9 (7-11)	11 (7-15)	9 (7-13)	9 (9-13)	8 (6-9)	8 (7-15)	9 (4-16)	9 (7-11)	9 (7-11)	7 (4-11)	10 (7-11)	11 (7-13)
ScvO ₂ , %	78 (71-80)	77 (73-80)	79 (76-82)	78 (71-84)	79 (66-81)	78 (75-83)	80 (75-83)	85 (83-88)	82 (79-85)	75 (73-79)	81 (71-84)	79 (74-83)
Lactate, mmol/L	2.1 (1.7-2.2)	2.2 (1.8-2.4)	2.1 (1.9-2.5)	2.3 (2.0-2.6)	2.0 (1.4-2.2)	2.1 (2.0-2.7)	1.9 (1.7-1.9)	2.4 (1.9-2.6)	2.0 (1.6-2.4)	2.2 (1.7-2.5)	2.5 (2.0-2.9)	2.1 (1.8-2.7)
Hct, %	42 (40-43)	41 (41-43)	42 (42-43)	43 (41-44)	43 (42-45)	42 (40-43)	43 (41-43)	44 (43-44)	42 (40-43)	42 (40-43)	41 (38-43)	42 (40-44)
BE, mEq/L	4 (3-5)	4 (3-4)	5 (3-5)	4 (3-4)	4 (3-5)	4 (3-5)	5 (4-7)	4 (3-5)	5 (4-6)	6 (3-6)	5 (4-6)	5 (3-6)
Pre-resuscitation												
MAP, mmHg	81 (67-92)	92 (87-108)	94 (85-98)	91 (76-96)	86 (67-100)	96 (81-109)	57 (51-66)	59 (50-80)	48 (45-51)	49 (40-55)	50 (47-51)	56 (53-60)
PPV, %	18 (15-21)	13 (12-22)	13 (12-19)	16 (15-19)	16 (13-21)	17 (15-18)	24 (19-27)	16 (12-19)	20 (10-32)	24 (13-28)	24 (19-28)	19 (17-27)
ScvO ₂ , %	60 (50-70)	51 (39-57)	57 (53-59)	62 (45-69)	51 (45-68)	56 (50-61)	54 (46-61)	59 (51-69)	43 (40-53)	44 (33-52)	41 (37-48)	43 (32-50)
Lactate, mmol/L	2.7 (2.6-3.3)	3.1 (2.7-3.3)	2.8 (2.2-3.3)	3.1 (2.8-3.4)	3.6 (2.8-5.4)	3.6 (3.2-3.9)	4.2 (3.2-4.6)	2.7 (2.4-3.8)	4.6 (3.8-5.2)	4.5 (3.8-5.7)	4.5 (4.1-6.1)	4.6 (3.9-6.1)
Hct, %	46 (41-48)	46 (43-48)	46 (43-46)	48 (44-50)	46 (41-49)	49 (42-51)	30 (27-32)	26 (25-28)	28 (26-30)	28 (26-31)	28 (27-28)	29 (26-30)

Table 1 Hemodynamic and laboratory data. Data are presented as median and IQR. The change in hemodynamic and laboratory data within the sepsis and hemorrhage groups at 15 and 60 min after resuscitation were analyzed using one-way ANOVA on ranks (* = $P < 0.05$ for comparisons with the 10 ml/kg group using the Dunn's method to correct for multiple comparisons). MAP = mean arterial pressure, PPV = pulse pressure variation, ScvO₂ = central venous saturation, Hct = hematocrit, BE = base excess, Δ = change in respective parameter, ns = no survivors (*Continued*)

	Sepsis										Hemorrhage									
	0 (n = 10)	10 (n = 9)	30 (n = 10)	50 (n = 10)	75 (n = 8)	100 (n = 8)	0 (n = 4)	10 (n = 7)	30 (n = 8)	50 (n = 8)	75 (n = 8)	100 (n = 8)	0 (n = 4)	10 (n = 7)	30 (n = 8)	50 (n = 8)	75 (n = 8)	100 (n = 8)		
Resus. volume ml/kg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
BE, mEq/L	-3 (-3- -1)	-3 (-4-2)	-2 (-4- -1)	-3 (-4- -2)	-5 (-8- -2)	-4 (-4- -1)	-1 (-3-0)	1 (-2-2)	-1 (-3-0)	-3 (-4- -1)	-2 (-4- -1)	-2 (-3-1)	-1 (-3-0)	1 (-2-2)	-1 (-3-0)	-3 (-4- -1)	-2 (-4- -1)	-2 (-3-1)		
15 min after resuscitation																				
ΔMAP, mmHg	-7 (-19 - -5)	-18 (-36 - -6)	-4 (-8 - -1)	0 (-8-6)*	-2 (-13-5)	-2 (-9-7)	ns	-7 (-8-0)	4 (1-6)	5 (3-9)	8 (1-26)*	2 (-4-14)	ns	-7 (-8-0)	4 (1-6)	5 (3-9)	8 (1-26)*	2 (-4-14)		
ΔPPV, %	-1 (-6-7)	4 (0-6)	3 (0-6)	1 (-10-3)	-4 (-7-6)	-4 (-6-1)	ns	0 (-3-3)	-4 (-11-2)	-8 (-16 - -2)	-11 (-13- -7)*	-7 (-9 - -2)	ns	0 (-3-3)	-4 (-11-2)	-8 (-16 - -2)	-11 (-13- -7)*	-7 (-9 - -2)		
ΔScvO ₂ , %	-9 (-14 - -1)	1 (-10-7)	-1 (-7-8)	-6 (-13-5)	6 (-15-15)	8 (-1-10)	ns	0 (-4-8)	9 (0-12)	9 (1-19)	13 (8-24)	19 (-1-26)	ns	0 (-4-8)	9 (0-12)	9 (1-19)	13 (8-24)	19 (-1-26)		
ΔLactate, mmol/L	0.3 (-0.3-0.6)	-0.2 (-0.5-0.3)	-0.4 (-1.1-0)	-0.7 (-1- -0.3)	-1.1 (-1.9 - -0.6)*	-1.4 (-1.8 - -1.0)*	ns	-0.1 (-0.7-0.2)	-1.0 (-1.6- -0.6)	-1.7 (-2.2- -0.9)	-1.9 (-2.8- -1.1)*	-2.4 (-2.8- -1.1)*	ns	-0.1 (-0.7-0.2)	-1.0 (-1.6- -0.6)	-1.7 (-2.2- -0.9)	-1.9 (-2.8- -1.1)*	-2.4 (-2.8- -1.1)*		
ΔHct, %	0 (-1-1)	-3 (-3 - -2)	-4 (-5 - -3)	-5 (-7- -5)*	-6 (-10-0)	-7 (-10 - -3)	ns	-2 (-3 - -1)	-6 (-8 - -5)	-9 (-11- -6)	-11 (-12- -10)*	-12 (-14 - -11)*	ns	-2 (-3 - -1)	-6 (-8 - -5)	-9 (-11- -6)	-11 (-12- -10)*	-12 (-14 - -11)*		
ΔBE, mEq/L	-3 (-3 - -1)	-1 (-2- -1)	1 (0-1)	2 (1-2)*	3 (2-5)*	3 (2-3)*	ns	0 (-1-1)	2 (1-4)	4 (2-5)*	4 (2-5)*	4 (2-5)*	ns	0 (-1-1)	2 (1-4)	4 (2-5)*	4 (2-5)*	4 (2-5)*		
60 min after resuscitation																				
ΔMAP, mmHg	-19 (-27-0)	-23 (-53- -16)	-14 (-23- -4)	-14 (-31-0)	-21 (-34 - -6)	-11 (-26- -8)	ns	-8 (-11-2)	4 (-3-8)	3 (-3-8)	10 (2-14)*	5 (-5-8)	ns	-8 (-11-2)	4 (-3-8)	3 (-3-8)	10 (2-14)*	5 (-5-8)		
ΔPPV, %	1 (-3-6)	4 (-1-7)	2 (-2-5)	0 (-5-5)	-2 (-11-5)	-3 (-8-3)	ns	0 (-4-6)	-1 (-15-7)	-5 (-10-4)	-7 (-14- -6)*	-7 (-17- -3)	ns	0 (-4-6)	-1 (-15-7)	-5 (-10-4)	-7 (-14- -6)*	-7 (-17- -3)		
ΔScvO ₂ , %	-19 (-27-12)	-14 (-16- -10)	-10 (-13 - -3)	-16 (-24 - -6)	-6 (-20-7)	-8 (-13-4)	ns	-1 (-13-2)	3 (-7-7)	4 (2-6)	10 (4-18)*	19 (-2-21)*	ns	-1 (-13-2)	3 (-7-7)	4 (2-6)	10 (4-18)*	19 (-2-21)*		
ΔLactate, mmol/L	1.3 (0.4-2.7)	0.7 (0.3-3.0)	-0.2 (-0.3-1.2)	0.8 (-0.1-1.3)	0 (-0.4-0.6)	-0.8 (-1.0-0)*	ns	0.5 (-0.6-1.8)	0.3 (-0.4-1.5)	-0.5 (-0.9-0.8)	-0.4 (-1.4-0.8)	-1.3 (-1.8-2.4)	ns	0.5 (-0.6-1.8)	0.3 (-0.4-1.5)	-0.5 (-0.9-0.8)	-0.4 (-1.4-0.8)	-1.3 (-1.8-2.4)		

Table 1 Hemodynamic and laboratory data. Data are presented as median and IQR. The change in hemodynamic and laboratory data within the sepsis and hemorrhage groups at 15 and 60 min after resuscitation were analyzed using one-way ANOVA on ranks (* = $P < 0.05$ for comparisons with the 10 ml/kg group using the Dunn's method to correct for multiple comparisons). MAP = mean arterial pressure, PPV = pulse pressure variation, ScvO2 = central venous saturation, Hct = hematocrit, BE = base excess, Δ = change in respective parameter, ns = no survivors (Continued)

	Sepsis										Hemorrhage			
	0 (n = 10)	10 (n = 9)	30 (n = 10)	50 (n = 10)	75 (n = 8)	100 (n = 8)	0 (n = 4)	10 (n = 7)	30 (n = 8)	50 (n = 8)	75 (n = 8)	100 (n = 8)		
Resus. volume ml/kg	0													
Δ Hct, %	-1 (-4-0)	-2 (-4--2)	-5 (-6--1)	-6 (-6--4)	-5 (-9--1)	-8 (-13--3)	ns	-5 (-6--3)	-6 (-8--4)	-9 (-11--7)	-11 (-12--10)*	-12 (-13--10)*		
Δ BE, mEq/L	-7 (-8--5)	-6 (-9--4)	-3 (-4--2)	-3 (-4--2)	-1 (-2--1)*	0 (-1--1)*	ns	-1 (-4-0)	-1 (-2-2)	1 (-2-4)	1 (-2-4)	2 (-4-3)		
Urine ml/kg/h	0.4 (0.3-0.5)	0.5 (0.4-0.7)	0.7 (0.6-0.9)	0.7 (0.5-0.9)	0.5 (0.3-0.8)	1.2 (0.8-1.4)*	ns	0.7 (0.6-0.9)	0.4 (0.3-0.8)	0.8 (0.6-1.4)	1.4 (0.9-1.7)	1.9 (1.6-2.2)*		

100 ml/kg compared to the group receiving 10 ml/kg ($P < 0.001$ for both). The urine production was higher in animals resuscitated with a dose of 100 ml/kg compared to those resuscitated with a dose of 10 ml/kg ($P = 0.022$). Arterial pH and $p\text{CO}_2$ did not differ between the groups at any of the time points (data not shown).

Hemorrhage

At the start of resuscitation, lactate and PPV had increased, and base excess, MAP, ScvO_2 , and hematocrit had decreased compared to baseline values ($P < 0.001$ for all). At 15 min after resuscitation, animals resuscitated with 75 and 100 ml/kg decreased in hematocrit ($P < 0.001$ for both groups) and lactate ($P = 0.013$ and $P = 0.014$, respectively) and increased in base excess ($P = 0.002$ for both groups) compared to the group receiving 10 ml/kg. At 60 min after resuscitation hematocrit decreased and ScvO_2 increased in the group receiving 100 ml/kg compared to the group receiving 10 ml/kg ($P = 0.007$ and $P = 0.021$, respectively). The urine production was higher in animals resuscitated with a dose of 100 ml/kg compared to those resuscitated with a dose of 10 ml/kg ($P = 0.018$). Arterial pH and $p\text{CO}_2$ did not differ between the groups at any of the time points (data not shown).

Plasma volumes

Sepsis

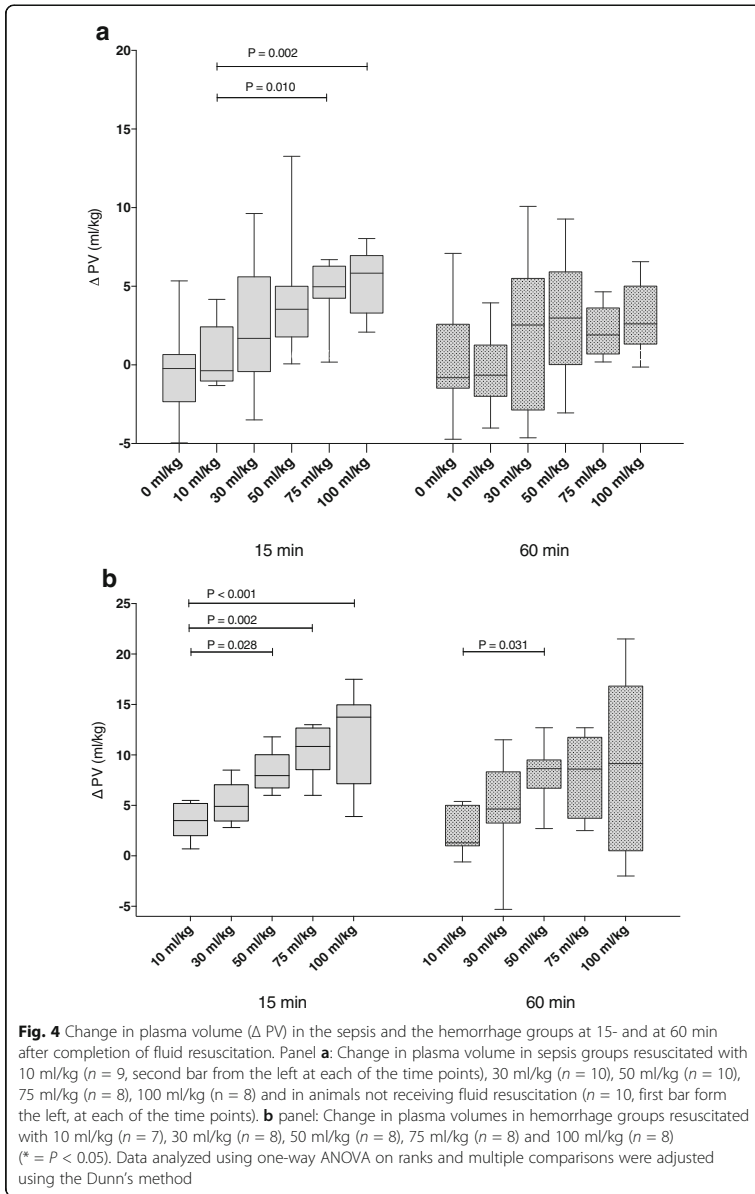
The baseline plasma volume in the sepsis group was 42.1 (39.5–46.4) ml/kg and decreased to 30.8 (28.1–33.7) ml/kg 4 h after the CLP procedure ($P < 0.001$). Corresponding blood volumes were 72.4 (68.2–79.5) ml/kg and 56.7 (52.6–60.4) ml/kg, respectively. At 15 min after resuscitation, there was a dose-dependent increase in the plasma volume (Fig. 4a). Calculated blood volume increased to at most 54.8 (52.5–57.7) ml/kg at a dose of 100 ml/kg. At 60 min after resuscitation, no differences in the plasma volume change could be detected ($P = 0.17$ for ANOVA).

Hemorrhage

The baseline plasma volume in the hemorrhage group was 41.9 (39.8–43.5) ml/kg and decreased to 30.6 (28.7–32.5) ml/kg 2.5 h after the hemorrhage ($P < 0.001$). The corresponding blood volumes were 71.1 (69.1–74.7) ml/kg and 42.5 (39.39–44.8) ml/kg, respectively. Volume of hemorrhage therefore corresponds to a 40% hemorrhage. At 15 min after resuscitation, the plasma volume increased dose-dependently (Fig. 4b). Blood volume at 15 min after resuscitation increased to at most 49.6 (45.3–56.4) ml/kg at a dose of 100 ml/kg. At 60 min after resuscitation, the 50 ml/kg group had increased in plasma volume compared to the 10 ml/kg group, whereas the 75 ml/kg and 100 ml/kg groups did not differ from the 10 ml/kg group (Fig. 4b).

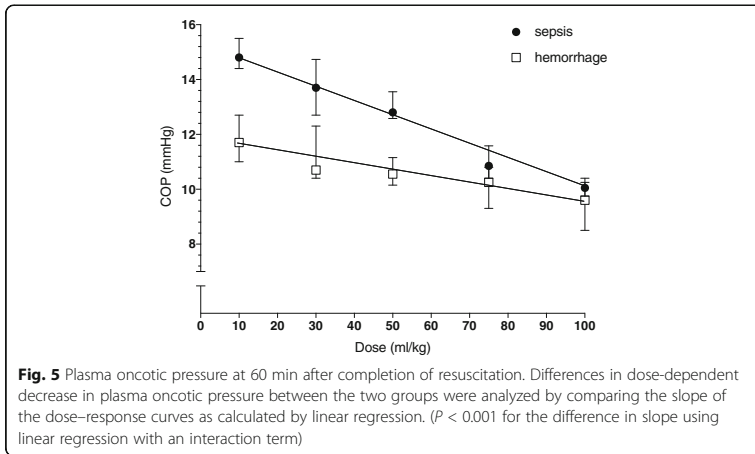
Sepsis versus hemorrhage

Plasma volume expansion as a percentage of the administered dose was assessed to test the hypothesis that efficacy of crystalloids as plasma volume expanders differs between the two inflammatory conditions. Average efficacy both at 15 and 60 min was lower in sepsis than in hemorrhage (15 min; 5.9 (2.5–8.8) % vs. 14.5 (12.1–20.0) %, $P < 0.001$, 60 min; 2.9 (–2.9)–8.3) % vs. 13.3 (8.3–19.0) %, $P < 0.001$, Mann–Whitney test). In sepsis, average efficacy decreased from 15 to 60 min ($P = 0.006$, Wilcoxon matched-pair signed ranks test), whereas it did not differ between the different time points in hemorrhage ($P = 0.108$).



Plasma oncotic pressure

In the sepsis and hemorrhage groups resuscitated with 10 ml/kg plasma oncotic pressures at 60 min after resuscitation were 14.8 (14.4–15.5) mmHg and 11.7 (11.0–12.7) mmHg, respectively, (Fig. 5). There was a dose-dependent decrease in colloid osmotic pressure in both conditions, which was more marked in sepsis than



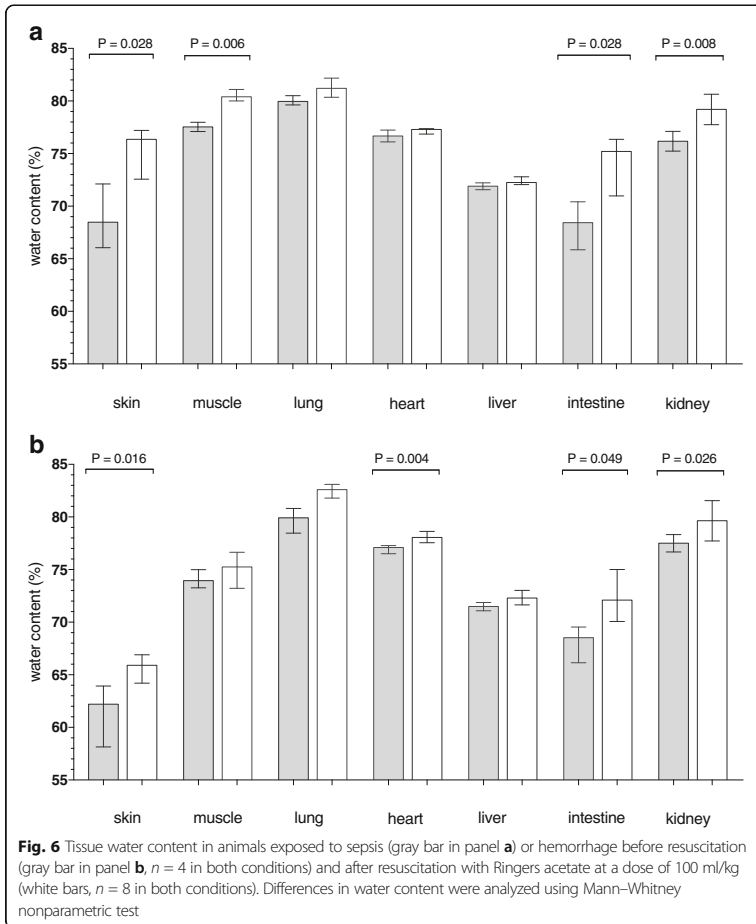
in hemorrhage ($P < 0.001$ for the difference in slope using linear regression with an interaction term). In sepsis, the plasma oncotic pressure was 10.1 (9.8–10.3) mmHg in the group resuscitated with a dose of 100 ml/kg. This corresponds to a 32% lower plasma oncotic pressure than in the 10 ml/kg group. At this time point, the plasma volume in animals resuscitated with 100 ml/kg had increased by about 10% relative to the plasma volume change observed in animals resuscitated with 10 ml/kg. In hemorrhage, the plasma oncotic pressure was 9.6 (8.5–10.4) mmHg in the group resuscitated with a dose of 100 ml/kg. This corresponds to a 20% lower plasma oncotic pressure than in the 10 ml/kg group. At this time point, the plasma volume in animals resuscitated with 100 ml/kg had increased by about 23% relative to the plasma volume change observed in animals resuscitated with 10 ml/kg.

Tissue water content

The tissue water content in skin, intestine, muscle and kidney was higher in resuscitated group than in the control group in sepsis (Fig. 6). The tissue water content in the skin, intestine, heart, and kidney was higher in the resuscitated group than in the control group in hemorrhage (Fig. 6). In both conditions water content appeared to increase the most in skin and intestines.

Discussion

Our results show that resuscitation with Ringers acetate in inflammation induced by abdominal sepsis increases plasma volume by about 7% of the infused dose early after resuscitation. In inflammation induced by hemorrhagic shock, Ringers acetate result in an increase in plasma volume by about 18% of the infused volume early after resuscitation. Calculated blood volumes after resuscitation did not reach baseline values even at the highest dose in any of the conditions. Resuscitation with Ringers acetate was associated with a dose-dependent decrease in the plasma oncotic pressure, which was more marked in abdominal sepsis.



Measurement of plasma volume using radiolabeled albumin is considered to be a golden standard and both baseline values and values after CLP in the present study are very similar to those reported in previous studies supporting the reliability of the methodology [14, 19, 20]. Sources of error, such as changes in the rate of extravasation of albumin secondary to increases in permeability, and amount of free iodine has been analyzed in detail previously [14] and it has been concluded that these sources of error are small during the present experimental conditions. Furthermore, our finding of reciprocal changes in hematocrit also supports the reliability of the plasma volume measurements.

Our finding of hypovolemia, increased lactate and a decreased MAP after CLP are in keeping with previous results and support that the model initiates an inflammatory response which mimics many features of human sepsis [14, 21]. Observational studies have shown that it is not uncommon that patients with septic shock receive more than 125 ml/kg during the first hours of resuscitation [22] suggesting that the investigated

doses are similar to those used in clinical practice. Also, hemorrhagic shock is suggested to initiate an inflammatory response, which shares many aspects of septic shock despite the differing etiologies [23] and our previous result showing increased levels of TNF- α before resuscitation using an identical protocol support the development of a shock induced inflammatory response in our model [18].

To our knowledge, this is the first study investigating the dose–response relationship of Ringers acetate as a volume expander. Our finding that Ringers acetate expands the plasma volume by about 7% of the infused volume early after resuscitation in sepsis is in agreement with previous studies evaluating effects of a single dose on plasma volume in experimental and clinical inflammation [13–15]. The present results extend the previous findings and indicate that potency appears to be similar across a wide range of doses immediately after resuscitation. However, 1 h after completion of resuscitation no difference in plasma volumes between the different doses could be detected. While this in part can be referred to low statistical power, it should also be noted that mortality appeared to be higher in animals receiving no resuscitation or the lower doses of Ringers acetate. Assuming that hypovolemia contributes to mortality, it is possible that this biased selection of animals available for plasma volume measurement in favor of those with less hypovolemia and/or inflammatory response. Such a bias may have overestimated potency of Ringers acetate in these groups.

Plasma volume expansion by 0.9% saline and Ringer acetate is often assumed to be equal and guidelines do not specify the type of crystalloid to use [1, 2] and, while studies comparing the two are sparse, a study on healthy humans has suggested that 0.9% saline is more potent than Ringers acetate [24]. In contrast, a previous study from our laboratory [13] using similar sepsis and hemorrhage protocols found that a single dose of 0.9% saline (32 ml/kg) increased plasma volume by 0.6 and 20% of the injected dose (corresponding results for Ringers acetate in the present study was 8 and 17%) suggesting that in rat sepsis, Ringers acetate is at least, equally potent as 0.9% saline. Future studies will have to address the relative potency of Ringers acetate and 0.9% saline in humans suffering from inflammatory conditions.

The finding that average efficacy was lower at 1 h align with a clinical study showing that crystalloid induced increases in cardiac output are transient and return to pre-infusion levels within 1–2 h [25]. The previous finding that immediate plasma volume expansion in less severe sepsis is about 20–30% of the infused volume contrasts to our results and suggests that efficacy in sepsis is not uniformly low and could be dependent on the severity of the inflammatory response [26, 27].

In hemorrhagic shock, Ringers acetate was a more potent plasma volume expander than in sepsis. Moreover, no decrease in efficacy during the first hour after resuscitation could be detected. The observation that normovolemia was not restored even at the highest dose of Ringers acetate, align with our previous finding that normovolemia was not achieved even at a dose of 135 ml/kg at 2 h after a hemorrhage of the same magnitude [18]. This contrasts to less severe hemorrhage, in which immediate resuscitation with crystalloids at a dose of about 4.5 the bleed volume restores normovolemia [14, 28]. Taken together our results suggest that normovolemia is very difficult to achieve using Ringers acetate in these two models of inflammation. As mentioned above, in clinical practice patients with sepsis may receive large amounts of crystalloids to correct hemodynamics. While we acknowledge that unresponsiveness to fluid resuscitation in sepsis is likely to be

multifactorial our results indicate that poor plasma volume expansion by crystalloids is a potential contributing factor.

Our finding that water content increased mainly in the intestines and skin after resuscitation supports that excess fluid is not uniformly distributed in different tissues and organs but is preferably distributed to organs with a high interstitial compliance [29]. While we did not measure interstitial and intravascular extracellular volumes changes after resuscitation previous studies suggest in experimental hemorrhagic shock the fluid is mainly distributed interstitially [30]. Of note is that no increase in tissue water content in the lungs could be detected after resuscitation. This finding contrasts to previous studies and could be related to lack of power [30].

The question may be asked how the lower efficacy of Ringers acetate as a plasma volume expander in sepsis than in hemorrhage may be explained? The vascular endothelium of all tissues except the brain is freely permeable to electrolytes already during normal conditions it is therefore highly unlikely that an inflammation induced increase in endothelial permeability for electrolytes could directly influence efficacy of crystalloids as plasma volume expanders per-se. However, hypovolemia in sepsis is secondary to redistribution of fluid from the intra- to the extravascular compartment in part due to increased permeability for colloids. This is in contrast to the hypovolemia in hemorrhage, which will initiate compensatory mechanisms to redistribute fluid in the opposite direction in an attempt to maintain normovolemia. Assuming that a crystalloid initially distributes in the extravascular space, the change in the ratio of intravascular to extravascular extracellular volumes is likely to contribute to the low potency of crystalloids in sepsis. Moreover, inflammation blunts homeostatic mechanisms that contribute to the plasma volume expansion observed in non-inflammatory conditions and by this mechanism contribute to the lower potency. Also inflammation-induced changes in the structure of the interstitial matrix may contribute to the low potency and duration of effect of crystalloids in severe systemic inflammation [31].

The result that plasma oncotic pressure decreased dose-dependently after hemorrhagic shock can be explained by the dilution of plasma proteins by Ringers acetate. This is in contrast to sepsis, in which, the decrease in plasma oncotic pressure was two times higher than what can be explained by dilution alone. This result suggests that Ringers acetate induce a dose-dependent increase in extravasation of albumin and/or a decrease in lymphatic return of albumin. At least two mechanisms may contribute to an increased extravasation of albumin in sepsis. Firstly, the increased water content in the interstitium prior to resuscitation secondary to vascular leak may have increased the volume of the distribution for albumin in the interstitium. This may have caused increased diffusion of albumin from the vessels to the interstitium. Secondly, increased permeability in sepsis is suggested to be secondary to an increased number of large pores [32] and/or to increased functional pore size due degradation of the glycocalyx [17]. Given that convective transport of albumin occurs through these pores, it is possible that convective transport of albumin due to a transiently increased transcapillary hydrostatic pressure during the distribution of crystalloids is larger in sepsis. Whatever the mechanism, this finding represents a previously unrecognized side-effect of crystalloid resuscitation in sepsis that is likely to contribute both to relatively poor potency and short duration of crystalloids as plasma volume expanders in sepsis.

Limitations

We acknowledge that our models suffer from several limitations. Most importantly, no empiric antibiotic therapy was initiated. Also vasoconstrictors and inotropes were not used in the sepsis model. We also acknowledge that a 40% hemorrhage would not normally be resuscitated using only crystalloids. Furthermore, the short observation time after resuscitation does not allow us to draw any conclusions about a more long-term dose-dependent effect on hemodynamics and more importantly on patient-important outcomes.

For standardization purposes all groups were resuscitated in 30 min and consequently flow rates of resuscitation fluids varied between the groups. While we cannot exclude this may have influenced our result, previous data indicate that flow rate does not influence plasma volume expansion by crystalloids [13].

Conclusions

We conclude that the efficacy of Ringers acetate as a plasma volume expander is context dependent. Plasma volume expansion is lower than previously realized across a wide range of doses and normovolemia may be impossible to achieve in sepsis. Resuscitation with Ringers acetate in sepsis induces a dose-dependent decrease in plasma oncotic pressure, which cannot be explained by dilution of plasma proteins only.

Abbreviations

ANOVA: Analysis of variance; CI: Confidence interval; CLP: Cecal ligation and puncture; IQR: Interquartile range; MAP: Mean arterial pressure; PP: Pulse pressure; PPV: Pulse pressure variation; PV: Plasma volume

Availability of data and materials

Data will be made available from the authors upon reasonable request.

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Authors' contributions

All authors participated in the conception and design of the study and in the critical revision of the manuscript for important intellectual content. PB and SS participated in data acquisition. PB, SS and AF performed the data analysis, interpreted the data. PB and produced the draft of the manuscript. All authors had full access to all of the data (including statistical reports and tables) in the study and can take responsibility for the integrity of the data and the accuracy of the data analysis. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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Paper III



STUDY PROTOCOL

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The importance of albumin infusion rate for plasma volume expansion following major abdominal surgery – AIR: study protocol for a randomised controlled trial

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Abstract

Background: Administration of fluids to restore normovolaemia is one of the most common therapeutic interventions performed peri-operatively and in the critically ill, but no study has evaluated the importance of infusion rate for the plasma volume-expanding effect of a resuscitation fluid. The present study is designed to test the hypothesis that a slow infusion of resuscitation fluid results in better plasma volume expansion than a rapid infusion.

Methods/design: The study is a single-centre, assessor-blinded, parallel-group, randomised prospective study. Patients over 40 years of age admitted to the post-operative care unit after a Whipple procedure or major gynaecological surgery and presenting with signs of hypovolaemia are eligible for inclusion. Patients are randomised in a 1:1 fashion with no stratification to either rapid (30 minutes) or slow (180 minutes) infusion of 5% albumin at a dose of 10 ml/kg ideal body weight. Plasma volume is measured using ¹²⁵I human serum albumin at baseline (prior to albumin infusion) as well as at 30 minutes and 180 minutes after infusion start. The primary endpoint is change in plasma volume from baseline to 180 minutes after the start of 5% albumin infusion. Secondary endpoints include the integral of plasma volume over time from baseline to 180 minutes after the start of the infusion and transcapillary escape rate of albumin (%/h) from 180 minutes to 240 minutes after the start of albumin infusion. In addition, diuresis, change in central venous oxygen saturation, lactate and blood pressure will be evaluated. A total of 70 patients will be included in the study, and the study has 80% power to detect a difference of 4 ml/kg in plasma volume expansion between the two groups.

Discussion: The present study is the first clinical investigation of the importance of infusion rate for the plasma volume-expanding effect of a resuscitation fluid.

Trial registration: EudraCT identifier: 2013-004446-42. Registration date: 20 December 2013.
ClinicalTrials.gov identifier: NCT02728921. Registration date: 31 March 2016.

Keywords: Albumin, Infusion rate, Plasma volume expansion, Permeability, Transcapillary escape rate

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Background

Major surgery initiates a systemic inflammatory response syndrome (SIRS), which disrupts the normal regulation of transcapillary fluid exchange with tissue oedema and hypovolaemia as a consequence. Hypovolaemia will amplify the inflammatory reaction by reducing cardiac output and oxygen delivery, which creates a vicious circle. Fluid therapy is therefore a cornerstone in the peri-operative treatment of patients undergoing major surgery as well as in patients with increased vascular leakage of other aetiologies. However, even if fluid therapy is life-saving, it is also associated with side effects such as further oedema formation, coagulopathy and further endothelial dysfunction. Several studies indicate that these side effects may adversely affect outcome following surgery [1, 2]. Also, in other patient groups with SIRS in the intensive care unit (ICU), it has been shown that fluid overload is associated with increased incidence of respiratory failure and worse outcome [3–6].

From a clinical perspective, it is therefore important that the fluid administered to antagonize hypovolaemia remains intravascular as far as possible. Colloids are macromolecules for which the vessel wall has a low permeability, and proponents of colloids argue that less volume is required for equal plasma volume compared with crystalloids. However, extravasation of colloids not only is a function of the vessel wall permeability but also is dependent on the volume of fluid that is filtered across the capillary wall, which in turn depends on the transcapillary hydrostatic pressure [7]. In addition, transient hypervolaemia induced by rapid administration of colloids may induce increased release of the permeability-increasing diuretic agent atrial natriuretic peptide and components of the endothelial glycocalyx [8, 9].

Taken together, these data indicate that slow administration may reduce extravasation of colloids by minimizing transient hypervolaemia and transient increases in hydrostatic pressure. This hypothesis is supported by results derived from two animal model studies of sepsis-induced systemic inflammatory response showing that the plasma volume expansion was greater in animals randomised to receive a slow infusion than in those randomised to receive a rapid infusion of the same volume of colloid [10, 11].

Fluid administration is one of the most common therapeutic interventions in ICUs and in post-operative units around the world [12], and a fluid bolus is a recommended method to assess fluid responsiveness. However, to date, no study has addressed the importance of infusion rates in a clinical setting. Although rapid correction of hypovolaemia makes intuitive sense, the need for further knowledge in this aspect of fluid

resuscitation was highlighted by the recent Fluid Expansion as Supportive Therapy trial, which showed a surprising increase in mortality following a rapid administration of resuscitation fluids compared with less aggressive fluid resuscitation [13]. Based on these considerations, the primary objective of the present study is to test the hypothesis that plasma volume expansion of a given volume of colloid is greater if fluid is administered slowly rather than rapidly.

Methods/design

We are conducting a single-centre, investigator-initiated, prospective, parallel-group, randomised study of two different albumin infusion rates in patients following major abdominal surgery. The study is approved by the regional ethical vetting board in Lund, Sweden (Dnr 2014/15), and will be conducted at Skåne University Hospital in Lund. Overviews of the trial design according to the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) statement and an overview of patient flow through the study are presented in Table 1 and Fig. 1, respectively. A SPIRIT checklist is provided in Additional file 1. Informed consent will be obtained non-consecutively from eligible participants when a member of the research team is available. The length of the study will be from signing of informed consent until 36 ± 7 days after the start of infusion of the test substance. All patients will be followed by a study investigator in a visit at the trial centre. All patients will be evaluated with regard to potential adverse effects by one of the investigators. The first patient was included on 18 June 2014. The protocol has been amended two times. The first amendment extended inclusion criteria to also include post-operative patients after major gynaecological surgery to promote recruitment. The second amendment changed the interim analysis plan by adding the Haybittle-Peto approach for the testing of efficacy. In addition, ongoing vasopressor and inotropic therapy was omitted as an exclusion criterion. The first amendment was made after 1 patient had been included, and the second was made after 24 patients had been included. The amendments were approved by both the regional ethical vetting board and the Swedish Medical Products Agency (MPA), and the current protocol version 1.3 2015-04-03 was approved by MPA 2015-05-27.

Inclusion criteria

Post-operative patients following non-emergent operation ad modum Whipple procedure or major gynaecological cancer surgery at the age of 40 years or older will be included if fulfilling the following criteria:

Table 1 Overview of enrolment, interventions and assessments according to the SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials) statement

Time point	Study period							
	Enrolment	Allocation	Post-allocation					Close-out
	-t ₁	0	t ₁	t ₂	t ₃	t ₄	t ₅	t ₆
Enrolment								
Eligibility screen	X							
Informed consent	X							
Allocation		X						
Interventions								
Infusion of 5% albumin (10 ml/kg) over 30 minutes				X				
Infusion of 5% albumin (10 ml/kg) over 180 minutes				X				
Assessments								
Plasma volume			X	X	X			
Transcapillary escape rate of albumin							X	
Haemodynamic data, haematocrit, lactate, ScvO ₂	X		X	X	X			
Post-operative complications			X	X	X	X	X	
Adverse events, serious adverse events			X	X	X	X	X	X

Abbreviations: ScvO₂ Central venous oxygen saturation, t₁ Baseline, t₂ Start of albumin infusion, t₃ 180 minutes after start of albumin infusion, t₄ 180–240 minutes after start of albumin infusion, t₅ 30 days after operation, t₆ 36 ± 7 days after inclusion or until resolution of any adverse event, serious adverse event or suspected unexpected serious adverse reaction

- Indication for fluid therapy as judged by the physician caring for the patient and at least one of the following criteria is fulfilled within 5 h after admission to the post-anaesthesia care unit (PACU):
 - Positive 'leg-raising test' (pulse pressure increase >9% or stroke volume increase by >10% as measured with cardiac ultrasound [14])
 - Central venous oxygen saturation (ScvO₂) <70%
 - Plasma lactate >2.0 mmol/L
 - Urine output <0.5 ml/kg in the hour prior to inclusion
 - Respiratory variation of the inferior vena cava >15% as measured by ultrasound [15, 16]
 - Systolic blood pressure <100 mmHg or mean arterial blood pressure <55 mmHg
- Written consent by the patient to participate in the study obtained prior to operation

Exclusion criteria

Patients fulfilling any of the following criteria will be excluded from the study:

- Hypersensitivity to the active drug or the tracer
- Signs of post-operative bleeding
- History of heart failure
- The physician caring for the patient considers that there are strong reasons to administer another fluid, or the same fluid but in another

way or in a different volume than stated in the protocol

- Pregnancy
- Clinical judgement by the investigator or the treating physician that the patient should not participate in the study for reasons other than described above

Informed consent and withdrawal

Patients scheduled for the operative procedures described above will be assessed for inclusion. If none of the pre-operative exclusion criteria are met, a member of the research team will provide both oral and written information before surgery (Additional file 2). All patients will be given the opportunity to ask questions about the study and will also be given sufficient time to decide whether to participate. Patients are informed of their right to withdraw from the study at any time. A patient who withdraws consent receives standard care. In addition, the patient/participant may be permanently withdrawn at the investigator's or the treating physician's discretion at any time if this is considered to be in the patient's best interest. Predefined permanent withdrawal criteria are as follows:

- Change of surgery procedure to other than operation ad modum Whipple or major gynaecological cancer surgery
- Serious violation of the study protocol, such as administration of other resuscitation fluid in a

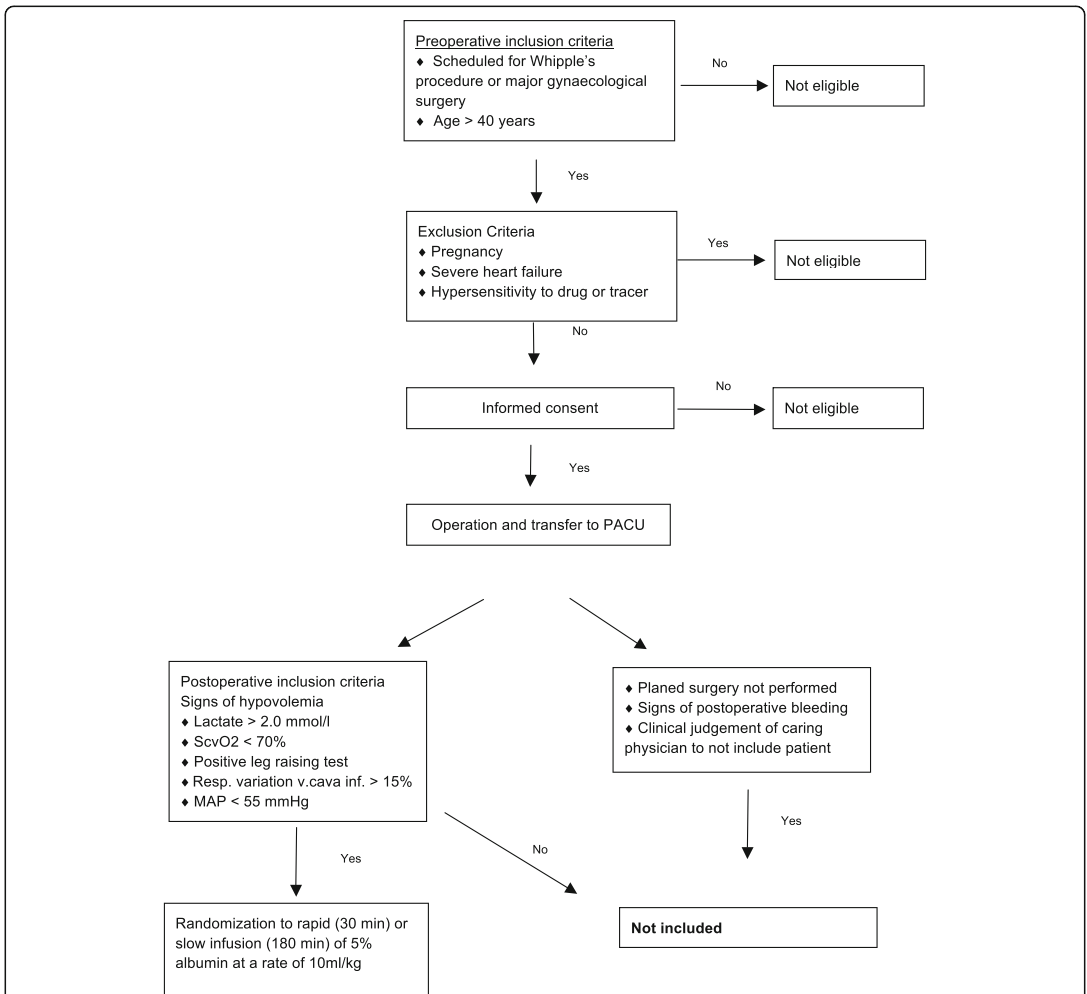


Fig. 1 Detailed enrolment and randomisation flowchart for Albumin Infusion Rate (AIR) study. PACU Post-anaesthesia care unit, ScvO₂ Central venous oxygen saturation, MAP Mean arterial pressure

volume >200 ml within 3 h after start of albumin infusion

3. Occurrence of known side effects, defined as serious adverse events (SAEs) listed in the summary of product characteristics of 5% albumin or SERALB-125* (IBA-CIS Bio International, Gif-Sur-Yvette, France) (Side effects that warrant permanent withdrawal include anaphylactic shock or pulmonary oedema.)

A record is kept of patients who consented in the pre-operative screening but were never enrolled (patient screening log).

Intra- and post-operative care of the patients

Eligible patients who have given consent to participate in the study will receive routine pre- and intra-operative care. Anaesthesia will be induced intravenously using propofol and maintained using either sevoflurane or desflurane. Patients will receive an epidural catheter for intra- and post-operative analgesia unless contraindicated. Fentanyl will be used as an analgesic during induction, and rocuronium will be used as a muscle relaxant. Crystalloids and colloids will be used as resuscitation fluids intra-operatively at the discretion of the attending anaesthetist. A haemoglobin level of 80–90 g/L will be the transfusion trigger. Post-

operative maintenance fluids will be a 2.5% or 5% glucose solution at a rate of 1 ml/kg/h. Analgesics may be given during the intervention phase of the study, and the rate of vasoactive agents may be adjusted to maintain mean arterial pressure >65 mmHg. No fluid other than maintenance and study fluids may be given during the intervention period.

Randomisation and blinding

Patients are screened for indications for fluid administration during the first 5 h after admission to the PACU as described above. Patients who fulfil inclusion criteria and meet no exclusion criteria will be randomised using a sealed envelope to either rapid or slow infusion of study drug. The randomisation and preparation of sealed envelopes are performed by an independent party (Clinical Research Unit, Skåne University Hospital, Lund). Randomisation was performed using a computerised random number generator. The research team is blinded to block size. Measurements of plasma volumes, transcapillary escape rate (TER) for albumin and post-operative follow-up will be performed by members of the research team who are blinded to the treatment allocation.

Study interventions

Patients randomised to a fast or slow infusion will receive 5% albumin at a dose of 10 ml/kg in 30 minutes or

180 minutes, respectively. Dose is based on ideal body weight [17]. At 240 minutes after the start of albumin infusion, the study protocol is completed, and the patient thereafter receives post-operative care according to local routine. Figure 2 shows a brief overview of the experimental protocol. Patients will be in lying position throughout study protocol time (240 minutes).

Primary outcome

The primary outcome in the study is change in plasma volume 180 minutes after the start of the albumin infusion.

Secondary outcomes

Secondary outcomes are differences in plasma volume over time (integral of plasma volume over time from the start of albumin infusion from plasma volume PV1 to PV3) and incidence of post-operative complications up to 30 days post-operatively (see Additional file 3 for definitions of complications). Other outcomes of interest are TER for albumin from 180–240 minutes after the start of albumin infusion, change in heart rate, change in central venous oxygen saturation, change in haemoglobin concentration in blood, change in blood pressure, change in central venous pressure, change in plasma lactate from the start of albumin infusion to 3 h after start of infusion, and diuresis from the start of albumin infusion to 3 h after the start of infusion.

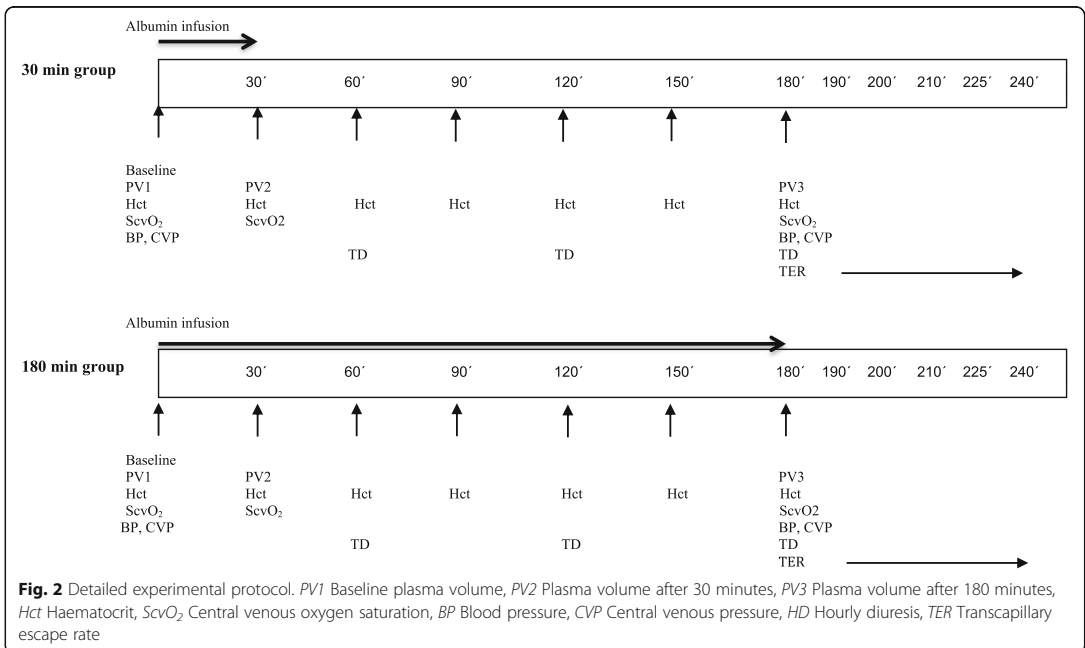


Fig. 2 Detailed experimental protocol. *PV1* Baseline plasma volume, *PV2* Plasma volume after 30 minutes, *PV3* Plasma volume after 180 minutes, *Hct* Haematocrit, *ScvO₂* Central venous oxygen saturation, *BP* Blood pressure, *CVP* Central venous pressure, *TD* Hourly diuresis, *TER* Transcapillary escape rate

In addition, exploratory analyses are planned. These analyses include measurement of effects of the different infusion rates on plasma concentration of markers of endothelial damage and on hormones involved in fluid homeostasis. For this purpose, blood samples are collected 5 minutes before centrifugation, and plasma samples will be stored following centrifugation at -80°C until analysis. We also intend to analyse to what extent included patients are hypovolaemic at inclusion ($>10\%$ lower than calculated normal blood volume [18]).

Measurements

Plasma volume is measured using ^{125}I human serum albumin (HSA) (SERALB-125[®]) in a total dose of at most 0.4 MBq. The dose administered to each patient is recorded in the case report form (CRF, Additional file 4). The dose in the syringes is determined from their increased weight multiplied by an activity concentration from a standard. This standard was prepared from a small sample from the ^{125}I -HSA vial added to a test tube and measured for both activity and weight. Blood samples are collected 5 minutes prior to injection of ^{125}I -HSA and 10 minutes after injection of ^{125}I -HSA for the plasma volume calculations. Plasma concentration is determined in a gamma counter (PerkinElmer 1480 Wizard; PerkinElmer, Waltham, MA, USA), and plasma volume is calculated by dividing the injected dose of ^{125}I -HSA by the change in concentration of ^{125}I -HSA in plasma at 10 minutes post-injection. Injected doses are corrected for remaining activity in the syringes.

Haematocrit is measured by colorimetric analysis using a blood gas analyser (Radiometer 850; Radiometer, Copenhagen, Denmark). Assuming that no blood loss occurs during the study period, changes in haematocrit will reflect changes in plasma volume. By this methodology, plasma volumes can be estimated every half-hour during the infusion of albumin. By measuring plasma volume with ^{125}I -HSA at three different points in time, the potential effect of alterations in small- to large-vessel haematocrit can be corrected [19]. The area under the plasma volume curve will then be calculated for each patient (plasma volume over time).

The TER for albumin is a measure of leakage of albumin from microvessels into the microvasculature. Changes in TER reflect changes in microvascular permeability and changes in parameters influencing convective transport of albumin such as transvascular hydrostatic pressure. Plasma concentration of ^{125}I -HSA is measured at 10 minutes, 30 minutes, 45 minutes and 60 minutes after the last injection of the tracer. Plasma concentration of ^{125}I -HSA as a function of time will be plotted in a log-linear plot. The slope of the line represents TER and will be expressed as the percentage decrease in

plasma concentration of ^{125}I -HSA per hour [20–22]. For a summary of measurements, please see Fig. 2.

Data collection and management

All study data will be recorded in CRFs for each patient, which are kept at the study site. Information on comorbidities, medications, results of pre-operative physical examinations, routine laboratory analysis results, American Society of Anesthesiologists physical status classifications, Physiological and Operative Severity Score for the enUmeration of Mortality and morbidity and Revised Cardiac Risk Index will be collected from the hospital electronic chart system. Peri-operative data will be collected from the anaesthesia chart, and data that will be registered include anaesthesia methods and drugs, type and volume of fluids, peri-operative bleeding, and diuresis. Inclusion criteria will be registered in CRFs before randomisation. Clinical haemodynamic evaluation will be performed and recorded at inclusion and at 180 minutes after the start of albumin infusion. Members of the research team have unlimited access to study data. The data collected in the present study will be available from the corresponding author on reasonable request. The auditing (see below) will include source data verification.

Sample size

Published SD values for plasma volume measured with the ^{125}I -HSA method are in the range of 4–7 ml/kg [22]. In the present study, we wish to compare differences in changes in plasma volume, and it is reasonable to assume that the SD is in the lower part of this range. In an experimental study, a 50% greater plasma volume expansion was found after slow administration of 5% albumin compared with a bolus dose of the same volume [10]. If we wish to detect a 40% difference in volume-expanding effect following administration of 10 ml/kg of 5% albumin, assuming that the SD of the plasma volume is 5, then about 30 patients in each group are required to obtain a power of 80%. To adjust for a slightly lower than expected treatment effect and for the possibility that patients may not complete the protocol, we intend to include a total of 70 patients: 35 patients in each arm. Should the number of patients who complete the protocol be <30 in any of the treatment groups, we plan to increase the sample size to maintain power.

Statistical analysis plan

The study will continue until the planned number of patients has been included, unless the interim analysis indicates that the study should be stopped. Results will be unblinded when all data have been collected. Only patients who received 90% or more of the intended dose

will be included in the analysis. The investigating team will perform the statistical analyses.

General analytical principles

1. Analysis will be performed on a per-protocol basis.
2. All hypothesis tests will be two-sided, with a maximal type I error risk of 0.05.
3. Subgroup analysis will be performed regardless of overall treatment efficacy.
4. Imputation will not be used to correct for missing data in the analysis.

Assessment of baseline variables

Baseline variables of patients fulfilling study protocol in the two study arms will be tabulated. Discrete variables will be reported as frequencies and percentages, and continuous variables will be reported as either means with SDs or medians with interquartile ranges.

Analysis of outcomes

The primary outcome will be analysed using Student's *t* test. Secondary outcomes will be analysed using Student's *t* test or the Mann-Whitney *U* test as appropriate. Differences between the groups will be reported as means with 95% confidence intervals. In the event of no difference between the groups with regard to primary outcome and a difference in the secondary outcome of plasma volume over time, we will interpret these results as supporting our hypothesis but that confirmatory studies are needed. With the exception of number of complications, all other outcomes are regarded as exploratory, and no emphasis will be placed on differences between the treatment groups, should the primary outcome and plasma volume over time outcome be negative.

Subgroup analysis

A sensitivity analysis using multivariate regression will be performed to assess if treatment effect is dependent on type of surgery and baseline plasma volume.

Auditing

The purpose of auditing and quality control is to ensure scientific integrity, data quality, the safety and integrity of the participating subjects, and that the study is compliant with the current versions of the Declaration of Helsinki, the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, good clinical practice and national regulations. The sponsor will delegate auditing to the Clinical Research Unit, Skåne University Hospital in Lund, an independent party that will perform on-site monitoring before, during and after the study. Considering that the patients will be treated according to normal routine

during major abdominal surgery, that the infusion rates and volumes are within the normal range, and the low frequency of adverse events (AEs) in an earlier large prospective randomised clinical trial comparing albumin with normal saline in an ICU setting, the study will be performed without the use of a data monitoring board [23].

Interim analysis

An independent statistician will perform an interim analysis for assessment of efficacy and futility after 36 patients have completed the protocol. The Haybittle-Peto approach will be used when testing for efficacy. If a difference with regard to the primary endpoint with $p \leq 0.001$ is detected, the study may be stopped. Futility will be assessed by simulating the remainder of the study multiple times using an SD of 5 and a difference in means of 4 ml/kg between the two groups. The results of each simulation will be combined with the obtained data. If the simulated data in combination with the observed data show a significant effect (two-sided Student's *t* test with an $\alpha < 0.05$) in less than 10% of the cases, the study will be stopped. Should the observed SD in interim analysis be higher than that used in the power calculation, the higher number will be used for the simulation. The principal investigator has the authority to stop the trial.

Harms

All patients are evaluated with regard to potential AEs or SAEs by one of the investigators, and potential AEs and SAEs are recorded in the CRF. A SAE is defined as an event that fulfils one or more of the following criteria: results in death, is life-threatening, requires prolongation of hospitalisation, results in persistent or significant disability or incapacity or any other important medical event. The investigator evaluating the patient at the end of the study is responsible for the treatment of any AE or SAE until resolution of the AE or SAE. Depending on the nature of the AE or SAE, the treatment will take place at either the trial centre, the local hospital or as an outpatient. If the responsible investigator/sponsor judges the SAE as being drug-related and unexpected, this must be promptly reported to the sponsor, which is responsible for reporting suspected unexpected serious adverse reactions to the Swedish MPA and the regional ethical vetting board.

Publication plan

The study is registered in the European Clinical Trials Database (EudraCT 2013-004446-42) and the ClinicalTrials.gov database (NCT02728921). Following completion of the trial, the main manuscript will be submitted to a peer-reviewed journal, regardless of the trial outcome. For publication of the main outcomes, the first figure presented will be a Consolidated Standards

of Reporting Trials (CONSORT) flowchart. The diagram will include the number of screened patients, the number of patients giving consent, the number of patients meeting all inclusion criteria, the number of patients randomised to each of the two treatment arms, and the number of patients completing the protocol in each of the treatment groups. The second figure will depict plasma volumes in the respective groups at baseline and at 30 minutes and 180 minutes after the start of albumin infusion. The first table will describe baseline demographics as detailed above. The second table will describe secondary outcomes. Authorship will be granted according to the criteria described by the International Committee of Medical Journal Editors [24].

Funding and sponsor

The AIR trial is funded by Region Skåne (Medical Training and Research Agreement [Avtal om Läkarutbildning och Forskning]), the Gyllenstiernska Krapperup Foundation and the Anna and Edwin Berger Foundation. Other than funding, the funders have no role in any aspect of the trial. The study principal investigator (PB) is also sponsor as delegated by Skåne University Hospital.

Discussion

The AIR study will provide clinical data on the importance of infusion rate for the plasma volume-expanding effect of colloids in patients with suspected hypovolaemia after major abdominal surgery. The decision to include patients subjected to a Whipple procedure or major gynaecological surgery is based on the clinical experience that these patients often appear hypovolaemic in the immediate post-operative period and thus are likely to require fluid resuscitation. Post-operative hypovolaemia in these patients is at least partly due to vascular leak secondary to the systemic inflammatory response initiated by the operation, and these patients are therefore likely to share pathophysiological mechanisms with other critically ill patients requiring fluid resuscitation [25]. Given the transient nature of the need for fluid resuscitation in the majority of these post-operative patients, the immediate post-operative period was believed to represent the optimal time point for our purposes.

Albumin is chosen as the study colloid because albumin is a naturally occurring colloid with very few contraindications in the current setting and with an excellent safety record [23]. The rates of infusion are chosen to differ as much as possible within the range of clinical practice for this patient category. In routine clinical practice, the anaesthesiologist caring for the patient decides how quickly 5% albumin is to be administered, and the rates and doses to be tested are within the limits normally used in our institution as well as

in others, and they do not differ from our clinical routine [26, 27].

Plasma volume measurement using ^{125}I -HSA is considered to be the gold standard for measurement of plasma volume and is used clinically in many hospitals worldwide. The radiation dose received by the patients due to participation in the study is approximately 0.12 mSv and is less than the background radiation that patients are naturally exposed to during a 6-month period, and also <0.1% of the dose used during radioiodine treatment of hyperthyroidism. Therefore, in accordance with World Health Organisation guidelines, patients will not receive treatment with potassium iodide to block thyroid uptake of radiolabelled iodine.

Measurement of TER for albumin is commonly used to evaluate vascular leak of albumin. After a bolus dose of ^{125}I -has, there is a steep decline in plasma concentration during the first 10 minutes, which is thought to represent mixing and distribution into a rapidly equilibrating space. After the first 10 minutes, the rate of decline in plasma concentration of ^{125}I -HSA reflects extravasation and a gradual increase in lymphatic return of the tracer. To minimize the influence of lymphatic return on the decrease in in plasma concentration of tracer, TER is measured during the first hour after injection. It could be argued that recirculation of ^{125}I -HSA from the two preceding plasma volume measurements (see Fig. 1) could influence the TER measurement. The magnitude of this influence during the 4-h experimental period can be modelled by assuming a TER value of 15%, a ratio of 1:4 between the intravascular and interstitial extracellular compartments. On the basis of these assumptions, it can be estimated that recirculation will have a negligible influence on a difference in TER between the two treatment groups (Additional file 5). This conclusion aligns with results from a similar analysis concerning repeated TER measurements in patients with sepsis [28].

Trial status

Recruitment has been completed. The interim analysis has been performed, and no reason to halt the study has been found. No SAEs have been registered to date.

Additional files

- Additional file 1:** SPIRIT Checklist. (DOC 99 kb)
- Additional file 2:** Consent form (in Swedish). (DOCX 32 kb)
- Additional file 3:** Definition of postoperative complications. (PDF 108 kb)
- Additional file 4:** Case report form (in Swedish). (DOCX 258 kb)
- Additional file 5:** Effect of tracer recirculation on measurement of transcapillary escape rate for albumin. (DOCX 158 kb)

Abbreviations

AE: Adverse event; AIR: Albumin Infusion Rate study; BP: Blood pressure; CONSORT: Consolidated Standards of Reporting Trials; CRF: Case report form; CVP: Central venous pressure; Hct: Haematocrit; HD: Hourly diuresis; HSA: Human serum albumin; ICU: Intensive care unit; MAP: Mean arterial pressure; MPA: Swedish Medical Products Agency; PACU: Post-anaesthesia care unit; PV: Plasma volume; SAE: Serious adverse event; ScvO₂: Central venous oxygen saturation; SIRS: Systemic inflammatory response syndrome; SPIRIT: Standard Protocol Items: Recommendations for Interventional Trials; TER: Transcapillary escape rate

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Authors' contributions

SS was responsible for study design, study coordination and conduct, and manuscript preparation. JB was responsible for study conduct and manuscript preparation. BB was responsible for study design and manuscript preparation. EL was responsible for study conduct and manuscript preparation. CMÖ was responsible for mathematical modelling and manuscript preparation. PK was responsible for study design and conduct as well as manuscript preparation. BT was responsible for study design, study conduct and manuscript preparation. PB was the principal investigator and sponsor and was responsible for study design, study coordination and conduct, and manuscript preparation. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Ethics approval and consent to participate

The trial will be conducted in accordance with the protocol, applicable regulatory requirements, the principle of good clinical practice and the Declaration of Helsinki. All patients will be given the opportunity to ask questions about the study and will also be given sufficient time to decide whether to participate in the study. Patients will be informed of their right to withdraw from the study at any time. The radiation dose received by the patients due to participation in the study is calculated to be small, and the protocol is approved by the local radiation committee (Strålskyddskommitté). As a consequence of participation in the study, the patients will be subjected to a blood loss of 86 ml. The study was first approved by the Swedish MPA on 9 February 2014.

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Paper IV



The effect of albumin infusion rate on plasma volume expansion- a randomized clinical trial

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Running title: Infusion rate and plasma volume expansion

ABSTRACT

Rationale: Optimal infusion rate of colloids in patients with suspected hypovolemia is unknown.

Objective: Test the hypothesis that a slow infusion of 5% albumin results in a better plasma volume expansion than a rapid infusion.

Methods and measurements: Patients showing signs of hypoperfusion after major abdominal surgery were randomized to intravenous infusion of 5% albumin at a dose of 10 ml/kg either slowly (180 minutes) or rapidly (30 minutes). Primary outcome was change in plasma volume, measured using radiolabelled albumin, from the start of infusion to 180 minutes after start of infusion, and secondary outcomes were change in area under the plasma volume curve and complications up to 30 days postoperatively. Data were analyzed per protocol.

Main results: A total of 34 and 31 patients completed the protocol in the slow and rapid groups, respectively. Change in plasma volume from start of infusion to 180 min did not differ between the slow and rapid infusion groups (6.7 ± 5.0 ml/kg vs. 6.5 ± 4.1 ml/kg; absolute difference, 0.2 ± 1.1 ml/kg [95%CI, -2.4 – 2.1], $P=0.89$). Change in plasma volume over time did not differ between the slow and rapid infusion groups (970 ± 119 ml*min/kg and 1226 ± 75 ml*min/kg, $P=0.08$). Number of complications did not differ between the slow and rapid infusion groups and was 8 and 6 in respective group ($P=0.77$).

Conclusions: A slow infusion of 5% albumin does not result in better plasma volume expansion than a rapid infusion in postoperative patients with suspected hypovolemia.

Key words: fluid therapy, plasma substitute, serum albumin

Trial registry number: EudraCT 2013-004446-42

INTRODUCTION

The systemic inflammatory response syndrome (SIRS) disrupts the normal regulation of transcapillary fluid exchange with tissue edema and hypovolemia as a consequence.¹ Fluid therapy is therefore a cornerstone in the perioperative treatment of patients subjected to major surgery as well as in patients suffering from increased vascular leakage of other etiologies. Although fluid therapy is life saving it is also associated with side effects such as further edema formation and compartment syndromes, which may impair organ function.^{2,3} Several studies indicate that such side effects may adversely affect outcome both in postoperative patients^{4,5} and in patients suffering from SIRS in the ICU.⁶⁻⁸

From a clinical perspective it is therefore important that the fluid administered to counteract hypovolemia as far as possible remains intravascular. Colloids are macromolecules for which the vessel wall has a low permeability and less volume is required for an equal plasma volume expansion compared to crystalloids.^{9,10} However, extravasation of colloids is not only a function of the vessel wall permeability, but is also dependent on the volume of fluid that is filtered across the capillary wall, which in turn depends on the trans-capillary hydrostatic pressure.¹¹ This indicates that a slow rate of infusion may reduce extravasation of colloids by minimizing transient hypervolemia and transient increases in hydrostatic pressure. This hypothesis is supported by studies in rodent models of sepsis showing that plasma volume expansion in experimental sepsis is greater after a slow infusion compared to a rapid infusion of the same volume of colloid.^{12,13}

Fluid administration is one of the most common therapeutic interventions in intensive care units and in postoperative units around the world and a fluid bolus is a recommended method to assess fluid responsiveness.¹⁴ However, recent surveys show that infusions rate of

resuscitation fluids are highly variable¹⁵ and no study has addressed the importance of infusion rates on plasma volume expansion in a clinical setting. While rapid correction of suspected hypovolemia makes intuitive sense, the need for further knowledge in this aspect of fluid resuscitation was highlighted by the recent FEAST trial showing a surprising increase in mortality following resuscitation using fluid boluses compared to less aggressive fluid resuscitation.¹⁶

Based on these considerations the primary objective of this study was to test the hypothesis that plasma volume expansion by a given volume of colloid is greater if fluid is administered slowly rather than rapidly.

METHODS

We conducted a single center, prospective randomized physiological trial of albumin administration at two different infusion rates to patients with suspected hypovolemia following major abdominal surgery. The study was approved by the regional ethical vetting board (# 2014/15), and was conducted at Skåne University Hospital, Lund, Sweden. Written informed consent was obtained from all subjects. The protocol was amended two times. The first amendment extended inclusion criteria to include postoperative patients after major gynecological cancer surgery (open ovarian and endometrial debulking surgery) to promote recruitment. The second amendment changed the interim analysis plan by adding the Haybittle-Peto approach for testing of efficacy. In addition, vasopressor and inotropic therapy was omitted as exclusion criteria. The first amendment was performed after 1 patient had been included and the second was performed after 24 patients had been included. The study was registered in the European Clinical Trials Database (EudraCT 2013-004446-42) and was

was authorized by the Swedish medical products agency to proceed on the 9th of February in 2014. The experimental protocol has been published.¹⁷

Inclusion criteria

Patients subjected to non-emergent Whipple operations or major gynecological cancer surgery ≥ 40 years of age were screened during the first 5 hours after admission to the post anesthesia care unit when a member of the research team was available. Inclusion criteria were: 1. Written consent. 2. Indication for fluid therapy as judged by the physician caring for the patient and at least one of the following criteria: a) positive "leg raising test" (pulse pressure increase $> 9\%$ ^{18, 19}); b) central venous oxygen saturation $< 70\%$; c) arterial lactate > 2.0 mmol/L; d) urine output < 0.5 ml/kg the hour prior to inclusion; e) respiratory variation of the inferior vena cava of more than 15% as measured by ultrasound ^{20, 21}; f) systolic blood pressure < 100 mmHg or mean arterial blood pressure < 55 mmHg.

Exclusion criteria

Patients fulfilling any of the following criteria were excluded. 1. Hypersensitivity to the active drug or the tracer. 2. Signs of postoperative bleeding. 3. History of the heart failure. 4. The physician caring for the patient considered that there were strong reasons to administrate another fluid or the same fluid, but in another way or in a different volume than stated in the protocol. 5. Pregnancy. 6. Clinical judgment by the investigator or the treating physician that the patient should not participate in the study for reasons other than described above. Predefined permanent withdrawal criteria included change of surgery procedure and the occurrence of serious adverse events.

Intra- and post-operative care of the patients

Included patients received routine pre- and intra-operative care. Briefly, anesthesia was induced intravenously using propofol and maintained using either sevoflurane or desflurane. Patients received an epidural catheter for intra- and post-operative analgesia unless contraindicated. Crystalloids and colloids were used as resuscitation fluids intra-operatively at the discretion of the attending anesthetist. A hemoglobin level of 80–90 g/L was the transfusion trigger. Analgesics were given as needed during the intervention phase of the study, and the rate of vasoactive agents was adjusted to maintain mean arterial pressure >65 mmHg. No fluid other than maintenance (2.5% or a 5% glucose with electrolytes at a rate of 1 ml/kg/h) and study fluids was given during the intervention period. The trial was audited by external monitors. Auditing included source data verification.

Intervention

Eligible patients were randomized as described previously [17] to receive 5% albumin at a dose of 10 ml/kg (ideal body weight ²²) in either 30 minutes or in 180 minutes.

Outcomes

The primary outcome was change in plasma volume from start to 180 minutes after the start of albumin infusion. Secondary outcomes were change in area under the plasma volume curve from start to 180 minutes after start of infusion of albumin and incidence of postoperative complications up to 30 days postoperatively. Other outcomes were transcapillary escape rate (TER) for albumin and changes in hemodynamic parameters, plasma concentration of hormones involved in fluid homeostasis, glycocalyx components and diuresis.

Measurements

Plasma volume was measured using ^{125}I -Human Serum Albumin (HSA) before start of the albumin infusion, at 30 minutes and at 180 minutes after start of the infusion and the change in area under the plasma volume curve was calculated using the trapezoid rule.^{17, 23} TER for albumin is a measure of leakage of albumin from microvessels into the interstitium and was measured after the last injection of ^{125}I -HSA at 180 min.²⁴⁻²⁷

Plasma concentrations of glypican-4 (Cloud-Clone Corp), hyaluronan (Echelon Biosciences), and Syndecan-1 (Diacclone), renin (IDS), copeptin (Brahms GmbH) and MR-proANP (Brahms GmbH) were determined by immunologic assays according to the manufacturer's instructions. Blood gas analysis and determination of hematocrit and plasma lactate were performed using a blood gas analyzer (Radiometer 850, Radiometer).

Statistical analysis

Published values of standard deviations for plasma volume measured using ^{125}I -HSA are in the range 4-7 ml/kg.²⁸ In an experimental study plasma volume expansion was found to be 6 ml/kg greater after slow administration of 12ml/kg of 5% albumin compared with a bolus dose of the same volume. To detect a 4 ml/kg difference in volume expanding effect following administration of 5% albumin, assuming that the standard deviation of the plasma volume is 5²⁸, about 30 patients in each group is required to obtain a power of 80% using a Student's t-test. To adjust for the possibility that patients did not complete the protocol, we aimed to include 35 patients in each arm. Statistical analysis was performed blinded to treatment allocation and was performed per protocol as described previously.¹⁷

RESULTS

Patients

A total of 70 patients were enrolled between the 18th of June 2014 and the 22nd of November 2016 and 35 patients were assigned to each treatment. Prior to the second amendment one patient that had given consent was not included due to ongoing vasopressor therapy. A total of 34 patients receiving the slow infusion and 31 patients receiving the rapid infusion were included in the analysis. For a CONSORT flowchart of patients see Figure 1. Pre-treatment characteristics are presented in Table 1. The two most common criteria for fluid administration were a positive passive leg raising test and an elevated arterial lactate. Baseline plasma volume was 47.9 ± 10.3 ml/kg and 47.5 ± 6.3 ml/kg in the slow and rapid groups, respectively (Figure 2). This corresponded to a calculated blood volume of 74.1 ± 15.9 ml/kg and 72.9 ± 10.3 ml/kg in the slow and rapid groups, respectively.

Outcomes

The increase in plasma volume from start to 180 minutes after start of infusion did not differ between the different infusion rates and was 6.7 ± 5.0 ml/kg and 6.5 ± 4.1 ml/kg in the slow and rapid infusion groups, respectively (absolute difference, 0.16 ± 1.1 [95%CI, -2.4 - 2.1], $P=0.89$). Change in area under the plasma volume curve over time did not differ between the different infusion rates and was 970 ± 119 ml*min/kg and 1226 ± 75 ml*min/kg, respectively in the slow and rapid groups, respectively (absolute difference, 256 ± 141 ml*min/kg [95%CI, -32 - 543], $P=0.08$) (Table 2). The number of patients with postoperative complications in the slow and rapid infusion groups were 8 and 6, respectively, and did not differ between the groups ($P=0.77$) (Table 2). Urine production was lower in the slow than in the rapid group whereas no treatment effect on TER, lactate, Hct or any of the hemodynamic parameters could be detected (Table 2).

Plasma concentration of the stable precursor fragment of ANP, mid regional pro-Atrial Natriuretic peptide (MR-proANP), increased more from start to 180 min after start of infusion in the rapid infusion group than in the slow infusion group whereas renin and copeptin, the latter reflecting vasopressin release, did not differ between the groups (Table 2). Glypican-4, a circulating component of the endothelial glycocalyx, increased more in the slow infusion group than in the rapid infusion group whereas no difference in the change in hyaluronan and syndecan-1 could be detected (Table 2).

The pre-planned sensitivity analysis demonstrated that a low baseline blood volume was associated with a higher increase in plasma volume from start to the 180 min time point (2-way ANCOVA, $P=0.003$, Supplement figure 2). The sensitivity analysis did not demonstrate an interaction between baseline blood volume and treatment effect ($P=0.38$, 2-way ANCOVA) or between type of surgery and treatment effect ($P=0.89$, 2-way ANCOVA). To further explore if the treatment effect was dependent on baseline blood volume, patients with baseline blood volume above and below median, respectively, were analyzed separately and the results aligned with the ANCOVA results (Supplement figure 3). Treatment effect in Whipple and gynecological surgery patients was also analyzed separately and no difference in the primary outcome could be demonstrated in either of the groups (Supplement figure 4). On a post hoc basis the correlation between changes in the area under the plasma volume curve and urine production was analyzed to further assess if the higher urine production reflected differences in change in preload. No interaction could be demonstrated (Pearson $r: 0.16$, $P=0.20$, Supplement figure 5).

DISCUSSION

No evidence was found to support the hypothesis that infusion rate of 5% albumin influences plasma volume expansion in patients with signs of hypoperfusion after major abdominal surgery. Moreover, infusion rate did not influence vascular leak, inadequate tissue perfusion or hemodynamic parameters. Urine production was lower and MR-proANP concentrations increased less in the slow infusion group than in the rapid infusion group.

The volume of fluid used in the present study is within the range of that used in previous studies investigating hemodynamic effects of fluid bolus therapy.^{15, 16, 29-31} Also, the rate of infusion in the rapid infusion group agrees with that commonly used for a fluid bolus^{25, 29-31} whereas rate of infusion in the slow infusion group was based on previous experimental studies and institutional practice.^{12, 13} Fluid boluses are commonly used to correct suspected hypovolemia in hemodynamically unstable patients and presumed benefits include a rapid correction of hypovolemia. In a recently published survey on global ICU fluid resuscitation practices in 2014, it was reported that about 22% of all fluid resuscitations in surgical ICU patients were performed using albumin.³² This aligns with a consensus statement on perioperative fluid therapy suggesting use of both crystalloids and colloids for major surgery³³ and by the suggestion in the Surviving Sepsis Campaign to consider use of albumin if the initial crystalloid resuscitation fails to establish hemodynamic stability. Note that the average patient in the present study had received about 4300 ml of crystalloid and 500 ml of colloid prior to inclusion. Taken together the above support the relevance of investigating the physiological response to administration of albumin.

As mentioned in the introduction, experimental data from a rat sepsis model suggest that plasma volume expansion for a given volume of colloid may be reduced when using a bolus

compared a slower infusion.^{12, 13} Our results of no difference in the primary outcome, ie change in plasma volume from start to 180 min after start of infusion, and a trend towards a higher area under the plasma volume curve in the rapid infusion group suggest that in this clinical setting a bolus approach, if anything, may result in a better plasma volume expansion the first hours after infusion. The reasons for the differences in results include species differences, but could also be related to etiology and/or severity of the SIRS reaction.

Plasma volume expansion of 5% albumin as a fraction of infused volume is shown to be in the range of 50-110 % immediately after infusion in previous studies using a similar methodology in postoperative or in septic patients.^{9, 10, 34} Our results extend these previous findings and show that the volume expanding effect persists for at least 2.5 hours after termination of infusion. In addition, the results from the sensitivity analysis suggest that the wide range of potencies for 5% albumin as a plasma volume expander reported previously, at least partly, may be explained by differences in baseline blood volumes.

Previous data have shown that transient hypervolemia induced by rapid administration of colloids may induce shedding of components of the endothelial glycocalyx, which is in turn associated with reduced endothelial barrier function.^{35, 36} We therefore hypothesized that rapid infusion could induce an increase in shed glycocalyx components and endothelial permeability. Surprisingly, a rapid infusion decreased plasma concentrations of glypican-4, but had no detectable effect on plasma concentrations of syndecan-1 and hyaluronan. Given that increased plasma glypican-4 has been shown to be associated with septic shock (unpublished observations) it could be hypothesized that rapid infusion was beneficial for endothelial integrity, potentially decreasing vascular leak of macromolecules. However, our result that transcapillary escape rate for albumin did not differ between the groups indicates

that such an effect, if present, is small. Our results align with results presented in a recent study in porcine endotoxemia in which different infusion rates of 5% albumin did not influence extravasation of albumin.³⁷

The result that diuresis was higher in the rapid infusion group is of interest given the emphasis that is placed on urine production both as an indication for fluid administration and to evaluate response to fluid.^{15, 19} The trend towards a higher area under the plasma volume curve in the rapid infusion group could be taken to indicate that the increased diuresis reflects a better preload during the observation period. However, the absence of correlation between change in area under the plasma volume curve and diuresis does not support such a notion and suggests that mechanisms other than differences in preload over time contribute to this result (see supplement figure 5). As indicated by our result of a higher plasma concentration of the stable ANP precursor fragment MR-pro ANP³⁸ such a mechanism could be release of endogenous diuretic hormones as a result of a transient increase in wall stress of the heart in the rapid infusion group.

Limitations and strengths

While the study was adequately powered to detect a treatment effect on the primary outcome we cannot exclude that an effect on the area under plasma volume curve could have been detected if the number of patients had been increased. Moreover, the observation time after administration of fluid was short and it is also possible that hemodynamic effects could have been detected at later time points.

The tissue trauma induced by major surgery initiates a stress response and a SIRS reaction that shares many similarities with sepsis including endothelial dysfunction and disruption of

microvascular function.^{39, 40} However, the low incidence of vasopressor use in our population in spite of epidural analgesia indicate a preserved vascular reactivity and suggest a less severe SIRS reaction compared to that observed in septic shock. Thus, the generalizability to patients with a more severe disturbance of vascular homeostasis such as in septic shock is uncertain. Similarly the results cannot be generalized to other resuscitation fluids such as crystalloids and hyperoncotic albumin solutions.

Strengths of the study include the low risk of bias due to the randomized design, publication of the study protocol and the blinded analysis of the outcomes. Moreover, the use of the gold standard for measurement of plasma volume²³ and auditing support the reliability and scientific integrity of the data.

Conclusions

The present study does not support our hypothesis that a slow infusion of a colloid results in a greater plasma volume expansion. The increased diuresis observed in the rapid infusion group is associated with increased secretion of atrial natriuretic factor. Whether different rates of fluid administration have long-term consequences on hemodynamics and fluid balance should be investigated in future studies.

DECLARATION OF INTERESTS

None of the authors have any completing interests.

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AUTHOR CONTRIBUTIONS

Conception and design: S.S., B.B., P.B.

Data collection: S.S., J.B., E.L., P.K., B.T., J.F., P.B.

Statistical analysis: S.S., C.Ö., P.B.

Drafting of manuscript: S.S., P.B.

Editing and approval of manuscript: All authors

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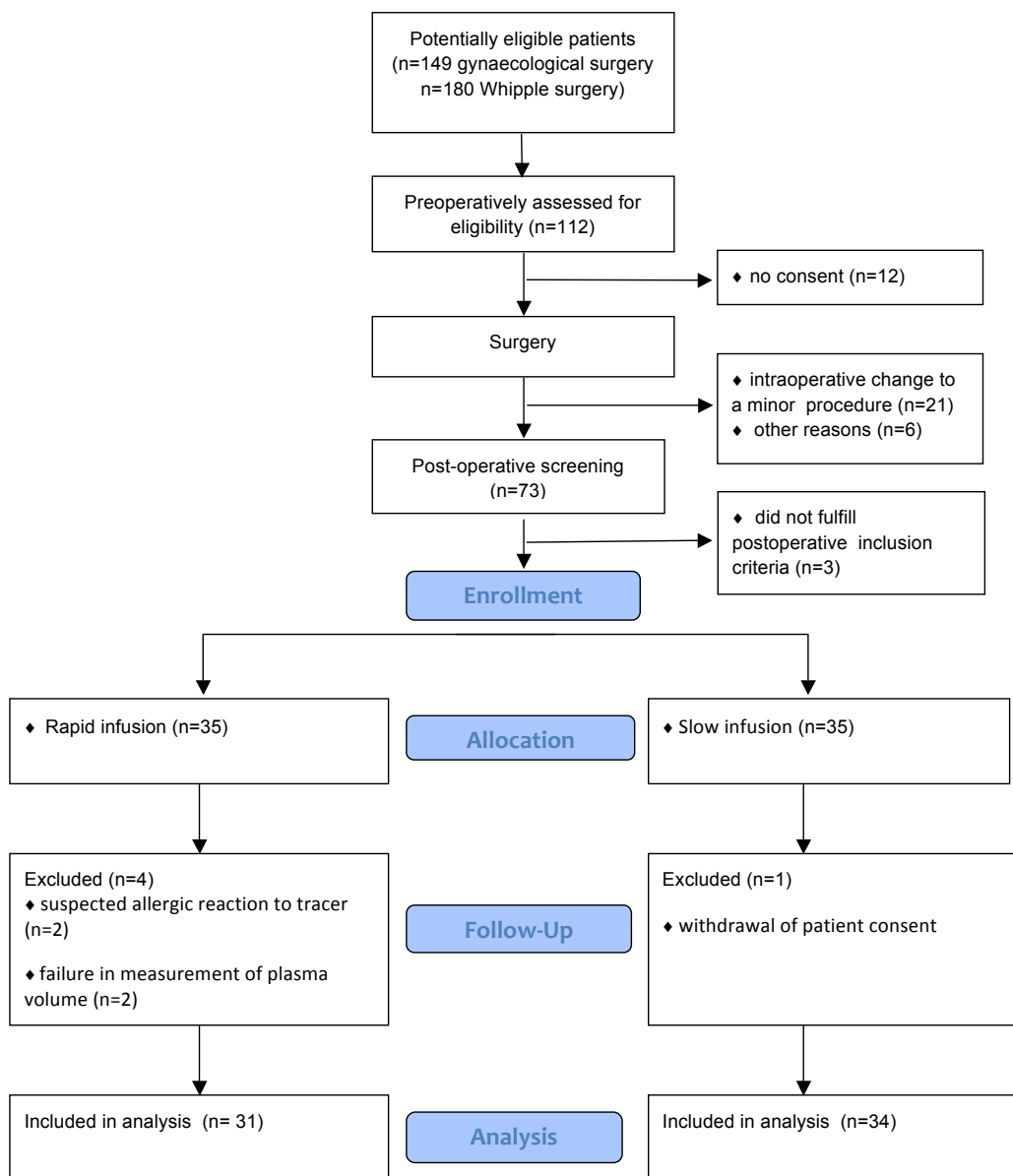


Fig. 1. CONSORT flowchart.

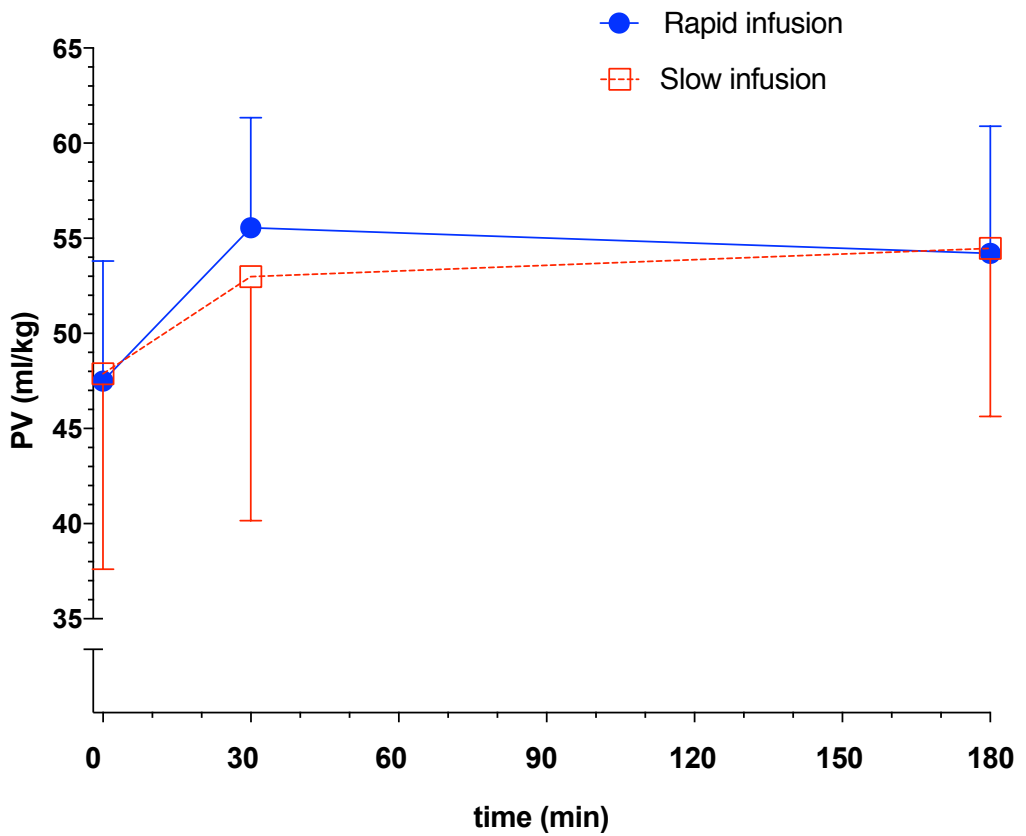


Fig. 2. Plasma volumes in the slow and the rapid infusion groups. Data are shown as mean and SD. Error bars for the rapid group points up and error bars for slow group points down.

Table 1. Demographics

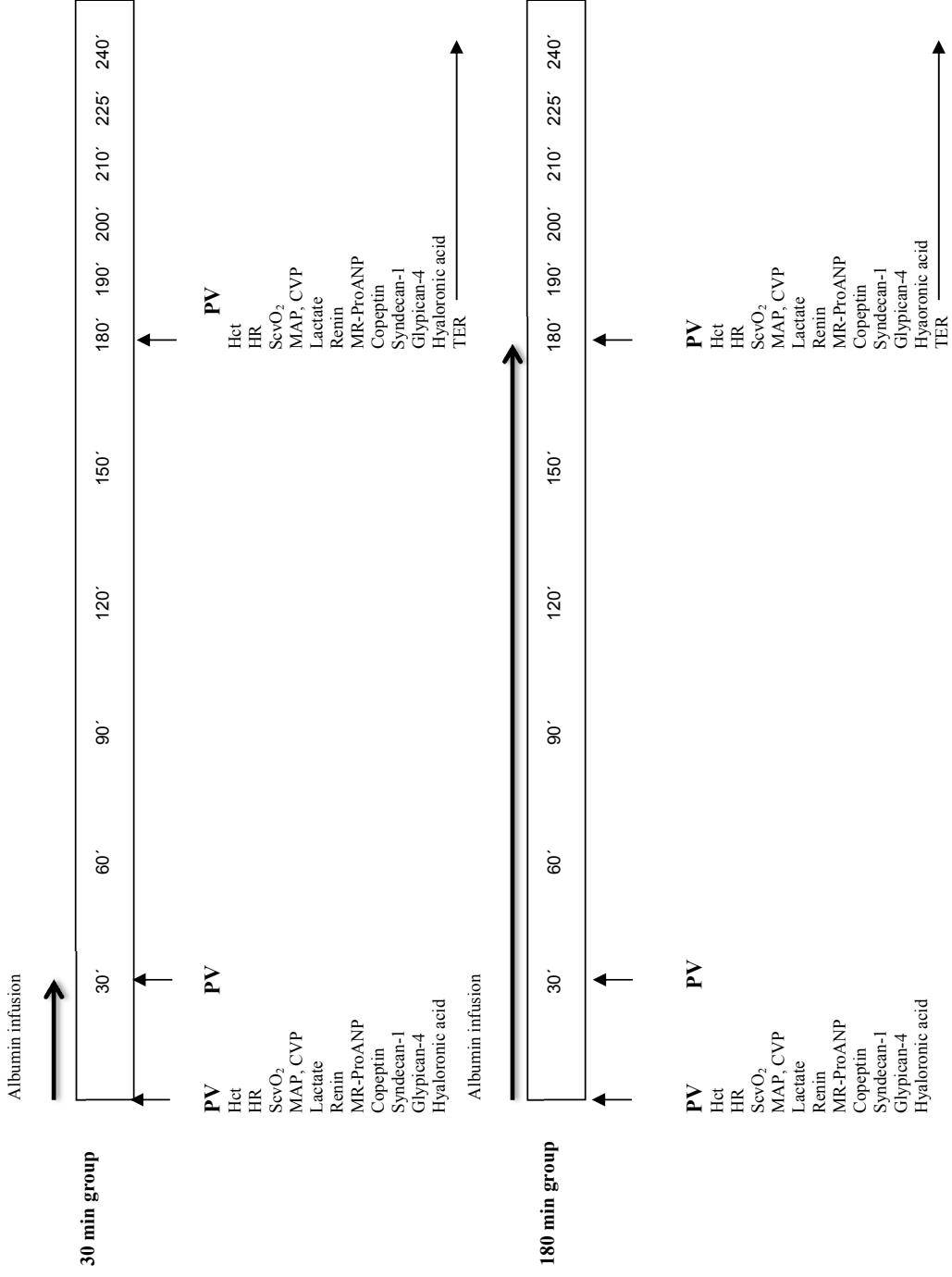
	Slow infusion (n=34)	Rapid infusion (n=31)
Gender (female)	26 (76)	16 (52)
Age, years	69 (65 - 74)	68 (55 - 74)
BMI, kg/m ²	25.4 (22.9 - 29.4)	25.3 (22.8 - 27.2)
ASA class 1	0	2 (6)
ASA class 2	22 (65)	21 (68)
ASA class 3	12 (35)	8 (26)
P-POSSUM		
Estimated morbidity, %	81.7 (70.9 - 94.4)	79.7 (67.5 - 89.4)
Estimated mortality, %	8.1 (4.9 - 20.1)	7.8 (4.1 - 13.6)
Inclusion criteria		
PLR	24 (71)	20 (65)
ScvO ₂	10 (29)	9 (29)
Lactate	21 (62)	20 (65)
Urine production	13 (38)	13 (42)
Systolic blood pressure	10 (29)	8 (26)
Co-morbidities		
Cerebrovascular	1 (3)	4 (13)
Lung disease	4 (12)	2 (6)
Insulin dependent diabetes	3 (9)	0
Ischemic heart disease	1 (3)	0
Malignancy		
Suspected	34 (100)	31 (100)
Confirmed	32 (94)	30 (97)
Operation time, min	366 (278 - 476)	418 (365 - 467)
Intraoperative bleeding, ml	550 (300 - 1000)	500 (300 - 1000)
Epidural analgesia	30 (88)	30 (97)
Intraoperative fluids		
Crystalloids, ml	4325 (4000 - 5250)	4250 (3500 - 5250)
Colloids, ml	500 (500 - 1000)	700 (250 - 1000)
Pre-treatment hemodynamics		
HR, beats per minute	85 (74 - 93)	88 (71 - 97)
Systolic blood pressure, mmHg	113 (95 - 124)	112 (99 - 129)
MAP, mmHg	75 (67 - 88)	77 (68 - 88)
CVP, cmH ₂ O	3 (0 - 7)	3 (-1 - 8)
Urine production, ml/kg/h	0.9 (0.4 - 2.1)	0.8 (0.5 - 1.0)
Pre-treatment laboratory data:		
Lactate, mmol/L	2.2 (1.6 - 3.0)	2.7 (1.5 - 3.2)
ScvO ₂ , %	73 (67 - 75)	73 (68 - 78)
Hct (%)	36 (34 - 38)	35 (32 - 39)
Glycocalyx components and hormones:		
Hyaluronan, ng/mL	158 (126 - 205)	174(132 - 208)
Syndekan-1, ng/mL	57 (33 - 102)	91 (54 - 190)
Glypican-4, ng/mL	13 (10 - 18)	13 (10 - 21)
Copeptin, pmol/L	133 (72 - 239)	113 (71 - 207)
MR-proANP, pmol/L	85 (63 - 108)	94 (71 - 139)
Renin, mU/L	44 (23 - 141)	40 (19 - 131)
Infusion of norepinephrine at inclusion	4 (12)	1 (3)

Data are presented as number (percent) or median (IQR). Abbreviations: IQR= interquartile range, BMI= body mass index, P-POSSUM= (Portsmouth) Physiological and Operative Severity Score for the enUmeration of Mortality and Morbidity, PLR= passive leg raising, ScvO₂= central venous saturation, HR= heart rate, MAP= mean arterial pressure, CVP= central venous pressure, BE= base excess, ScvO₂= central venous saturation, Hct= hematocrit, MR-pro-ANP = mid-regional pro-atrial natriuretic peptide.

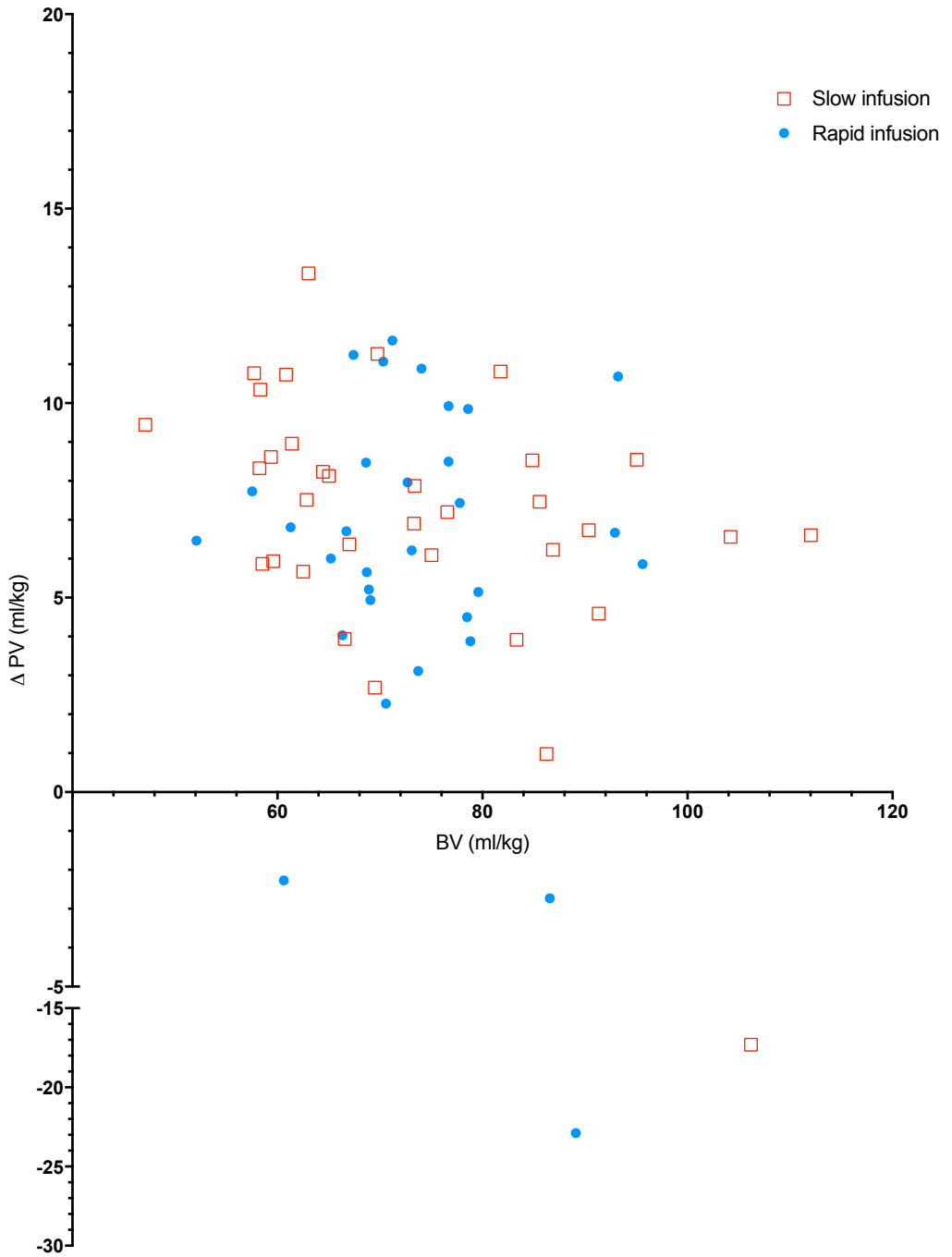
Table 2. Secondary and other outcomes

	Slow infusion (n = 34)	Rapid infusion (n = 31)	Absolute difference/risk reduction (95% CI)	P Value
Secondary outcomes				
Change in plasma volume over time, ml/ml/kg	970 ± 119	1226 ± 75	256 (-32 - 543)	P = 0.08
Post-operative complications, number	8	6	2 (0.6 - 1.6)	P = 0.766
Other hemodynamic outcomes				
TER, %	5.2 ± 2.9	5.2 ± 3.0	0.1 (-1.0 - 1.5)	P = 0.95
ΔHR, beats/min	-1 ± 11	0 ± 10	0 (-5 - 5)	P = 0.81
ΔSevO ₂ , %	0 ± 6	2 ± 9	2 (-3 - 6)	P = 0.99
ΔHet, %	-4 ± 2	-4 ± 2	0 (-1 - 1)	P = 0.82
ΔMAP, mmHg	4 ± 12	4 ± 13	0 (-5 - 6)	P = 0.96
ΔCVP, cmH ₂ O	2 ± 3	1 ± 3	1 (-2 - 1)	P = 0.32
Δlactate, mmol/L	-0.4 ± 0.8	-0.2 ± 1.2	0.2 (-0.3 - 0.7)	P = 0.71
Urine production 0-180 min, ml/kg/h	0.7 ± 0.3	1.1 ± 0.8	0.4 (0.1 - 0.7)	P = 0.009
Glycocalyx components and hormones				
ΔHyaluronic acid	-4.6 ± 9.7	15.3 ± 14.9	19.9 (-14.8 - 54.6)	P = 0.26
ΔSyndekan-1	15.3 ± 13.3	20.3 ± 17.5	5.1 (-38.4 - 48.5)	P = 0.82
ΔGlypican-4	1.1 ± 0.9	-2.4 ± 1.5	3.5 (-7.3 - 0)	P = 0.048
ΔCopeptin, pmol/L	-73.9 (-148.6 - [-27.4])	-59.3 (-129.8 - 40.6])	14.6 (-29.9 - 33.8)	P = 0.88
ΔRenin mU/L	-14.3 (-55.1 - [-5.3])	-22.4 (-78.9 - [-7.4])	8.1 (-24.1 - 6)	P = 0.36
ΔMR-proANP, pmol/L	20.6 (9.2 - 34.6)	45.1 (30.5 - 71.5)	24.5 (12.6 - 34.8)	P < 0.001

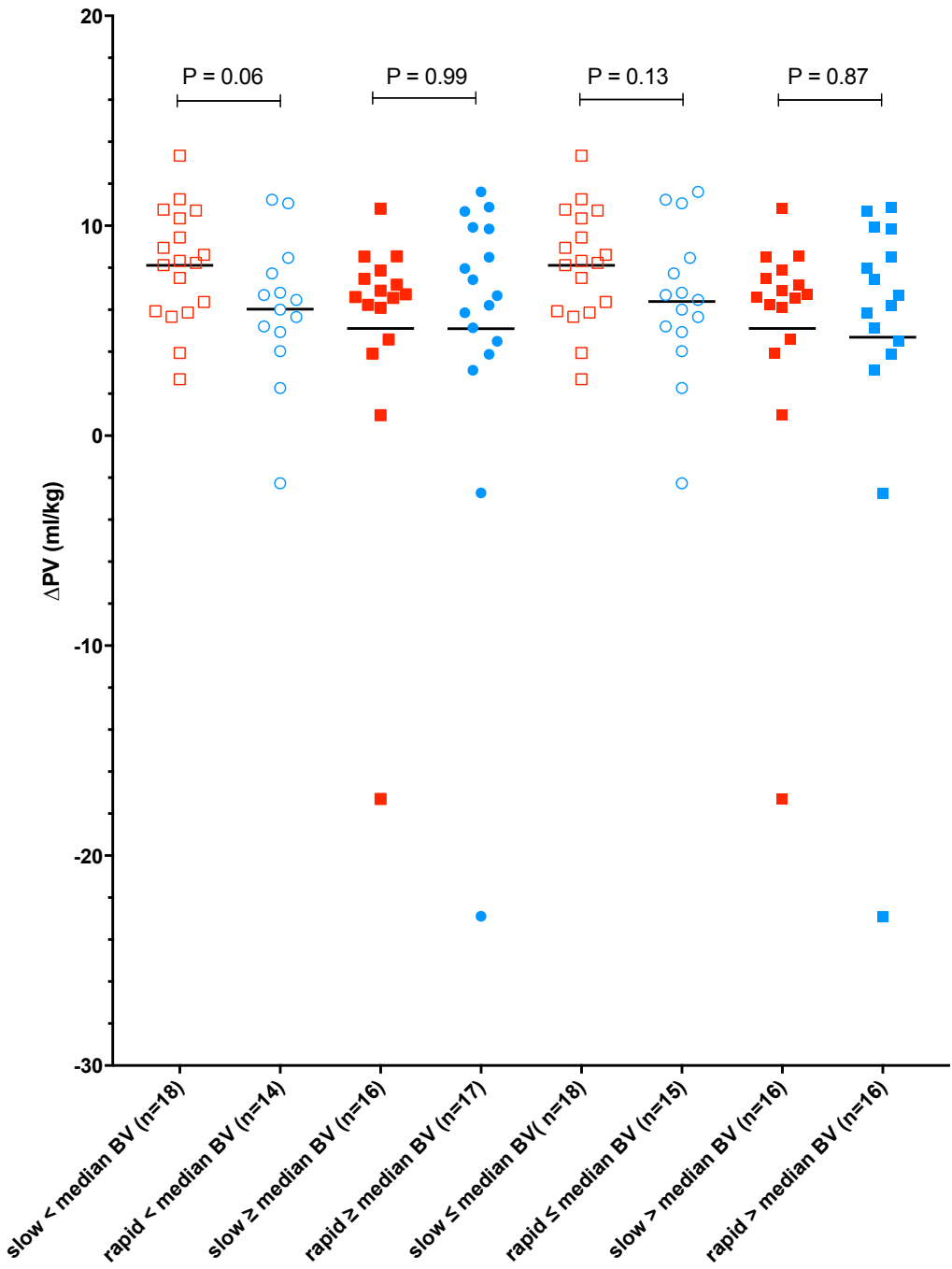
Data are presented as mean ± SD or median with IQR. Abbreviations: TER = transcapillary escape rate, Δ = change from baseline to 180 after start of infusion, HR = heart rate, SevO₂ = central venous saturation, Het = hematocrit, MAP = mean arterial pressure, CVP = central venous pressure, MR-pro-ANP = mid-regional pro-atrial natriuretic peptide. Fisher's exact test and Student's t-test were used for the analysis as appropriate.



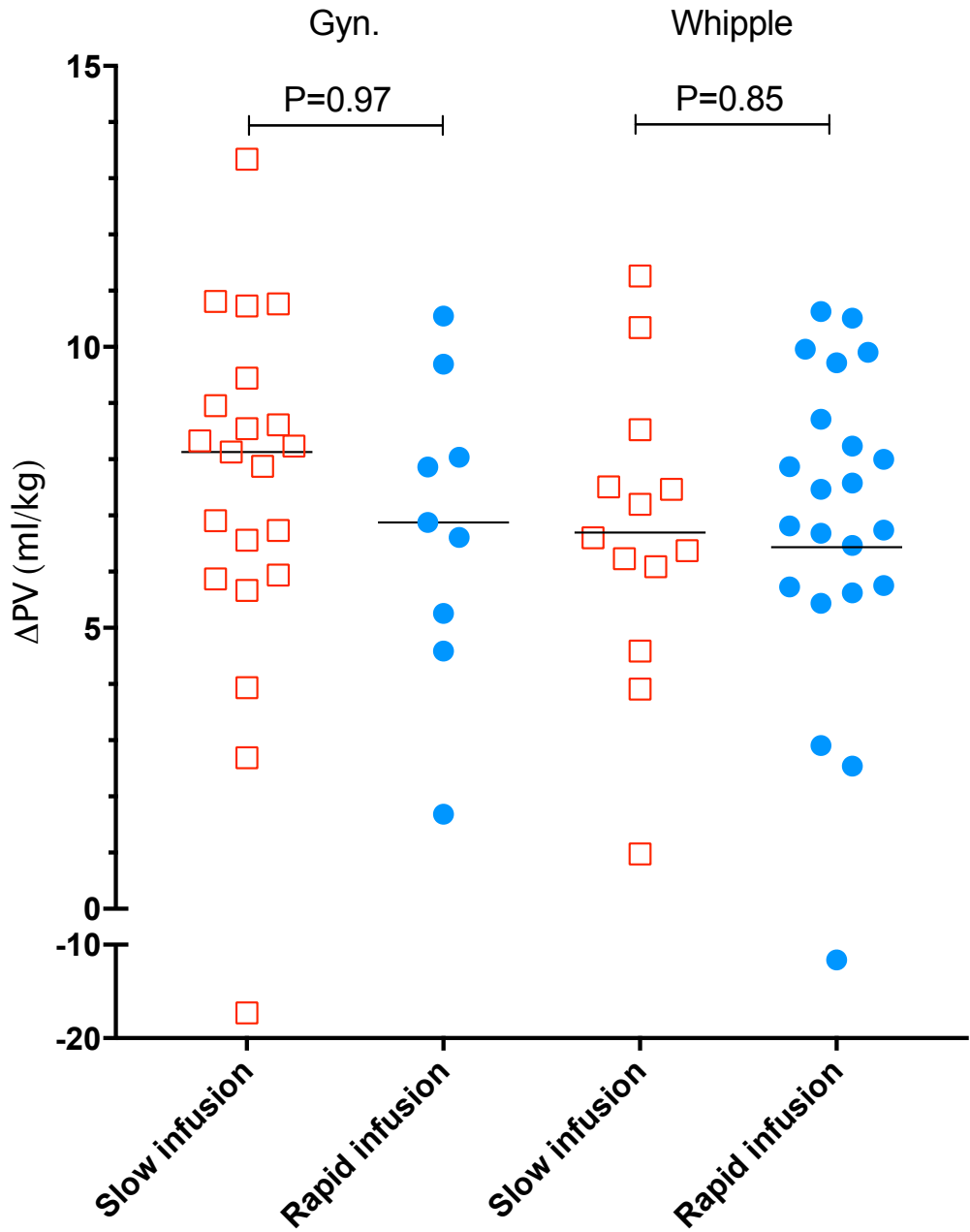
Supplement fig. 1. Overview of the study protocol. PV = plasma volume, Hct = hematocrit, HR = heart rate, SevO₂ = central venous oxygen saturation, MAP = mean arterial blood pressure, CVP = central venous pressure, TER = transcapillary escape rate for albumin.



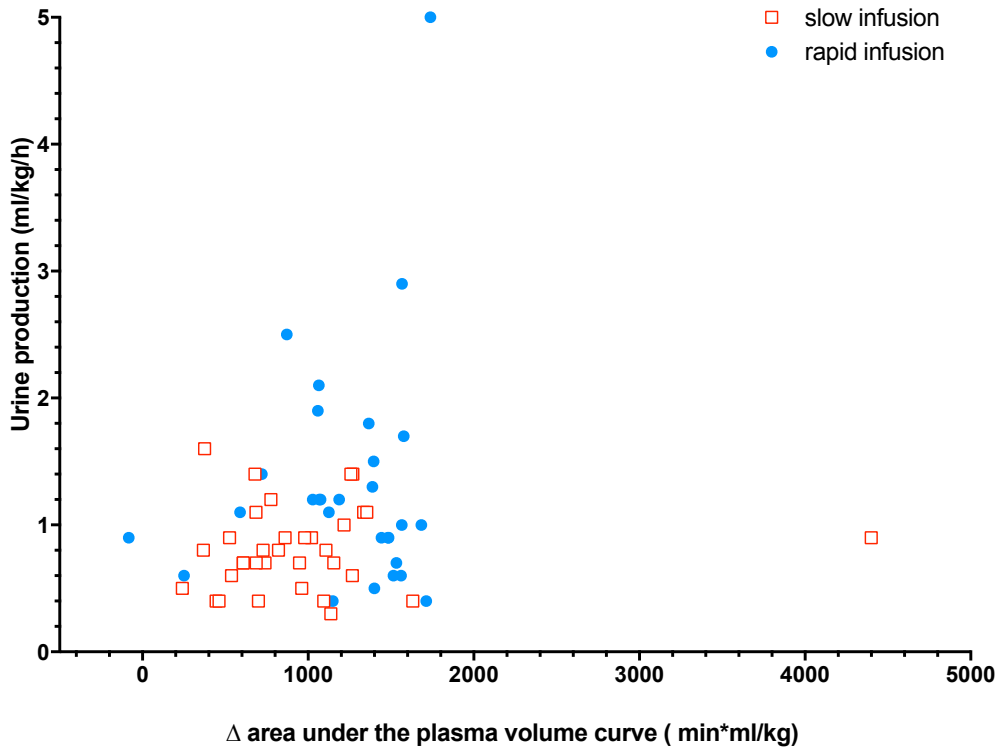
Supplement fig. 2. Plot of change in plasma volume from start to 180 minutes after start of the infusion of albumin in respective treatment groups.



Supplement fig. 3. Change in plasma volume from start to 180 minutes after start of the infusion of albumin in patients with baseline blood volume above or below median. Because the cohort could not be divided in two equally large groups results for both possible divisions are presented. Outcomes within each baseline blood volume were analysed using unpaired Student's test without adjustment for multiple comparisons.



Supplement fig. 4. Change in plasma volume from start to 180 minutes after start of the infusion of albumin in which data for patients subjected to Whipple or major gynaecological cancer surgery (Gyn.) are presented separately. Outcomes within each type of surgery were analysed using unpaired Student's test without adjustment for multiple comparisons.



Supplement fig. 5. Diuresis from start to 180 minutes after start of the infusion of albumin as a function of change in area under the plasma volume curve.

