Title: **An assessment of possible interventions to optimise haemodynamics in patients receiving major surgery**

Fumitaka Yanase MD

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Australian and New Zealand Intensive Care Research Centre (ANZIC-RC)
School of Public Health and Preventive Medicine
Faculty of Medicine, Nursing and Health Sciences
Monash University, Melbourne, Australia

Primary supervisor: Professor Rinaldo Bellomo
Secondary supervisor: Professor Michael Bailey

Table of Contents

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Notice1

Summary

Haemodynamic instability, typically represented by hypotension, is common in patients after surgery. Intravenous fluid (crystalloid and/or colloid) and vasopressor and/or inotropic drugs are the most common treatments for such hypotension. The goal of such treatment is to optimise haemodynamics (blood pressure, heart rate, central venous pressure, and cardiac output) in order to maintain patient safety and improve vital organ perfusion. However, there are very few controlled studies of these intervention to demonstrate their physiological efficacy and new interventions have not been consistently and widely considered or explored in this field (Chapter 1). Therefore, as part of this thesis, I led the conduct of several controlled studies in this field. The aim was to define the physiological effects of different approaches to fluid therapy and their impact on haemodynamics and to explore novel strategies to modulate the physiological response to surgery with the goal of improving haemodynamic and clinical outcomes.

Firstly, I led an examination of the haemodynamic effects induced by body temperature albumin infusion compared with room temperature albumin infusion in patients after cardiac surgery (warm vs. cold 4% albumin, and warm vs. cold 20% albumin [Chapter 2 and 3]). This assessment was performed because warm fluid infusion had been reported to induce greater increases cardiac index (cardiac output divided by body surface area) than room temperature fluid in healthy volunteers.

Secondly, as slower fluid infusion is theoretically beneficial and may induce different and longer lasting haemodynamic effects, I led a comparison of the haemodynamic

effects of slower 4% albumin infusion with those of a rapid infusion of the same fluid in patients after cardiac surgery (Chapter 4).

Thirdly, as albumin containing fluids and especially those with a high concentration of albumin are believed (but not proven) to have a longer lasting haemodyamic effect than crystalloids, I led a study to compare the haemodynamic effect of different types of fluid (crystalloid vs 4% albumin vs 20% albumin) in the same patient group (Chapter 5).

Fourthly, in addition to the fluid intervention, preliminary evidence had suggested that, in septic patients, high dose intravenous vitamin C might decrease the need for vasopressor drugs. Thus, I led a randomized double-blind study of the effect of high dose intravenous vitamin C in patients with vasoplegia after cardiac surgery (Chapter 6), and assessed its safety (Chapter 7)

Fifthly and finally, preliminary evidence had suggested that a glycocalyx protection strategy using dexamethasone and albumin in patients undergoing major abdominal surgery might achieve haemodynamic stability and decrease complications after such surgery. Thus, in addition to a systematic review of the normal range of glycocalyx biomarkers (Chapter 8), I led a multicentre randomized controlled study in patients undergoing major abdominal surgery to test whether such glycocalyx protection strategy could achieve the proposed physiological effects on haemodynamic stability and clinical benefits (Chapter 9).

In summary, in patients having major surgery, I led a substantial and novel body of work focussed on understanding the effects of modulating choice, speed and temperature of fluid therapy on haemodynamics. Moreover, I led two randomised controlled trials to explore two novel promising interventions (high dose intravenous vitamin C and glycocalyx protection therapy with dexamethasone and albumin) to optimise haemodynamic and improve clinical outcomes. The findings of my study provide the most extensive set of controlled studies in this field and deliver the necessary evidence-based evidence for continued improvements in the haemodynamc optimisation therapy of patients after major surgery.

Thesis including published works declaration

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes nine original papers published in peer reviewed journals. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the student, working within the ANZIC-RC and Department of Epidemiology and Preventive Medicine, under the supervision of Professor Rinaldo Bellomo.

Thesis Chapter	Publication title	Status	Extent of candidate's contribution	Co-authors names and co-authors' contribution	Monash students
2	Comparison of the Hemodynamic and Temperature Effects of a 500-mL Bolus of 4% Albumin at Room Versus Body Temperature in Cardiac Surgery Patients	Published	60% (concept, data collection, manuscript writing)	Laurent Bitker, Luca Lucchetta, Thummaporn Naorungroj, Salvatore L. Cutuli, Eduardo A. Osawa, Emmanuel Canet, Anthony Wilson, Glenn M. Eastwood, Michael Bailey, Rinaldo Bellomo. All contributed 4% each to the manuscript	No
3	Temperature and haemodynamic effects of a 100 ml bolus of 20% albumin at room vs. body temperature in cardiac surgery patients	Published	65% (concept, data collection, manuscript writing)	Salvatore L. Cutuli, Thummaporn Naorungroj, Laurent Bitker, Alessandro Belletti, Anthony Wilson, Glenn M. Eastwood, Rinaldo Bellomo. All contributed 5% each to the manuscript	No
4	Rapid 500 ml albumin bolus vs rapid 200 ml bolus followed by slower continuous infusion in post-cardiac surgery patients: a pilot before-after study.	Published	80% (concept, data collection, manuscript writing)	Thummaporn Naorungroj, Salvatore L Cutuli, Glenn M Eastwood, Rinaldo Bellomo. All contributed 5% each to the manuscript	No
5	A comparison of the hemodynamic effects of fluid bolus therapy with crystalloids vs. 4% albumin and vs. 20%	Published	70% (concept, data collection, manuscript writing)	Salvatore L Cutuli, Thummaporn Naorungroj, Laurent Bitker, Anthony Wilson, Glenn M Eastwood,	No

	albumin in patients after cardiac surgery			Rinaldo Bellomo. All contributed 5% each to the manuscript	
6	A Pilot, Double-Blind, Randomized, Controlled Trial of High-Dose Intravenous Vitamin C for Vasoplegia After Cardiac Surgery	Published	61% (concept, data collection, manuscript writing)	Laurent Bitker, Lara Hessels, Eduardo Osawa, Thummaporn Naorungroj, Salvatore L. Cutuli, Paul J. Young, Jay Ritzema, Georgia Hill, Charlotte Latimer-Bell, Anna Hunt, Glenn M. Eastwood, Andrew Hilton, Rinaldo Bellomo. All contributed 3% each to the manuscript	No
7	Harm of IV High-Dose Vitamin C Therapy in Adult Patients: A Scoping Review	Published	65% (concept, data collection, manuscript writing)	Tomoko Fujii, Thummaporn Naorungroj, Alessandro Belletti, Nora Luethi, Anitra C. Carr, Paul J. Young, Rinaldo Bellomo. All contributed 5% each to the manuscript	No
7	Efficacy and Safety of Parenteral High-Dose Vitamin C Therapy in Pediatric Patients: A Scoping Review	Published	70% (concept, data collection, manuscript writing)	Sainath Raman, Thummaporn Naorungroj, Avril McCarthy, Michele Cree, Luregn J. Schlapbach, Rinaldo Bellomo. All contributed 5% each to the manuscript	No
8	Glycocalyx damage biomarkers in healthy controls, abdominal surgery, and sepsis: a scoping review	Published	80% (concept, data collection, manuscript writing)	Thummaporn Naorungroj, Rinaldo Bellomo. All contributed 10% each to the manuscript	No
9	A randomized, multicenter, open-label, blinded endpoint, phase 2, feasibility, efficacy and safety trial of preoperative microvascular protection in patients undergoing major abdominal surgery	Published	60% (concept, data collection, manuscript writing)	Shervin H Tosif, Leonid Churilov, Ken Yee, Rinaldo Bellomo, Kerry Gunn, Chang Kim, Camilla Krizhanovskii, Robert G Hahn, Bernhard Riedel, Laurence Weinberg. All contributed 4% each to the manuscript	No

Fumitaka Yanase 26 August 2021

Supervisor: Rinaldo Bellomo 26 August 2021

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Chapter 1: Novel treatments to optimise haemodynamic instability patients after major surgery: optimising fluid temperature, optimising fluid infusion speed, treatment with high dose vitamin C and delivery of glycocalyx protection therapy.

Haemodynamic instability during and after major surgery, and key interventions to attenuate it.

More than 300 million surgical operations are performed every year in the world and the rate of complications remains high (1, 2). Haemodynamic instability (hypotension or tachycardia or both) is one of the major complications, which develop in the perioperative period. For example, one study involving 15,000 patients reported that intraoperative hypotension defined as a systolic blood pressure below 80 mmHg was observed in 41% of patients who underwent non-cardiac surgery (3). Moreover, hypotension defined as at least one episode of systolic blood pressure more than 20% below the pre-operative systolic blood pressure was observed in 93% of such patients. These observations are relevant to the post-operative period as well as the intraoperative period, where such hypotension is similarly very common.

Although there is no universal consensus definition of perioperative hypotension (3), a low blood pressure below baseline is associated with postoperative complications, such as acute kidney injury, brain ischemia or myocardial injury (4, 5). A systematic review concluded that when patients' mean arterial pressure is below 80 mmHg for 10 minutes or more, the risk of organ injury increases significantly (6). This study also found that the risk incrementally increased as blood pressure became progressively lower. Therefore, it is important for clinicians to understand the

mechanisms of such hypotension and to better define approaches to its prevention and to its treatment.

The pathophysiology of perioperative hypotension is likely multifactorial because, among other factors, haemodynamic status is affected by anaesthetic drugs, the patient's position during the operation, blood losses or tissue injury associated inflammation. In general, blood pressure is determined by, cardiac preload, cardiac index and cardiac afterload. Both preload and afterload are profoundly affected by vascular tone. Cardiac preload is decreased by venous vasodilation (anaesthetic drugs and surgical inflammation), decreased intravascular volume (bleeding, perioperative fasting and capillary leakiness with fluid transudation from the circulation into the interstitial tissues, possibly due to glycocalyx injury) or low venous return (high intra-thoracic pressure induced by mechanical ventilation). In addition, cardiac output may fall because of decreased heart rate or stroke volume (anaesthetic drugs or neuraxial anaesthesia). Cardiac afterload is decreased by reduced vascular tone (anaesthetic drugs and surgical inflammation) and, possibly, by endothelial glycocalyx injury (7). Therefore, to optimise haemodynamic status, clinicians have investigated several treatments including fluid bolus therapy (preload correction) or catecholamine therapy (cardiac index or cardiac afterload correction).

Crystalloid-based fluid bolus therapy

To optimise cardiac preload, crystalloid fluid bolus therapy (by rapid infusion) is widely used in most perioperative clinical settings. Crystalloids are solutions of water and electrolytes that can be isotonic, hypotonic or hypertonic compared to human plasma. Such fluids are widely used as a "maintenance fluid therapy" during and

after surgery because of their easy availability and low costs (8). There are two main crystalloid solutions that are used in the clinical setting: normal saline and balanced crystalloids. Normal saline (0.9% sodium chloride solution) is the most traditional fluid. However, because its strong ion difference is zero and its pH is acidic (pH=5.5), it is not considered “physiological” (8). On the other hand, balanced crystalloids contain buffers, such as lactate, gluconate, acetate or bicarbonate, to maintain a physiological strong ion difference and pH. Therefore, balanced crystalloids may have several advantages in terms of acid-base balance effects and, possibly, clinical outcomes.

One double-blind randomised controlled study reported that, in 60 patients undergoing major abdominal surgery, a balanced crystalloid solution (sodium 140 mmol/l, potassium 5.0 mmol/l, calcium 2.5 mmol/l, magnesium 1.5 mmol/l, chloride 108 mmol/l, acetate 45 mmol/l) showed more physiological base excess values, and a decreased requirement for vasopressors compared to normal saline (9). In ICU patients, use of balanced crystalloid (lactated Ringer’s solution or Plasmalyte) showed a reduction in the study’s composite outcome (death, renal replacement therapy or persistent renal dysfunction) compared to normal saline in a cluster cross over trial involving 15800 patients (10). Therefore, fluid bolus therapy with balanced crystalloid is widely used to treat hemodynamic instability in both the operating theatre and the ICU.

Colloid fluid bolus therapy

In fluid therapy, the term “colloid” is used to describe a solution that contains larger molecular weight solutes beyond electrolytes. These solutes (albumin or starch or

gelatin) carry an oncotic pressure effect (colloid osmotic pressure). Colloid solutions used to treat patients are composed of semisynthetic preparations (hydroxyethyl starch [HES] or gelatin) or human plasma proteins (human albumin) which, under normal conditions, are expected not to pass through semipermeable vessel walls, and, under conditions of capillary leakage, may attenuate the consequences of fluid transudation. Thus, they theoretically should achieve greater intravascular volume expansion and should restore and maintain blood pressure and cardiac output more effectively than crystalloid fluids. Therefore, some clinicians prefer to use colloid solutions for patients with high capillary leakage, such as sepsis, or in patients with oedema (11).

HES solutions are synthetic colloids, which are made from potato or corn starch, and are cheaper than human albumin solution. HES 6% (600 kiloDalton / 0.75 molar substitution) has a volume effect of 100 percent whereas that of crystalloids is only 30% (12). Therefore, HES has been widely used to treat intraoperative hypotension or to maintain intravascular volume in response to bleeding. However, HES has now been shown in randomised trials to lead to several unfavourable outcomes. In patients with sepsis, for example, HES 6% (130 kiloDalton /0.42 molar substitution) showed an increased risk of renal replacement therapy and mortality compared to Ringer's acetate solution (13). In addition, compared to normal saline, HES 6% (130 kiloDalton /0.4 molar substitution) demonstrated an increased risk of needing renal replacement therapy (14). In patients undergoing major abdominal surgery, administration of a HES 6% (130 kiloDalton / 0.4 molar substitution) versus normal saline to provide goal-directed fluid therapy did not reduce the composite outcome of death or major postoperative complications, and, again, showed harmful effects on

kidney function (postoperative acute kidney injury [relative risk 1.34, 95% CI 1.00-1.80]) (15). Due to the findings of these randomised trials, HES solutions are now restricted in North America and Europe (16) and almost never used in Australia and New Zealand (ANZ).

Compared to other colloids, human albumin is regarded as a more physiological solution. This is because albumin is a normal component of human blood. As albumin is the major determinant of plasma oncotic pressure and because it has a long half-life (15 days), 5% albumin solution has a volume effect of 70%. Worldwide, there are two different concentrations of human albumin solutions available, one is iso-oncotic albumin (4% or 5% human albumin) and the other is hyper-oncotic human albumin (20% or 25% human albumin). Hyper-oncotic albumin solution is also almost chloride free.

In addition to its proposed intravascular volume effect, albumin has other theoretical benefits, such as working as antioxidant, protecting the endothelial glycocalyx layer, or carrying endogenous or exogenous molecules (16). However, although giving albumin solutions is rational, albumin is not an initial treatment for hypotension because its cost is higher than that of crystalloids or HES solutions. However, in Australia, albumin is provided free to hospitals as a blood product. In non-randomised studies of patients undergoing cardiac surgery, use of HES and 5% albumin solution was associated with an increase of blood loss within 24 hours after the surgery possibly because of impaired haemostasis due to haemodilution (17). What is more, in a multicentre randomised control trial of ICU patients involving close to 7,000 individuals (18), use of 4% albumin could not deliver any advantage

compared to normal saline, for example, in terms of mortality or need of renal replacement therapy. In summary, although 4% albumin has some theoretical benefits, its clinical effects have not been shown to be significant.

Only a few studies have compared hyper-oncotic albumin with other fluids in patients having major surgery. This is unfortunate because hyper-oncotic albumin, may help absorb tissue oedema and achieve a great volume expansion effect with much less volume. One study showed that 200 ml of 20% albumin achieved 430 ml of volume expansion effect in patients with sepsis and 500 ml in non-septic patients (19). In a single centre, before-and-after trial, 20% albumin fluid resuscitation of patients after cardiac surgery showed a less positive fluid balance and reduced requirements for noradrenaline (20). Moreover, in patients with sepsis, 20% albumin fluid resuscitation was associated with decreased fluid requirements, a less positive fluid balance, and higher proportion of patients discharged alive from ICU (21). However, because there has not been a large, multicentre, randomised control trial, the clinical utility and physiological effects in cardiac surgery patients of hyper-oncotic albumin remain controversial.

Vasopressors, inotropes and goal directed haemodynamic therapy

Vasopressors drugs (metaraminol, phenylephrine, noradrenaline or vasopressin) and inotropes (dobutamine or milrinone) are also used to treat intraoperative hypotension. There is no study, however, that has compared vasopressors with inotropes in patients undergoing abdominal or cardiac surgery because their effects are completely different. Vasopressors increase vessel tone, induce vasoconstriction, increase cardiac afterload and increase blood pressure. In contrast,

inotropes increase cardiac contractility and, in some cases, heart rate to increase cardiac index. Therefore, to optimise haemodynamics, so-called goal directed haemodynamic management has been proposed (22).

Goal directed haemodynamic management was introduced because of the notion that postoperative complications may be related to inadequate oxygen delivery and tissue hypoperfusion (23). The concept behind goal directed haemodynamic management is that it is vital to achieve adequate oxygen delivery by appropriate haemodynamic management. Figure 1 shows one example of goal directed therapy (22). The first step is to give fluid bolus therapy (FBT) to optimise cardiac preload. If the patient's cardiac preload is low, stroke volume increases with FBT. When stroke volume does not change after fluid bolus, vasopressors or inotropes are commenced according to the mean arterial pressure and cardiac index.

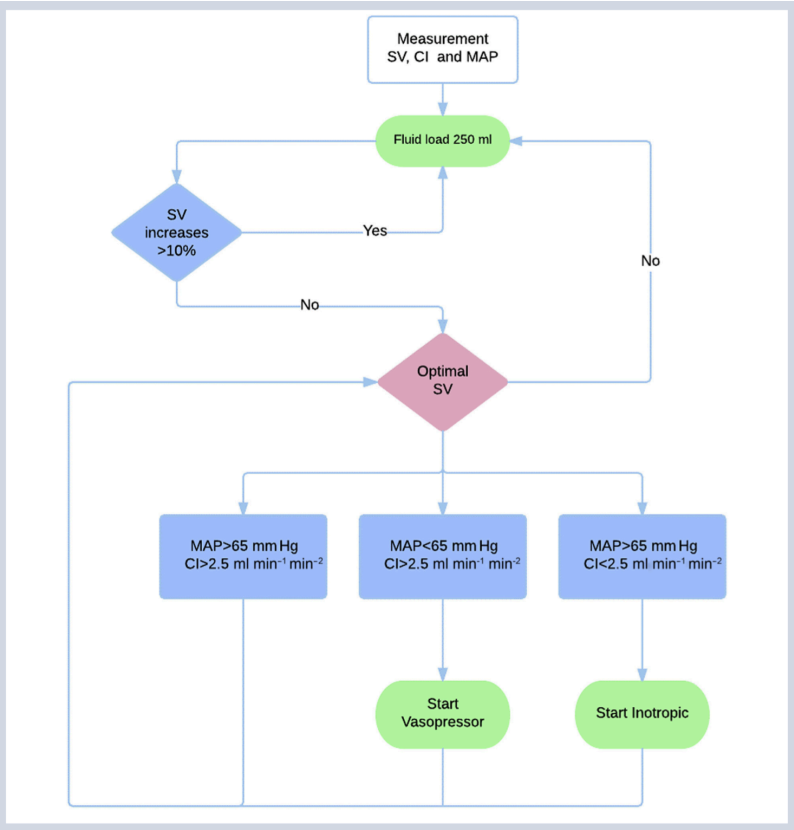

Figure 1. Example of Goal directed therapy algorithm, CI denotes cardiac index, MAP denotes mean arterial pressure and SV denotes stroke volume. (Figure from reference 22)

A meta-analysis found that perioperative goal directed haemodynamic management reduced pneumonia, acute kidney injury, wound infection and hospital length of stay (24). However, there are several limitations to this assessment. In particular, this meta-analysis included different types of surgery (general surgery, cardiac surgery or other surgeries), and each study used different “goal directed treatment” approaches. There was also a lack of well-defined endpoints. Finally, there were different types of fluid for fluid bolus approaches (crystalloids or colloids) and different types of invasive haemodynamic monitoring (pulmonary artery catheter, arterial waveform analysis or transoesophageal echocardiography). In addition, the largest multicentre, randomised, observer-blinded trial that compared goal directed treatment with standard treatment in high-risk patients aged 50 years or older and undergoing major gastrointestinal surgery concluded that the goal directed treatment did not reduced postoperative complications or 30-day mortality (25).

In summary, crystalloid or colloid fluid bolus therapy (preload optimisation), vasopressor (vital organ perfusion pressure optimisation, afterload optimisation), and/or inotropes (cardiac index optimisation) are used to treat perioperative haemodynamic instability and these treatments are the main components of goal directed haemodynamic treatment. However, several aspects of such therapy remain unknown. For example, if fluid bolus therapy (FBT) is given, the effects of type of fluid, fluid temperature and speed of fluid administration have not been studied. If

vasopressor therapy is administered, the effect of agents that might improve the effectiveness of such therapy (such as high dose intravenous vitamin C) have not been studied in post-surgical patients. Finally, surgery may be associated with damage to the endothelial glycocalyx and such a damage may lead to fluid extravasation into the tissues. However, glycocalyx protection strategies, which may help optimise post-surgical haemodynamic instability, have not been studied.

Warm fluid bolus and its potential haemodynamic benefits

One possible treatment to optimise the effect of fluid bolus therapy (FBT) might be to give body temperature (instead of room temperature) fluids (so called “warm” fluids). Body temperature is an important factor that regulates blood pressure or cardiac output. In intensive care units or hospital wards, intravenous fluids, such as Hartmann’s solution or 4% albumin solution, are stored at room temperature (usually 18-22 °C) and given rapidly, which possibly causes transient falls in body temperature and haemodynamic changes. However, in contrast, in many operating theatres, they are warmed and given at body temperature to avoid possible hypothermia related complications, such as surgical site infection, coagulopathy or shivering (26). These temperature changes induced by different fluid temperature might cause different haemodynamic changes in healthy volunteers or patients in ICU.

In healthy volunteers, large volume (20% of calculated extracellular volume, equivalent to 1790 to 3270 ml) infusion of cold (18 °C) Ringer’s acetate solution over 45 minutes achieved higher mean arterial pressure and lower heart rate compared to warm (36 °C) Ringer’s acetate infusion (27). Another study compared the

haemodynamic effects of 500 ml of warm (38 °C) acetated Ringer's solution infused over 15 minutes with those of cold (22 °C) acetated Ringer's solution in healthy volunteers (28). The study concluded that the cardiac index was lower (mean difference [95% confidence interval] -0.05 [-0.09 to -0.02] L/min/m^2), mean arterial pressure was higher (3.42 [2.86 to 3.97] mmHg) and heart late was lower (-2.13 [-2.62 to -1.65] /min) in the cold fluid group.

In critically ill patients, the only assessment of the haemodynamic effects of body temperature changes relate to accidental hypothermia and are not directly relevant (29). On the other hand, in older vascular surgery patients, a mean core temperature of 35.3 °C led to a lower heart rate (72 vs 78 /min, however, $P>0.05$) and a higher systolic blood pressure (160 vs 137 mmHg, $P<0.05$) compared to the warm temperature group (mean core temperature of 36.7 °C) (30). However, such studies do not address the transient hypothermia likely to be induced by cold fluid administration. In particular, no study has compared the haemodynamic effect of fluid boluses at different temperatures in patients after cardiac surgery. Therefore, as part of my thesis, we led before-after studies in such patients to assess whether a change in fluid temperature would alter the haemodynamic effect of fluid bolus therapy in cardiac surgery patients with haemodynamic instability.

In chapter 2, I report the haemodynamic effect of room temperature (cold) 4% albumin 500 ml fluid bolus therapy in patients after cardiac surgery and compare its effects with those of body temperature (warm) 4% albumin fluid bolus therapy (31). This study found that warm 4% albumin infusion achieved faster return of mean arterial pressure to target levels and higher mean pulmonary artery pressures, while

preventing the blood temperature drop seen with the administration of room temperature albumin fluid bolus therapy.

Also, I led the conduct of the first human before-and-after research that compared the haemodynamic effects of different temperature (room temperature vs. body temperature) for hyper-oncotic (20%) albumin fluid (100 ml bolus) therapy in patients after cardiac surgery (Chapter 3). Relevant to fluid bolus therapy, 20% albumin has several theoretical advantages. As it is hyper-oncotic, 20% albumin may trigger fluid movement from the extravascular space into intravascular space and could achieve significant positive haemodynamic changes with small infusion volumes. This study found that compared with room temperature 20% albumin, treatment with body temperature 20% albumin prevented the blood temperature fall associated with fluid bolus therapy, and increased mean pulmonary artery pressure. However, there was no difference in cardiac index or mean arterial pressure changes between the two groups.

Fluid infusion rate

The next potentially important variable that might affect the impact of fluid bolus therapy on haemodynamics is fluid infusion speed. Theoretically, slow fluid infusion might have advantages in achieving a higher blood pressure or cardiac index compared to fast infusion because of its differential effect on the glycocalyx. The glycocalyx layer plays an important role in maintaining intravascular volume (see Chapter 8). The glycocalyx membrane, which covers the internal wall of small vessels helps regulate fluid movements from the intravascular space to extravascular space (32). The glycocalyx is known to be damaged by the release of

atrial natriuretic peptide, which is synthesised and released from cardiac muscle cells during hypervolaemia (such as after a fluid bolus) (33). Therefore, fast fluid infusion may cause transient hypervolaemia followed by a release of atrial natriuretic peptide, which results in glycocalyx injury and fluid movement from the intravascular space to extravascular space. In contrast, slower fluid infusion might prevent this vicious cycle and could maintain intravascular volume.

In a sepsis model in rats, slow fluid infusion (over 3 hours) of 5% albumin (12 ml/kg), hydroxyethyl starch (12 ml/kg) and 4% gelatin (12 ml/kg) showed greater plasma expanding effects than fast infusion (same volumes over 15 minutes) (34). However, this study also reported that when normal saline (48 ml/kg) was given by fast speed or slow speed, there was no difference in its plasma expanding effects measured by radioisotope methods. In addition, although there was no statistical difference due to the small sample size (8 to 12 rats in each group), mean arterial pressure tended to be higher in the slow infusion group for all fluid types. Moreover, the same group of investigators reported the results of another study that focused on a septic guinea pig model (35). The authors administered 6% dextran (12 ml/kg) or 5% human albumin (12 ml/kg) by fast infusion (over 15 minutes) or slow infusion (over 3 hours). These investigators concluded that, for both fluids, (dextran and 5% albumin), slow infusion achieved a greater plasma volume expansion effects. Therefore, experimental research supports the view that slow infusion might induce greater haemodynamic changes because its plasma expansion effects should be greater.

Despite such, no human study has reported the haemodynamic effects induced by different infusion rates in cardiac surgery patients. Accordingly, I led a study of the

haemodynamic effects of different intravenous fluid infusion speed in patients after cardiac surgery (Chapter 4). Although slow fluid infusion may achieve a greater intravascular volume expansion effect compared to fast fluid infusion, it may not achieve prompt hemodynamic stability. Therefore, small rapid fluid bolus followed by continuous infusion may combine the advantage of a rapid response with those associated with a sustained effect. Thus, we conducted a before-and-after trial to compare the haemodynamic effects induced by rapid 4% albumin bolus therapy (500 ml over a few minutes) vs. 4% albumin small fluid bolus followed by continuous infusion (200 ml over a few minutes followed by 300 ml over 30 minutes) (combined group). This study showed that the overall number of MAP-responders (defined as >10% increase from the baseline) or CI-responders (defined as >15% increase from the baseline) did not differ between the two groups at 30 minutes. However, a rapid FBT of 500 ml 4% albumin achieved a greater number of immediate CI-responders, and higher overall MAP, CVP, mean PAP values than a slower combined FBT. Taken together, these findings imply that, contrary to animal studies, rapid FBT is haemodynamically superior to combined FBT.

Crystalloid vs iso-oncotic albumin vs hyper-oncotic albumin.

Other possible treatment is giving colloid solution. Crystalloids and colloids are widely used for fluid resuscitation in the operating theatre and the intensive care unit. And as we discussed, balanced crystalloids (Hartmann's solution or Plasmalyte) or human albumin are the most common fluids used in clinical practice in Australia because these fluids have fewer theoretical disadvantages compared to normal saline or hydroxyethyl starch. According to physiological theory, crystalloids are expected to have shorter intravascular half-life and move from the intravascular

space to extravascular space because they do not contain albumin or other materials that maintain oncotic pressure. On the other hand, there are two different types of human albumin solution, iso-oncotic (4%) albumin and hyper-oncotic (20%) albumin. Theoretically, 100% volume of iso-oncotic albumin remains in the intravascular space and hyper-oncotic albumin expands the intravascular space by more than 100% of infused volume because its hyper-oncotic pressure may absorb extravascular fluid back to intravascular space. However, there is no study to compare the hemodynamic effects of the three different fluid types in cardiac surgery patients in the intensive care unit. Therefore, I led a study that compared the haemodynamic effects associated with rapid infusion of 500 ml of crystalloid fluid bolus therapy vs 500 ml of 4% albumin fluid bolus therapy vs 100 ml of 20% albumin fluid bolus therapy in patients after cardiac surgery (Chapter 5). In this study, we found that mean cardiac index changes or mean arterial pressure changes were almost identical (0.4 [0.4] L/min/m^2 and 11 [10] mmHg with crystalloids, 0.4 [0.5] L/min/m^2 and 12 [9] mmHg with 4% albumin, and 0.3 [0.4] L/min/m^2 9 [6] mmHg with 20% albumin). However, there was a time-group interaction of mean arterial pressure changes between the crystalloid group and the 4% albumin group, and between the crystalloid group and the 20 % albumin group, which implied that the crystalloid group showed a faster mean arterial pressure reduction over time than the 4% or 20% albumin group. Also, patients treated with crystalloid fluid bolus therapy showed a faster decline in central venous pressure and perfusion pressure. Finally, 20% albumin fluid bolus therapy attenuated the fall in temperature induced by room-temperature crystalloid fluid bolus therapy.

Use of high-dose intravenous Vitamin C and its rationale for haemodynamic instability

Another possible treatment of haemodynamic instability is one that might increase vessel responsiveness to endogenously released vasoactive hormones such as noradrenaline and vasopressin. Such an effect has been proposed for high dose intravenous vitamin C. Thus, vitamin C (ascorbic acid) is a water soluble micronutrient that for several reasons could be used to treat haemodynamic instability secondary to vasoplegia. As humans cannot synthesise endogenous vitamin C, they need to ingest exogenous vitamin C to prevent vitamin C deficiency or scurvy (36). On the other hand, animals with L-gulono-γ-lactone oxidase can synthesise endogenous vitamin C. For example, goats are known to synthesise vitamin C (2-4 g/day) with their livers and can increase vitamin C synthesis significantly under stress conditions (37). However, as humans cannot synthesise endogenous vitamin C, up to 88% of patients with sepsis or those after cardiac surgery have low vitamin C levels (38, 39). Therefore, giving supplemental intravenous vitamin C to such patients appears rational. Additionally, vitamin C has several potential advantages for the treatment of critically ill patients, especially in relation to radical oxygen species generation (38).

Vasodilation after cardiac surgery and sepsis appears related to overwhelming reactive oxygen species (ROS) production (28). For cardiac surgery patients, the triggers for ROS production are multifactorial, and include surgical stress and ischemia reperfusion injury by cardio-pulmonary bypass (CPB) (40), and, in sepsis, the major driver may be the uncontrolled inflammatory response to infection (41). When ROS are over-produced, they activate several biological pathways, which can

be injurious to humans, including vasodilation, endothelial dysfunction and/or microvascular dysfunction (42). In this setting, attenuating such ROS response may be clinically useful. In this regard, intravenous high-dose vitamin C may be an effective agent to diminish the intensity of the ROS response. Vitamin C works as an oxygen radial scavenger, and serves as an electron donor, which helps reducing ROS levels (43). In addition, vitamin C can generate other oxygen radical scavengers, such as glutathione (44).

In addition to the above effects, vitamin C may affect the synthesis of catecholamines, which regulate the level of vasoconstriction and cardiac output. In keeping with this notion, impaired catecholamine synthesis has been reported in critically ill patients with low vitamin C blood levels (45, 46). In a pig model, noradrenaline levels fell when animals were fed a vitamin C deficient diet (47). Furthermore, vitamin C is a cofactor for dopamine beta-hydroxylase, which converts dopamine to noradrenaline (48). Finally, vitamin C enhances the synthesis of L-DOPA, which is a dopamine precursor (49). Therefore, vitamin C appears important to the synthesis of catecholamines.

Vitamin C also enhances vasopressin synthesis. Vasopressin is a peptide hormone synthesised in the hypothalamus and secreted by the posterior pituitary (50). When blood pressure falls or intravascular volume decreases, vasopressin is released. Vasopressin then binds to receptors on vascular smooth muscle cell to maintain vascular tone or to receptors in the kidney collecting duct to restore water reabsorption (51). Vitamin C is a cofactor for the copper-containing enzyme peptidylglycine α-amidating monooxygenase (PAM), which is essential to the

synthesis of vasopressin (52). Moreover, the pituitary gland, which is the site of vasopressin synthesis, shows the highest vitamin C concentration in the human body (53). Therefore, critically ill patients with low vitamin C levels may fail to produce sufficient amounts of vasopressin and giving vitamin C intravenously may help restore such production to the necessary higher levels (54).

Vitamin C for Sepsis

The first study reporting the use of high-dose intravenous vitamin C for the treatment of sepsis was published in 1989 (55). The paper included 16 septic patients with acute respiratory distress syndrome (ARDS) assigned to the antioxidant treatment group (vitamin C 4g/day, N acetylcysteine, selenium and vitamin E) and 16 sepsis patients with ARDS assigned to the usual care group. The trial concluded that the mortality of the antioxidant treatment group was lower than that of the standard treatment group (37% vs. 71%, $P<0.01$). However, no further studies followed until 25 years later. In 2014, a small randomised controlled trial compared the effect of different doses of intravenous vitamin C (8 patients, 200 mg/kg/day vs. 8 patients, 50 mg/kg/day vs. 8 patients, placebo) (56). The authors concluded that the two vitamin C treatment groups achieved supranormal plasma vitamin C revels, reductions in sequential organ failure assessment (SOFA) scores, and reductions in pro-inflammatory biomarkers (C-reactive protein and procalcitonin) compared to the placebo group without any adverse event (56).

In 2017, an additional study supportive of the use of vitamin C in patients with sepsis or septic shock was published (57). This retrospective single-centre, before-and-after study compared 47 sepsis patients who were treated with vitamin C at 6 g/day,

thiamine at 400 mg/day and hydrocortisone at 200 mg/day with 47 historical controls who had received usual treatment. The study concluded that there was a significant reduction in mortality for the vitamin C group (8.5% vs 40.4%, P<0.001) and a shorter duration from randomisation to the cessation of vasopressors (18.3 ± 9.8 vs. 54.9 ± 28.4, P<0.001). However, because this study was a single centre, retrospective, before-and-after study, the results could only be considered hypothesis-generating.

Vitamin C for cardiac surgery

There have been several studies of vitamin C for patients undergoing cardiac surgery. However, they all had major limitations, such as variable route of vitamin C (oral or intravenous) or main outcomes (most of them targeted postoperative arterial fibrillation and not vasopressor use or blood pressure). A matched control study gave oral vitamin C (2 g/day before the surgery and 0.5 g/day after the surgery) to patients undergoing on-pump cardiac surgery and the results showed a reduction of postoperative atrial fibrillation compared to the control group (7/43 [16.3%] vs. 15/43 [34.9%], P=0.048) (58). Of two other randomised controlled trials, one study gave a beta-blocker and oral vitamin C (2 g the night before surgery and 2 g/day postoperatively) (59) and the other gave intravenous vitamin C 2 g 3 hours before cardio-pulmonary bypass (60). Both showed a reduction in atrial fibrillation in the vitamin C group. However, the largest randomised controlled trial that compared oral vitamin C 2g the night before surgery and 2 g/day oral vitamin C postoperatively vs. placebo concluded that the rate of postoperative atrial fibrillation was not different between the two groups (30.3% in the vitamin C group vs. 30.2% in the control group [P=0.985]) (61).

Regrettably, these studies gave vitamin C orally or as a small dose of intravenous vitamin C, which was not enough to achieve the supranormal vitamin C levels likely needed in such critically ill patients. Moreover, vitamin C absorption after oral intake is limited by the capacity of its enteral transporter (sodium-vitamin C transporter-1). Healthy volunteers who received oral vitamin C 1.25 g showed markedly lower peak plasma concentration compared to those received intravenous vitamin C 1.25 g (134.8 ± 20.6 μmol/L vs. 885 ± 201.2 μmol/L) (62), implying that, if supranormal levels are aimed for, treatment with vitamin C should be intravenous. In addition, to achieve persistent normal vitamin C levels in critically ill patients, 3-6 g/day of vitamin C are estimated as minimal requirements (63). Only one controlled study gave high dose intravenous vitamin C (250 mg/kg/day) preoperatively to patients undergoing cardio-pulmonary bypass and reported that, compared to the control group, the high dose vitamin C group showed a lower creatine kinase myocardial band (CK-MB) and an increased cardiac index (64). However, because this study was not a double-blind randomised controlled trial, it was also considered hypothesis generating only. Thus, high dose intravenous vitamin C carries a strong biological rationale and a body of preliminary data that justify the conduct of further pilot double-blind randomised controlled trials

Therefore, I led the conduct of a pilot, double-blinded, randomised controlled trial to examine the haemodynamic and physiological efficacy of intravenous high-dose vitamin C (6 g/day intravenously) in patients with vasoplegia (marked vasodilatation leading to hypotension and treatment with vasoactive drug infusions) after cardiac surgery (Chapter 6) (65). The study, the first of its kind, randomised 50 patients with

post cardiac surgery vasoplegia to either 1.5 g of intravenous vitamin C 6 hourly (6g/day) or placebo. It found the active intervention (high-dose IV vitamin C) did not achieve any reduction in the duration of vasoplegia, total vasopressor dose, or intensive care unit admission or any other favourable physiological or clinical effect compared to placebo. The lack of efficacy in this setting suggested that the high dose vitamin C therapy proposed was ineffective. This observation may imply that, no matter the dose, vitamin C is simply not able to modify vessel tone in a clinically meaningful way in this setting (or indeed any other setting). However, it may potentially also imply that a much greater dose of vitamin C may be need to obtain a clinically meaningful effect. In this regard, if much larger doses (mega-dose vitamin C) are to be tested, there needs to be evidence of their safety.

The safety of mega-dose vitamin C

Although the efficacy of intravenous vitamin C has been re-evaluated, its potential for harm has been a “neglected” topic (66). People can buy vitamin C over the counter and this vitamin is considered safe at low doses. However, at higher doses and if taken over a prolonged period, vitamin C may lead to significant adverse events, such as oxalate nephropathy or point of care glucose measurement errors. Critically ill patients need 3-6 g/day to maintain physiological vitamin C level (63). In addition, as described, in a previous study, intravenous vitamin C 6 g/day, which is high dose in clinical setting, was required to achieve faster shock resolution (57). In theory, the incidence of adverse events of intravenous high dose vitamin C may be higher than that of low dose vitamin C or oral intake. However, as the incidence of adverse events is very rare, most of the studies are case reports and no comprehensive review of its toxicity exists. In addition, when considering the intravenous vitamin C

literature, the reporting if adverse effects is even more limited. Finally, when considering both high dose and very high dose (so-called mega-dose) intravenous vitamin C therapy, no systematic assessment has ever been undertaken.

Therefore, I decided to systematically review the literature and focus on all reported harmful effects or adverse events related to the use of intravenous high dose or mega dose vitamin C (at least ≥6 g/day or 75 mg/kg/day or 3 g/m^2/day) in both adult (more than 18 year old) and paediatric (less than 18 year old) patients (Chapter 7) (67, 68).

In the adult study, I identified 74 studies, including nine double blind randomized controlled trials, which reported harm associated with high dose vitamin C from more than 8000 screened studies (67). Among the patients included in the manuscripts, 2081 received intravenous high dose vitamin C. And among the nine double blind randomized controlled trials, adverse events were reported in three studies with an event rate per patient for high-dose vitamin C identical to placebo group in one study, numerically lower in another study, and numerically higher in the third study. Six double-blind randomised controlled trials reported no adverse event in either group. Also, several cases of oxalate nephropathy, hypernatremia, haemolysis in glucose-6-phosphate dehydrogenase deficiency patients, glucometer error and kidney stones were also reported.

In the paediatric review, I identified 12 eligible studies from 1364 articles, which included 194 patients receiving intravenous high dose vitamin C (68). Four studies were double-blind randomized controlled trials, and no clinical efficacy outcome was

reported in favour of or against vitamin C. Furthermore, no adverse event or signal of harm was reported with high-dose vitamin C.

In summary, these studies found that there was no evidence that giving high dose or mega dose intravenous vitamin C is harmful and that, in fact, its side effect profile is the same as that placebo in both paediatric and adult patients. These findings led to two key publications, which are reported in two separate studies for adults and children (Chapter 7) (67, 68).

Components of glycocalyx layer

Another possible haemodynamic optimisation approach is to deliver an improved glycocalyx protection strategy to decrease fluid movement from the intravascular to the extravascular space. The glycocalyx is a thin membrane that covers the luminal side of blood vessels and has multiple functions to maintain the microvascular environment (Figure 2). It regulates vascular tone, controls fluid movement from the intravascular space to the extravascular space, and inhibits microvascular thrombosis or leukocyte adhesion (69, 70). When patients are critically ill, such as during sepsis, after cardiac surgery or trauma, the glycocalyx is damaged, its components shed into the blood, and these functions are lost.

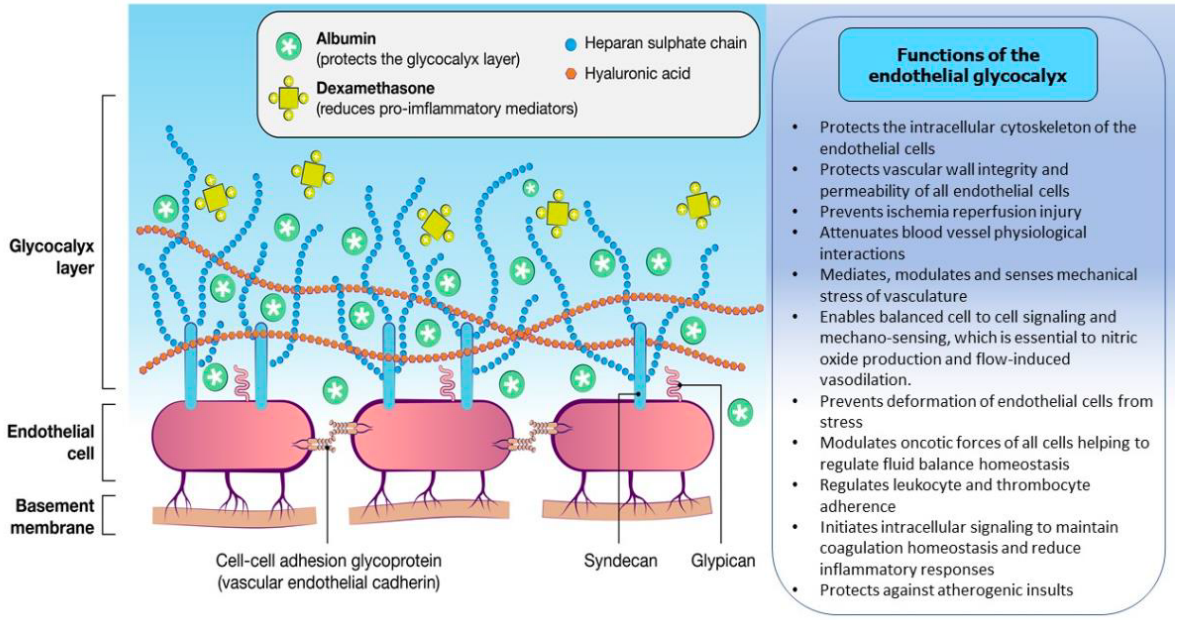

Figure 2. Structure and functions of glycocalyx membrane (from Chapter 9)

The thickness of the glycocalyx layer varies according to measurement techniques or type of vessels (71). It was first identified by electron microscopy in 1966, and, currently, its thickness in humans is estimated at 0.5 to 5.0 μm (72-75). The glycocalyx layer is composed of proteoglycans (syndecan 1), glycoproteins bound by sialic acid such as glycosaminoglycans (hyaluronan, heparan sulfate or chondroitin sulfate) and plasma proteins (albumin, fibrinogen, fibronectin, antithrombin III or thrombomodulin) (Figure 2) (32, 76). Proteoglycans are believed to act as the backbone of the glycocalyx and to connect the glycocalyx to endothelial cells. Moreover, one or more glycosaminoglycans are attached to proteoglycans (Figure 2). Glycosaminoglycans are negatively charged and electrically interact with and resist the movement of plasma proteins (75).

Functions of glycocalyx

The glycocalyx has several important physiological functions. The glycocalyx layer works as a negatively charged mesh layer that regulates fluid movement from the intravascular space to extravascular space (70). This negatively charged mesh layer limits the movement of negatively charged plasma proteins and proteins, which are larger than 70-kDa. This effect creates an albumin concentration gap and an oncotic pressure gradient between the intravascular space and the extravascular space, which works to maintain intravascular volume (69). In addition, the glycocalyx plays an important role as an anticoagulant and an antiadhesive layer to help prevent clots in small vessels (71). The glycocalyx also regulates nitric oxide production by shear stress to control vascular tone and protects endothelial cells from reactive oxygen species (32).

Diagnosis of glycocalyx injury or loss

In animal models, intravital microscopy has been considered the “gold standard” for glycocalyx injury or loss or damage. However, this technique is not suitable for human studies because it can only be used in anesthetised animal models and it needs fluorescent markers (77). Thus, there are mainly two visual methods to detect glycocalyx damage in the clinical setting. One methods is by orthogonal polarisation spectral imaging and the other is by sidestream dark field imaging (78). The sidestream dark field imaging technique uses subinguinal or oral microvessels for assessment of glycocalyx damage and it estimates thickness of the glycocalyx from the perfused boundary region (78, 79) (Figure 3 and 4). However, these technique can measure only a limited area of microvessels, for example, the oral or sublingual area, and there is a concern regarding inter-observer variability (32, 78, 79).

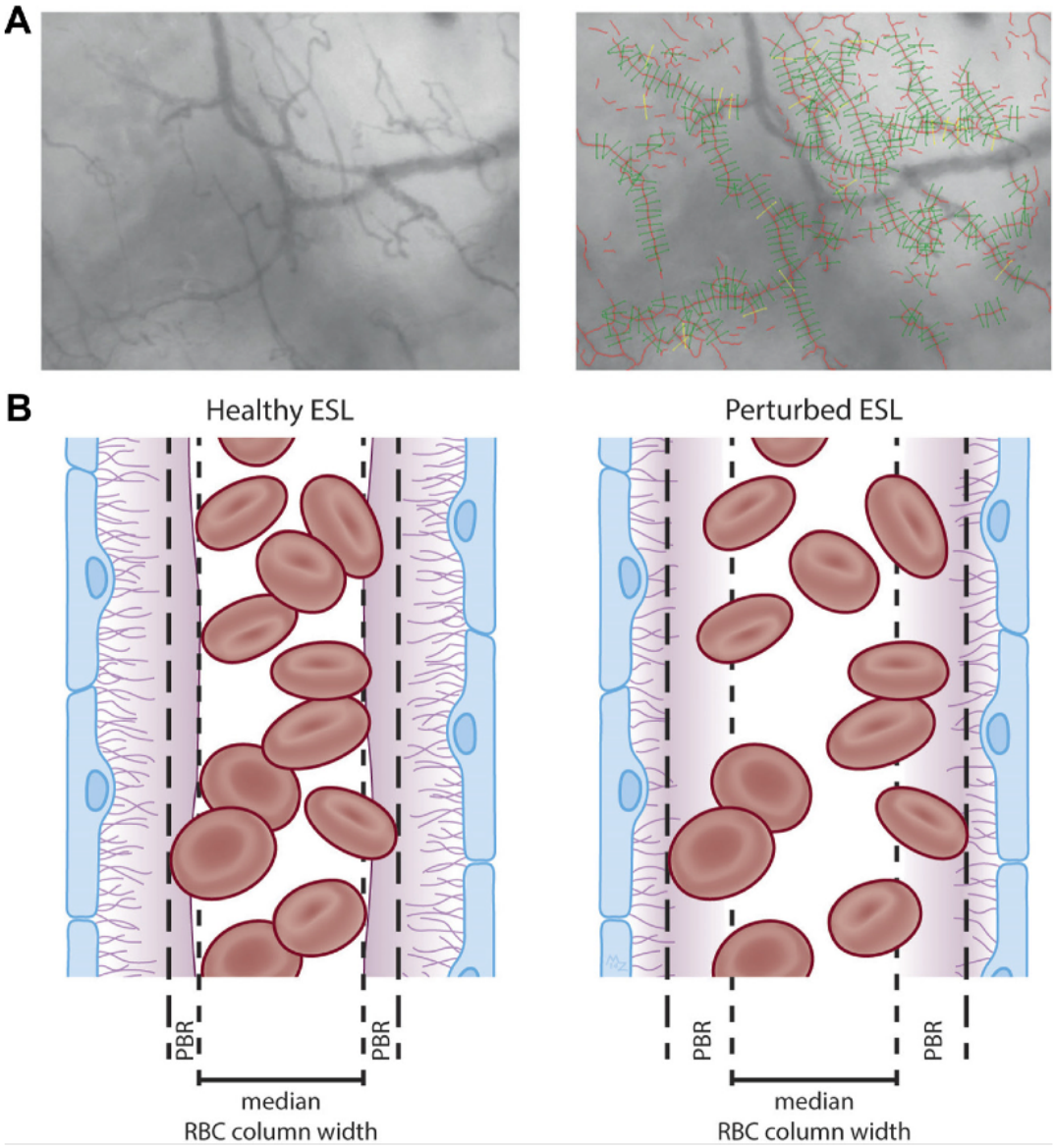

Figure 3. Sidestream darkfield imaging to assess glycocalyx thickness. Figure from reference (78) . When glycocalyx layer is damaged and becomes thin, the perfused boundary region becomes wide.

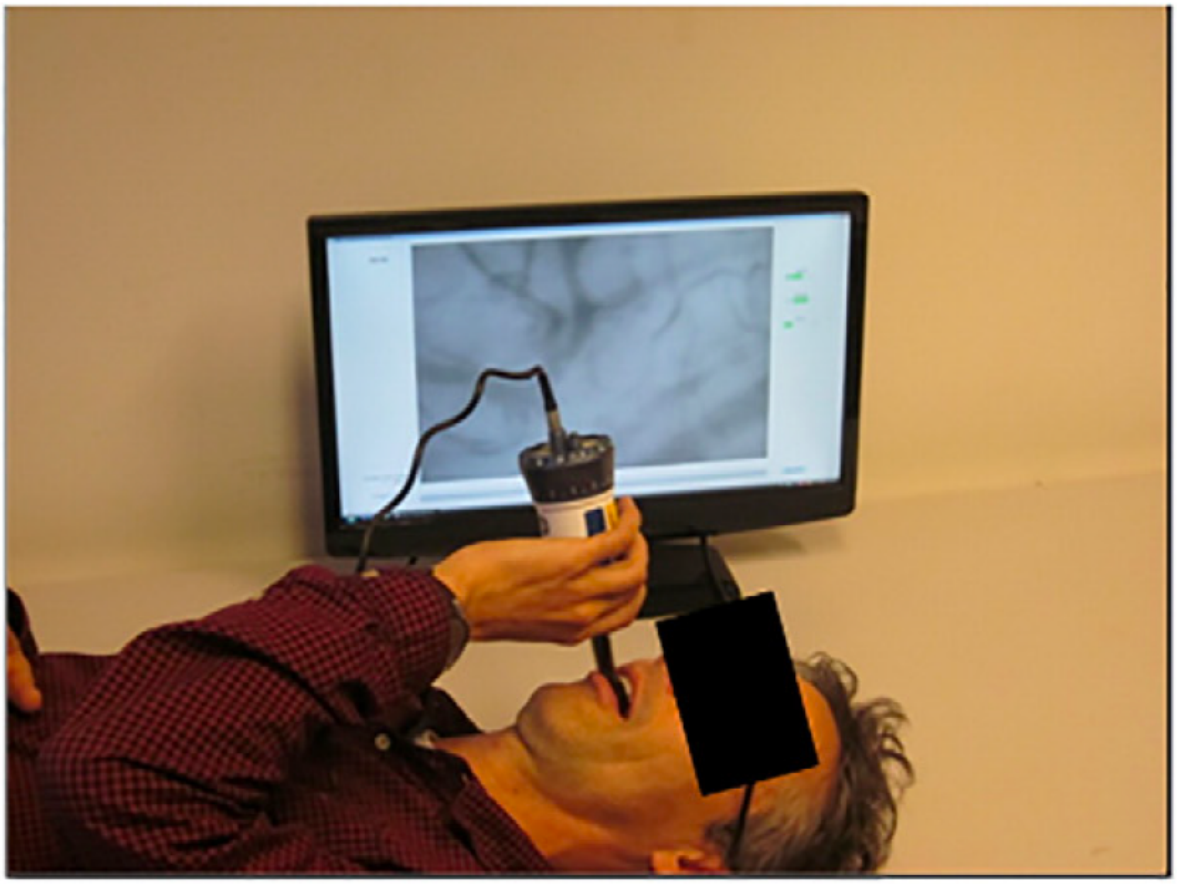

Figure 4. Sidestream dark field measurements using oral mucosa (from reference (79)).

Thus, glycocalyx biomarkers are widely used to assess glycocalyx damage in the clinical settings because they are easy to measure in the patient's blood without inter-observer variation. When the glycocalyx membrane is damaged, its components (syndencan-1, hyaluronan, heparan sulfate or chondroitin sulphate) are shed into the bloodstream and investigators can measure elevated biomarker levels using commercially available enzyme-linked immunosorbent assay (ELISA) kits.

Causes of Glycocalyx destructions

There are many factors that can induce glycocalyceal injury, such as surgical inflammation, ischaemia-reperfusion injury, sepsis, hypervolaemia or hyperglycaemia.

Surgical inflammation and/or ischaemia-reperfusion causes glycocalyceal injury. For example, cardiac surgery can induces inflammation and ischaemia-reperfusion injury. One study found that both patients after off-pump cardiac surgery and patients after on-pump cardiac surgery demonstrated significant increases of syndecan-1 and heparan sulfate levels compared to their preoperative values (80).

Also, patients with sepsis are known to show elevated glycocalyx biomarker levels compared with healthy volunteers. One study compared syndecan-1 levels and heparan sulfate levels in healthy volunteers vs those of post abdominal surgery patients and those seen in sepsis (81). The study concluded that both biomarker levels were markedly higher in the abdominal surgery group and the sepsis group.

Additionally, atrial natriuretic peptide, which is released from the atrium in response to hypervolaemia-associated stretch, is known to cause glycocalyceal damage. In patient undergoing elective surgery with good cardiopulmonary health, an acute volume loading (20 ml/kg of 6% hydroxyethyl starch [HES] infused over 15 minutes) showed higher glycocalyx biomarker levels (syndecan-1, heparan sulfate and hyaluronan) than in acute normovolaemic haemodilution group (500 ml/m^2 patient's blood removal and 6% hydroxyethyl starch infusion at the same rate) (82). Finally, in addition to the acute care settings, other chronic conditions, such as dyslipidaemia or diabetes, increases glycocalyx biomarker levels (83).

Protective Treatment of the Glycocalyx

There are several possible treatments to protect the glycocalyx. Anti-inflammatory treatments (anti-tumour necrosis factor-alpha [TNFα] antibodies and steroids) are known to reduce glycocalyx damage in animal models or healthy volunteers. In healthy volunteers who received endotoxin injection, TNFα receptor blockade (etanercept intramuscular injection) showed decreased glycocalyx thickness reduction on orthogonal polarization spectroscopy and a less hyaluronan increase in the blood stream compared to the control group (84). Also, steroid (hydrocortisone 100 mg injection) therapy reduced glycocalyx biomarker increases (syndecan 1 and heparan sulfate) after cardiac surgery compared to the placebo group (85).

On the other hand, the injection of glycocalyx components, such as albumin, antithrombin III or hyaluronan, may also protect the glycocalyx from injury. In a Guinea pig heart ischemia model, Bretschneider's solution (histidine-tryptophan-ketoglutarate [HTK]) with augmentation with 1% human albumin) achieved attenuated reduction of the glycocalyx layer in coronary arteries compared with the isolated Bretschneider's solution group (86). Also, other glycocalyx components (antithrombin III and hyaluronan) are protective to glycocalyx (83).

In summary, the glycocalyx is composed of several proteins, covers the internal wall of vessels, and has an important role in regulating fluid movement and vascular tone. Inflammation causes glycocalyx destruction and the damage is mainly assessed by biomarker levels in the clinical setting. Finally, anti-inflammatory treatment and infusion of glycocalyx components appear protective to the glycocalyx. However,

there is no “clinical gold standard” to assess glycocalyx damage and the clinical effects of glycocalyx protection strategies have not been systematically assessed in patients undergoing abdominal surgery. Therefore, in Chapter 8, I report the finding of a scoping review to address these questions (87). I performed a scoping review of all literature on glycocalyx biomarker values in healthy volunteers, abdominal surgery patients and sepsis patients. I found that syndecan 1 was the most frequently measured biomarker in the three above conditions and that, in clinical research, glycocalyx damage biomarkers values have been essentially defined by the measurement of syndecan 1. However, even among healthy volunteers, there are wide variations in syndecan 1 levels according to the assay kits being used. For instance, in healthy volunteers, there was a 195-fold difference in concentration according to the assay used to measure syndecan 1 values. Therefore, in this study, the finding demonstrated that, to avoid misleading results, it is important to report on biomarker directional changes and their percentage decrease or increase from baseline using the same method.

Albumin and dexamethasone for glycocalyx in abdominal surgery

As discussed, albumin and corticosteroids appear to protect the glycocalyx from damage and these agents might be a potential treatment to protect the glycocalyx, prevent fluid extravasation and intravascular fluid depletion and, thereby, optimise haemodynamic status. However, all research studies indicating such protective effects only included animal models, healthy volunteers, or cardiac surgery patients (83-86). In contrast, there was no research that included patients undergoing abdominal surgery. Moreover, the clinical efficacy of such glycocalyx protection strategies is unknown. Therefore, I led the conduct of a randomised, multicentre,

open-label, blinded endpoint assessment trial of preoperative microvascular glycocalyx protection in patients undergoing major abdominal surgery to assess the biological, haemodynamic, physiological and clinical efficacy of combined dexamethasone and albumin treatment compared to standard care (Chapter 9).

The trial enrolled 72 patients undergoing major abdominal surgery (liver, pancreas or colon surgery) and assigned them either to the dexamethasone and albumin treatment group or the usual care group. We found that the intervention could not reduce postoperative syndecan-1 levels or modify haemodynamics. However, we also found that it significantly reduced heparan sulfate and C-reactive protein levels. Additionally, it reduced postoperative pulmonary complications and was not associated with any adverse event. As this trial was a pilot, phase 2 trial with only 72 patients, its findings have limited statistical robustness and are only hypothesis generating. However, these results imply that the intervention is likely safe and feasible and justify its investigation in future phase 3 studies.

Chapter 2: Warm vs. Cold 4% albumin fluid bolus therapy in cardiac surgery patients

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Original Research

Comparison of the Hemodynamic and Temperature Effects of a 500-mL Bolus of 4% Albumin at Room Versus Body Temperature in Cardiac Surgery Patients

Fumitaka Yanase, MD[*,†], Laurent Bitker, MD[*,‡], Luca Lucchetta, MD[*], Thummaporn Naorungroj, MD[*,§], Salvatore L. Cutuli, MD[*,‖,¶], Eduardo A. Osawa, MD[*], Emmanuel Canet, MD[*], Anthony Wilson, MD[*], Glenn M. Eastwood, PhD[*], Michael Bailey, PhD[†], Rinaldo Bellomo, MD, PhD[*,†,**,1]

[*]*Department of Intensive Care, Austin Hospital, Melbourne, Victoria, Australia*
[†]*Australian and New Zealand Intensive Care Research Centre (ANZIC-RC), School of Public Health and Preventive Medicine, Monash University, Melbourne, Victoria, Australia*
[‡]*Service de médecine intensive et réanimation, hôpital de la Croix Rousse, Hospices Civils de Lyon, Lyon, France*
[§]*Department of Intensive Care, Siriraj Hospital, Mahidol University, Bangkok, Thailand*
[‖]*Dipartimento di Scienze dell'emergenza, anestesiologiche e della Rianimazione, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy*
[¶]*Università Cattolica del Sacro Cuore, Rome, Italy*
[**]*Centre for Integrated Critical Care, School of Medicine, University of Melbourne, Parkville, Victoria, Australia*

Objective: To compare the hemodynamic effect of room temperature (cold) 4% albumin fluid bolus therapy (FBT) with body temperature (warm) albumin FBT.
Design: Prospective, before-after trial.
Setting: A tertiary intensive care unit (ICU).
Participants: Sixty ventilated, post-cardiac surgery patients prescribed with 4% albumin FBT.
Intervention: Cold or warm 4% albumin 500 ml FBT.
Measurements and Main Results: We recorded hemodynamic parameters before and for 30 minutes after FBT. Cardiac index (CI) and mean arterial pressure (MAP) responses were defined by a CI increase >15% and a MAP increase >10%, respectively. Immediately after FBT, median [interquartile range] core temperature changed by -0.3 [-0.4; -0.3] ˚C with cold albumin vs. 0.0 [0.0; 0.1]˚C with warm albumin (P<0.001). The median CI increase was 0.3 [0.0; 0.5] L/min/m2 with 14 CI-responders (47%) in both groups (P>0.99). The median immediate MAP increase was 9 [3; 15] mmHg with cold albumin vs. 11 [5; 13] mmHg with warm albumin (P=0.79), with a MAP-response in 16 vs. 17 patients (P=0.99). There was an interaction between group and time for MAP (P=0.002), mean pulmonary artery pressure (PAP) (P=0.002) and core temperature (P<0.001). In the cold albumin group, after the initial response, MAP and mean PAP decreased more slowly than with warm albumin and, after the initial fall, core temperature increased toward baseline.
Conclusion: In postoperative cardiac surgery patients, warm albumin FBT prevents the decrease in core temperature and, after an initial similar increase, is associated with a faster return of MAP and mean PAP toward baseline.

Key Words: fluid bolus therapy; iso-oncotic albumin; fluid temperature; postoperative care; post cardiac surgery

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[1]*Address reprint requests to* Rinaldo Bellomo, MD, PhD, Intensive Care Research, Austin Health, Studley Road, Heidelberg, Victoria, Australia, 3084.
E-mail address: rinaldo.bellomo@austin.org.au (R. Bellomo).

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1053-0770/

HYPOTENSION IS a common complication after cardiac surgery, likely due to hypovolemia or vasoplegia.[1-3] Crystalloid or colloid fluid bolus therapy (FBT) is commonly given to restore cardiac index and increase blood pressure.[4] However, it is unclear whether fluid temperature may modulate the effects of colloid FBT in this setting.

Intravenous fluids are sometimes warmed in the operating room to avoid hypothermia.[5] In contrast, they are usually given at room temperature in the intensive care unit (ICU). Previous studies in volunteers found that room temperature (cold) fluid reduced body temperature and that body temperature (warm) fluid increased cardiac index (CI) to a greater extent.[6,7] On the other hand, cold fluid may cause norepinephrine release through its temperature effect and achieve a greater increase in mean arterial pressure (MAP).[6,8] In addition, postoperative hypothermia induced by cold fluid could contribute to coagulopathy, infection, or shivering.[9] However, no study has reported the comparative temperature and hemodynamic impact of intravenous colloids administered at different temperatures in any patient group and in patients after cardiac surgery in particular. In cardiac surgery patients, the use of 4% albumin solution is relatively common in Australia and New Zealand.[4] Thus, comparing cold versus warm 4% albumin FBT is clinically relevant.

Accordingly, the authors conducted a study of the hemodynamic effects of a 500-mL bolus of room-temperature (cold) 4% albumin compared with a 500-mL bolus of body-temperature (warm) 4% albumin in postoperative cardiac surgery patients. They aimed to test the hypothesis that the body temperature, CI, and MAP response would differ significantly between the 2 interventions.

Methods

This study was approved by the ethics committee in Austin Hospital in Melbourne, Australia (reference number LNR/16/Austin/358). The need for informed consent was waived because of the observational nature of the study.

Study Design

This single-center, prospective, before-after study was part of a research program aimed at understanding the effect of FBT after cardiac surgery from December 2016 to December 2019. The authors included adult patients (>18 years old) who were admitted to the intensive care unit (ICU) after on-pump cardiac surgery, required mechanical ventilation support, and were prescribed 500 mL of 4% albumin FBT. FBT was prescribed based on a clinical indication. The authors only included patients who needed FBT in the first 12 hours of ICU admission and had a pulmonary artery catheter to measure cardiac index. They obtained data in 30 patients who received cold 4% albumin FBT in the first period (December 2016 to September 2018), and thereafter in 30 patients who received warm 4% albumin FBT (September 2018 to December 2019).

If any major confounder that may have affected hemodynamic impact (Appendix 1) became necessary during the observation period, patients were excluded from analysis and the authors recorded the reason. However, when patients met minor confounders (Appendix 2), recording was continued and the data were used for analysis. Other exclusion criteria were patients who were known to be pregnant or who required mechanical hemodynamic support, such as intra-aortic balloon counterpulsation or extracorporeal membrane oxygenation. Furthermore, because a member of the research team needed to observe the full study period from 3 minutes before FBT to 30 minutes after FBT, to record hemodynamic confounders, the authors included patients admitted to the ICU who required FBT between 11 AM and 5 PM on weekdays.

Patient care, including vasoactive drug use, indication for fluid bolus, or mechanical ventilation setting, was based on usual care as prescribed by the attending clinicians.

Data Collection

Philips IntelliView MP70 monitors (Philips Healthcare, Best, Netherlands) were used for all patients, and the authors downloaded hemodynamic data on a second-by-second basis using Medicollector logging software (Medicollector LCC, Boston, MA). Systolic arterial pressure (SAP), diastolic arterial pressure (DAP), MAP, central venous pressure (CVP), systolic pulmonary arterial pressure (PAP), diastolic PAP, mean PAP, heart rate (HR), CI, and peripheral O_2 saturation (Spo_2) were recorded.

These hemodynamic measurements were referenced to the phlebostatic level, and the research team confirmed them before data collection. Systolic arterial pressure, diastolic arterial pressure, and MAP were recorded from the radial or brachial artery catheter, and CVP, systolic PAP, diastolic PAP, and mean PAP were recorded from the PA catheter (Edwards Lifesciences, Irvine, CA). The position of the PA catheter was confirmed via chest radiograph at ICU admission. CI was measured by continuous or intermittent technique depending on the type of PA catheter. When patients did not have a continuous cardiac output PA catheter, the research team performed intermittent measurements for 4 time points (before FBT, immediately [0 minutes] after FBT, 15 minutes after FBT, and 30 minutes after FBT), using a 10-mL normal saline bolus. Baseline hemodynamic parameters were recorded at least 3 minutes before the FBT. The authors also recorded mechanical ventilator setting, catecholamine level, and sedative drugs at the time of inclusion.

Fluid Bolus Therapy

FBT was administered according to the clinical team decision. The clinical team were asked to administer 500 mL of 4% albumin solution. Clinicians were also asked to record the reason for the FBT. Albumin was infused at room temperature (cold) in the first- half period and warmed to body temperature

(between 37˚C and 40˚C) by fluid warming devices (enFlow, Vital Signs, Inc GE Healthcare Company, Totowa, NJ; or HOTLINE, Smith Medical ASD, Inc, Minneapolis, MN) in the second- half period. After data on the risk of aluminum release from enFlow device were published, the authors used the HOTLINE system for all patients.[10] The ICU was climate controlled, and ambient temperature was maintained at 18˚C to 20˚C. All fluid boluses were infused rapidly using a hand pump infusion system with a compressible reservoir as is usual care in this ICU. There was no guideline for the speed of the fluid boluses; however, clinicians were encouraged to administer such FBT in less than 30 minutes.[11]

Hemodynamic Response Definitions

Patients were defined as CI- responsive (CI-R) if their CI increased by >15% above baseline, and as immediate CI-R if their CI increased by >15% above baseline immediately after the FBT.[12] Patients were defined as MAP- responsive (MAP-R) if MAP increased by >10% above baseline, and as immediate MAP-R if MAP increased by >10% above baseline immediately after FBT.

The authors defined time to dissipation of FBT effect as the time when the patient's CI was within 5% of baseline (CI dissipation) or the patient's MAP was within 3 mmHg of baseline for at least 2 consecutive minutes (MAP dissipation).[12]

The authors also defined a perfusion pressure as the difference between MAP and CVP.[13]

The primary hypothesis was that the CI and MAP response would differ between the different temperature FBT groups.

Power Calculation

Based on data from the ICU, the standard deviations for CI and MAP were estimated at 0.65 L/min/m^2 and 10 mmHg, respectively. With 30 patients per group, the authors estimated that this study would have an 80% power (2-sided p value of 0.05) to detect differences between the 2 groups equivalent to 73% of the standard deviation (equivalent to a 7.3 mmHg overall MAP difference and a 0.47 L/min/m^2 overall CI difference). The authors considered that differences of this magnitude would be of clinical importance.

Statistical Analysis

Analysis was performed using the R software, version 3.5.2 (R Foundation for Statistical Computing, Vienna, Austria). All data were initially assessed for normality. Categorical data were reported as counts (percentages), and continuous data were reported as median (interquartile range). All baseline characteristics were compared between the cold albumin group and the warm albumin group, using the Mann-Whitney U test for continuous variables and Fisher exact test for categorical variables. Hemodynamic variables collected during the observation period (either absolute values or relative change from baseline) were analyzed between the cold albumin group and the warm albumin group, using linear mixed- effects models, accounting for within- subject repeated measures, and treating time as a continuous variable. When a study group effect or an interaction between time and the study group was significant, the authors performed post-hoc analysis to examine the significance of the difference at each time point, accounting for the alpha inflation risk using the Tukey adjustment method. Spearman correlation was applied to evaluate the relationship between CI change and MAP change immediately after FBT. A 2-sided p value below 0.05 was considered as significant.

Results

Patients Characteristics

Of the 108 eligible patients, 60 patients without hemodynamic major confounders (30 in each group) were analyzed, and 48 patients were excluded mainly because of changes in norepinephrine dose or sedative drugs (Supplementary Fig 1).

The characteristics of the study patients at baseline were well balanced and are shown in Table 1. More than half of the surgeries were on-pump coronary artery bypass graft, and the median duration of cardio-pulmonary bypass time was similar between the 2 groups. Patient characteristics at the time of fluid bolus were also well balanced and are presented in Supplementary Table 1. More than 90% of the patients were sedated with propofol. Norepinephrine and milrinone were used in fewer than one-third of patients.

The baseline hemodynamic characteristics are presented in Table 2. There were no statistical differences in baseline MAP, mean PAP, CVP, HR, or CI between the 2 groups. Median baseline blood temperature was also the same in the 2 groups (36.4˚C [35.9˚C, 37.1˚C] in the cold albumin group *v* 36.5˚C [35.9˚C, 37.1˚C] in the warm albumin group, p = 0.83).

FBT Description

Median duration of 4% albumin infusion was 6.3 (4.4, 10.8) minutes in the cold albumin group and 5.4 (4.2, 6.9) minutes in the warm albumin group (p = 0.12) (Table 3). Hypotension was the main trigger for FBT in the 2 groups (18 patients [60%] in the cold albumin group and 23 patients [77%] in the warm albumin group, p = 0.42).

CI Changes After FBT

For CI, the study group effect or the interaction test between time and study group was not significant (Fig. 1 and 2). Immediately after FBT, median CI increased by 0.3 (0.0, 0.5) L/min/m^2 in the cold albumin group and 0.3 (0.0, 0.5) L/min/m^2 in the warm albumin group. Median CI was still 0.2 L/min/m^2 greater than baseline at 30 minutes after FBT in both groups (Supplementary Tables 2 and 3).

Immediate CI-R was observed in 14 patients (47%) in each group (Table 3 and Supplementary Table 4). In the immediate CI-R group, 4 patients (29%) showed dissipation of their CI response at 30 minutes after FBT in the room- temperature

Table 1
Baseline Characteristics of Study Patients

	All Patients N = 60	Cold 4%Albumin n = 30	Warm 4% Albumin n = 30	p
Age, y	66 (60-71)	66 (61-70)	70 (57-73)	0.89
Sex (male)	48 (80%)	25 (83%)	23 (77%)	0.75
Body mass index, kg/m^2	28.3 (26.1-32.2)	27.9 (26.1-32.1)	28.7 (26.4-32.1)	0.75
Comorbidities				
Atrial fibrillation	5 (8%)	3 (10%)	2 (7%)	>0.99
COPD	4 (7%)	3 (10%)	1 (3%)	0.61
Chronic kidney disease	4 (7%)	2 (7%)	2 (7%)	>0.99
Diabetes mellitus	21 (35%)	9 (30%)	12 (40%)	0.59
Hypertension	35 (58%)	19 (63%)	16 (53%)	0.60
Ischemic heart disease	45 (75%)	26 (87%)	19 (63%)	0.07
APACHE III score	40 (35-50)	42 (38-50)	36 (31-50)	0.16
EuroSCORE	4 (2-6)	3 (2-6)	4 (3-7)	0.34
Type of surgery				0.19
On-pump CABG	38 (63%)	22 (73%)	16 (53%)	-
Valve	13 (22%)	4 (13%)	9 (30%)	-
CABG + valve	7 (12%)	4 (13%)	3 (10%)	-
Other	2 (3%)	0 (0%)	2 (7%)	-
CPB duration, min	113 (93-134)	116 (103-149)	113 (88-127)	0.26
Aorta clamp duration, min	92 (70-108)	93 (85-119)	81 (69-100)	0.14
Post-CPB TEE assessment				
Left ventricular dysfunction	9 (15%)	6 (22%)	3 (11%)	0.30
Right ventricular dysfunction	4 (7%)	2 (7%)	2 (7%)	>0.99

Data are median (interquartile range) or count (percentage).
p values reflect the between-groups comparison.
Abbreviations: APACHE, acute physiology and chronic health evaluation score; CABG, coronary artery bypass graft; COPD, chronic obstructive pulmonary disease; CPB, cardio-pulmonary bypass; EuroSCORE, European system for cardiac operative risk evaluation; TEE, transesophageal echocardiography.

group compared with 2 (14%) in the warm- albumin group (p = 0.79) (Table 3 and Supplementary Table 4). In total, 22 (73%) patients in the cold group and 21 (70%) in the warm group showed CI response at least once during the observation period (p > 0.99).

MAP Changes After FBT

There was a significant interaction between time and study group, and the warm group showed a more rapid MAP decrease than the cold albumin group after initial increase (p = 0.002 in

Table 2
Baseline Hemodynamics

	All Patients N = 60	Cold 4%Albumin n = 30	Warm 4% Albumin n = 30	p
Mean arterial pressure, mmHg	69 (63-74)	70 (62-77)	68 (64-74)	0.90
Systolic arterial pressure, mmHg	98 (92-109)	99 (92-109)	97 (92-108)	0.71
Diastolic arterial pressure, mmHg	55 (50-60)	55 (50-60)	54 (51-59)	0.73
Pulse pressure, mmHg	44 (35-54)	43 (35-55)	44 (37-52)	0.76
Mean PAP, mmHg	20 (17-24)	20 (17-22)	20 (18-25)	0.34
Systolic PAP, mmHg	28 (23-32)	27 (24-32)	29 (23-34)	0.45
Diastolic PAP, mmHg	14 (12-18)	14 (12-17)	15 (12-20)	0.44
Central venous pressure, mmHg	7 (5-12)	8 (6-12)	7 (5-11)	0.58
Heart rate, beats/min	86 (80-90)	85 (80-90)	87 (80-88)	0.95
Cardiac index, L/min/m^2	2.2 (1.9-2.4)	2.2 (1.9-2.4)	2.2 (1.9-2.4)	0.71
Stroke volume index, mL/m^2	25 (22-29)	24 (22-29)	26 (22-28)	0.67
SVRi, dyn.s/cm^5/m^2	2,304 (1,962-2,627)	2,327 (1,894-2,772)	2,297 (2,022-2,533)	0.98
Systemic perfusion pressure, mmHg	61 (56-66)	61 (55-67)	60 (58-64)	0.79
Blood temperature,°C	36.4 (35.9-37.1)	36.4 (35.9-37.1)	36.5 (35.9-37.1)	0.83

Data are median (interquartile range).
p Values reflect the between-groups comparison.
Abbreviations: CI, cardiac index; PAP, pulmonary arterial pressure; SVRi, systemic vascular resistance index.

Table 3
Fluid Bolus Characteristics and Hemodynamic Response

	All Patients N = 60	Cold 4%Albumin n = 30	Warm 4% Albumin n = 30	p
Time from ICU admission to FBT, h	1.5 (0.9-2.4)	1.3 (0.6-2.1)	1.6 (0.9-2.7)	0.31
Fluid bolus indication				0.42
Low cardiac output	12 (20%)	8 (27%)	4 (13%)	-
Low filling pressures	6 (10%)	3 (10%)	3 (10%)	-
Hypotension	41 (68%)	18 (60%)	23 (77%)	-
To decrease vasopressor	1 (2%)	1 (3%)	0 (0%)	-
Duration of fluid bolus infusion, min	5.7 (4.4-7.9)	6.3 (4.4-10.8)	5.4 (4.2-6.9)	0.12
CI response*				
At end of bolus administration	28 (47%)	14 (47%)	14 (47%)	>0.99
At 15 min of bolus administration	30 (53%)†	12 (44%)†	18 (60%)	0.29
At 30 min of bolus administration	25 (42%)	11 (37%)	14 (47%)	0.60
MAP response‡				
At end of bolus administration	33 (55%)	16 (53%)	17 (57%)	>0.99
At 15 min of bolus administration	24 (40%)	13 (43%)	11 (37%)	0.79
At 30 min of bolus administration	22 (37%)	13 (43%)	9 (30%)	0.42
CVP response§	50 (83%)	25 (83%)	25 (83%)	>0.99
Perfusion pressure response‖	33 (55%)	16 (53%)	17 (57%)	>0.99
Occurrence of a confounding event¶				
Minor event	9 (15%)	6 (20%)	3 (10%)	0.47

Data are median (interquartile range) or count (percentage).
p values reflect the between-groups comparison.
Abbreviations: CI, cardiac index; CVP, central venous pressure; FBT, fluid bolus therapy; ICU, intensive care unit; MAP, mean arterial pressure.
* Defined as an increase >15% of baseline value.
† Three patients in the cold albumin group were not measured CI at 15 minutes after FBT.
‡ Defined as an increase >10% of baseline value.
§ Defined as +2 mmHg increase in CVP from baseline value, at the end of the bolus.
‖ Defined as 5% mmHg increase in perfusion pressure from baseline value, at the end of the bolus.
¶ Refer to Appendix 2 for definitions.

absolute MAP value and p = 0.002 in relative change from baseline) (Fig. 1 and 2). As a consequence, the proportion of patients with an MAP response was consistently greater with cold albumin FBT (Supplementary Fig 2).

Median MAP increased by 9 (3, 15) mmHg immediately after FBT and by 4 (2, 11) mmHg 30 minutes after cold albumin infusion, and by 11 (5, 13) mmHg immediately after FBT and by 3 (0, 8) mmHg 30 minutes after warm albumin infusion (p = 0.79 and p = 0.24, respectively) (Table 2 and Supplementary Table 3).

Sixteen patients (53%) in the cold albumin group and 17 (57%) in the warm albumin group showed an immediate MAP-R (p > 0.99) (Table 3). Among immediate MAP-responders, 5 patients (31%) showed MAP-R dissipation in the cold albumin group compared with 9 patients (53%) in the warm albumin group (p = 0.30) (Supplementary Table 4); and 18 (60%) patients in the cold group and 21 (70%) patients in the warm group showed MAP-R at least 1 time during the observation period (p = 0.59).

Other Hemodynamic Changes and Temperature Changes

There was an interaction between time and study group in core temperature (p < 0.001 in absolute value and p < 0.001 in relative change) such that the cold albumin group showed an immediate decrease in temperature and increased blood temperature over time, while warm albumin FBT-maintained core temperature was stable throughout (Fig. 1 and 2). In detail, a study group effect was significant on blood temperature (p < 0.001 in relative change), and the warm group showed higher blood temperature during the observation period. Median blood temperature decreased by 0.3˚C immediately after cold albumin FBT but did not change with warm albumin FBT (p < 0.001). However, 30 minutes after FBT, it returned to baseline in the room- temperature albumin group and increased by 0.1˚C in the warm- albumin group (p = 0.053) (Fig 2 and Supplementary Table 3).

There was an interaction between time and study group in mean PAP (p = 0.002 in absolute value and p = 0.002 in relative change) such that mean PAP decreased more rapidly in the warm- albumin group (Fig. 1 and 2). There was no significant study group or time interaction in CVP. Individual hemodynamic changes are summarized in Supplementary Figures 3 and 4.

Other Outcomes

Length of ICU stay, length of hospital stay, or in-hospital mortality was identical between the 2 groups (Supplementary Table 5). There was no significant correlation between the percent of CI increase and the percent of MAP increase from baseline immediately after FBT in the cold- albumin group (p = 0.99) or in the warm- albumin group (p = 0.25) (Supplementary Fig 5).

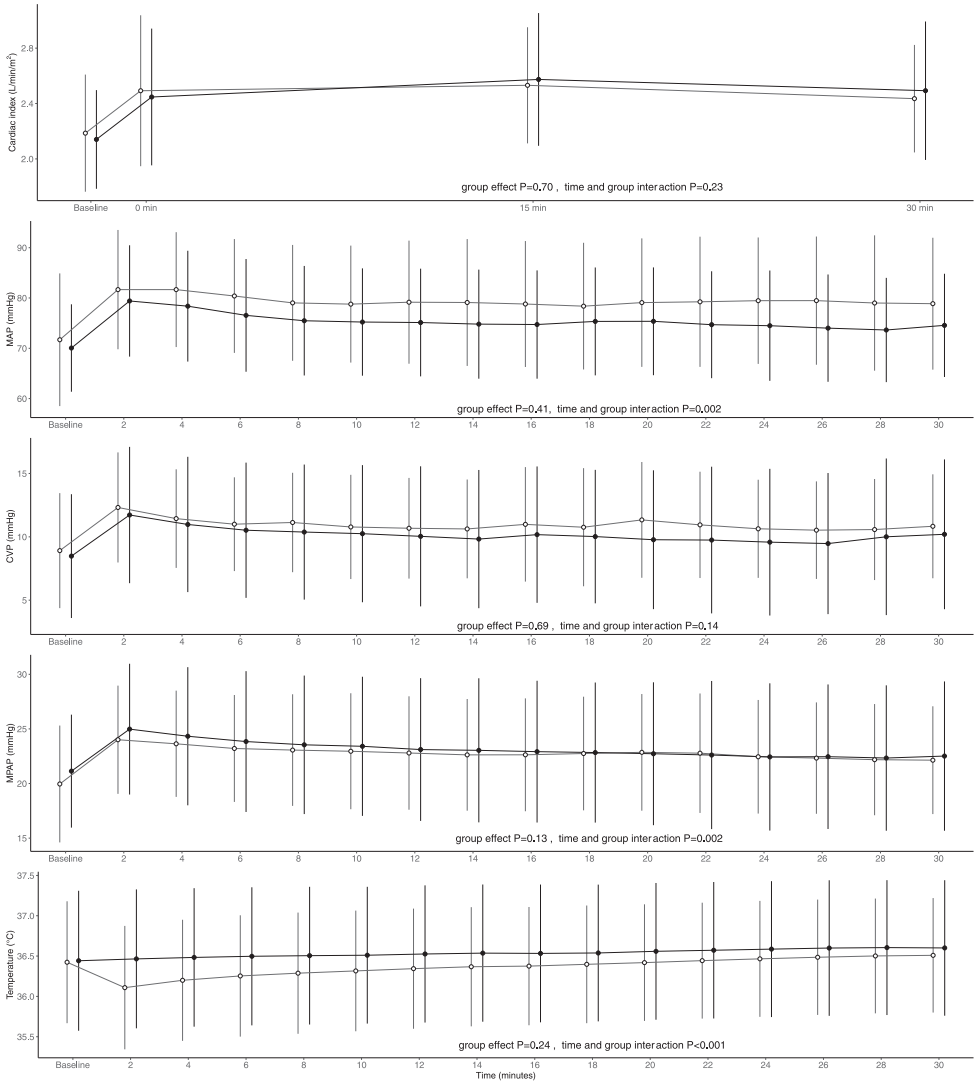

Fig 1. Comparison of the hemodynamic response (absolute value) to a 500-mL room-temperature (cold) 4% albumin fluid bolus (*white*) and to a 500-mL warm 4% albumin fluid bolus (*black*). Data are shown as mean with standard deviation, measured at baseline, and over 30 minutes after the end of the fluid bolus administration. Cardiac index was only reported at 3 time points after the end of bolus administration owing to specific measurement requirements. The asterisk represents a significant difference ($p < 0.05$) at this time point between the cold 4% albumin group (*white*) and the warm 4% albumin group (*black*) adjusted for repeated measurements in a given individual.

Discussion

Key Findings

The authors performed a prospective before-and-after study comparing the hemodynamic and temperature effects of a room temperature (cold) or body temperature (warm) 500-mL bolus of 4% albumin in postoperative cardiac surgery patients using second-to-second measurements. They observed that almost half of the patients in each group achieved an immediate CI-R, with fewer than one-third of these immediate CI-responders showing effect dissipation after 30 minutes. However, the main reason for FBT was hypotension in both groups, and only half of the study patients

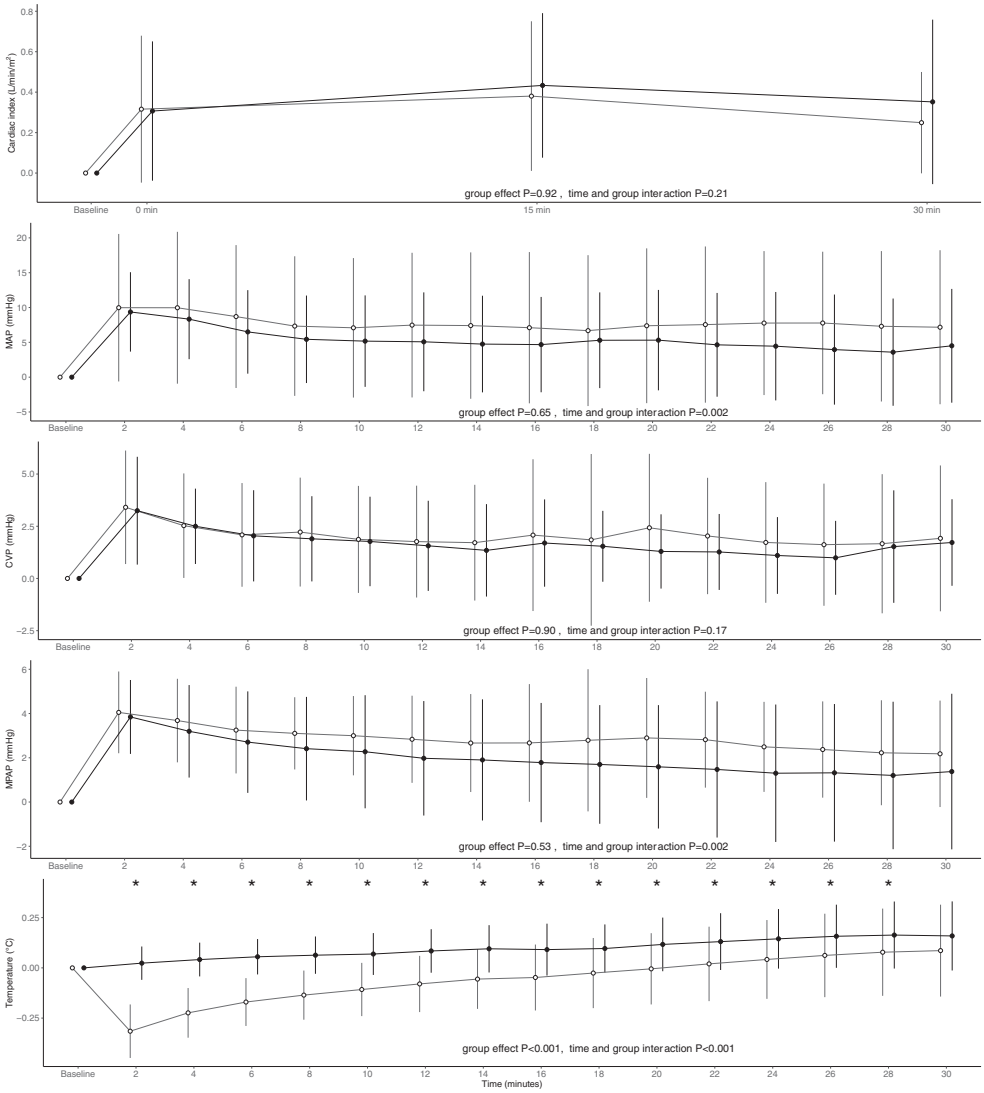

Fig 2. Comparison of the hemodynamic response (as absolute change from baseline) to a 500-mL bolus of room temperature (cold) 4% albumin (*white*) versus a 500-mL bolus of warm 4% albumin (*black*). Data are shown as mean with standard deviation, measured at baseline, and over 30 minutes after the end of the fluid bolus administration. Cardiac index was only reported at 3 time points after the end of bolus administration owing to specific measurement requirements. The asterisk represents a significant difference ($p < 0.05$) at this time point between the cold 4% albumin group (*white*) and the warm 4% albumin group (*black*) adjusted for repeated measurements in a given individual.

in each group achieved an immediate MAP-R, with one-third to one-half developing MAP effect dissipation. Cold- albumin FBT decreased core temperature by 0.3˚C immediately after the bolus and by 0.1˚C 15 minutes after the bolus. With a return to baseline at 30 minutes. No such effect was seen with warm- albumin FBT. There were no differences in clinical outcomes.

Relationship to Previous Studies

To the authors' knowledge, no study had compared the temperature and hemodynamic effects of cold 4% albumin FBT and warm 4% albumin FBT in any patient population, including cardiac surgery. Only 2 studies have reported a hemodynamic

comparison of room- temperature crystalloid (18˚C to 22˚C) infusion and warm (36˚C to 38˚C)- crystalloid infusion in young healthy volunteers.[6,7] One of the studies that measured CI concluded that room- temperature fluid decreased HR and increased MAP. However, warm fluid increased the CI by a greater extent.[7] The 2 studies that examined temperature- measured skin or rectal temperature instead of blood temperature, as in the authors' study. Importantly, the authors' study did not find an HR effect in either group, as most patients were in a paced rhythm. This aspect of cardiac physiology might have affected CI responsiveness to FBT. In addition, propofol sedation may have affected the release of endogenous norepinephrine in response to cold fluid.[8,14]

However, even in this setting, there were significant interactions between fluid temperature and time for core temperature, MAP, and mean PAP. Core temperature remained stable with warm albumin but decreased immediately and then increased with cold albumin and only recovered to equivalent values by 30 minutes. In association with such changes, and despite equivalent changes in CI, MAP demonstrated a different response pattern. With cold albumin showing a slower MAP decrease over time, an effect also seen for mean PAP. These findings suggested a greater degree of peripheral or pulmonary vasoconstriction, possibly due to endogenous norepinephrine release in the cold- albumin group.[8] Atrial natriuretic peptide can also play a role in maintaining intravascular volume because it contributes to shedding of the glycocalyx, shortens the intravascular time of FBT, and is released when patients receive FBT or are exposed to lower temperatures.[15,16] However, the findings of the effect of cold albumin on MAP and PAP do not support an effective potential role of atrial natriuretic peptide in counteracting a likely effect of norepinephrine.

Study implications

The authors' findings imply that, in postcardiac surgery patients, a 500 mL bolus of cold 4% albumin or warm 4% albumin have equivalent CI effects, but a different pattern of MAP response with less reduction with cold albumin after an initial increase. Moreover, their findings imply that warm 4% albumin FBT can achieve these hemodynamic changes without a decrease in core temperature and with a faster reduction in mean PAP after its initial increase. These changes in PAP are small in magnitude but may be beneficial in patients with pulmonary hypertension or right ventricular dysfunction. Furthermore, they imply that a cold albumin can be expected to induce a decrease of 0.3˚C in core temperature that continues for up to 30 minutes after the bolus.

Strengths and Limitations

The authors' study was the first to provide a detailed core temperature and hemodynamic comparison of the effect of FBT with a room- temperature (cold) 4% albumin versus a body- temperature (warm) 4% albumin FBT in patients after cardiac surgery without major confounders for 30 minutes, assessed during a total of 1,800 minutes of observation. Using 4% albumin, 500 mL, for FBT after cardiac surgery reflects common clinical practice in many ICUs in Australia and New Zealand.[4] The authors obtained data on core temperature by means of PA catheters, which provide a clinical standard to measure core temperature. They measured all variables (except CI) on a second-by-second basis, providing the most high-fidelity data set on the comparative effects of these 2 approaches to the choice of fluid temperature for FBT. In addition, to the authors' knowledge, this study was the largest prospective cohort study that used 4% albumin FBT in patients after cardiac surgery.

The authors acknowledge some limitations. Their study was a single-center study and included a limited number of patients. However, the single-center nature of the study diminished the impact of unit-specific confounders, allowing a more accurate assessment of the fluid temperature effect. The FBT occurred at different speeds. However, the infusion speed did not affect the proportion of fluid responders when given within 30 minutes.[17] The limited number of patients reflected the logistically demanding nature of detailed hemodynamic monitoring and the need to exclude patients when significant confounding events precluded an accurate assessment of such effects. Moreover, this was the first research to evaluate the hemodynamic effects, particularly in MAP and mean PAP, of 4% albumin FBT delivered at two different temperatures in patients after cardiac surgery. The authors measured CI intermittently for some patients. Their data (CI measurement at 4 time points) might be underpowered to show a statistically different but small CI change because a previous study measured CI every 20 second for 15 minutes (75 time points) to show a small (0.05 L/min/m^2) difference.[7] However, the CI effect was identical, suggesting that if any such differences were missed, they would have been negligible in magnitude.

Conclusion

In conclusion, cold- albumin FBT immediately lowers core temperature by 0.3˚C compared with warm albumin, and the effect continues for up to 30 minutes. Such effect is associated with no differences in CI responsiveness but is associated with significantly different patterns of MAP and mean PAP changes. Furthermore, although these effects are of limited magnitude and probably of limited clinical relevance, they suggest that in hypothermic patients, the administration of warm albumin may be more physiologically rational as it attenuates the risk of further hypothermia.

Conflict of Interest

F.Y. received a scholarship for a PhD course at Monash University from Japan Student Services Organization and Endeavour scholarship. The funding agencies had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1053/j.jvca.2020.06.045.

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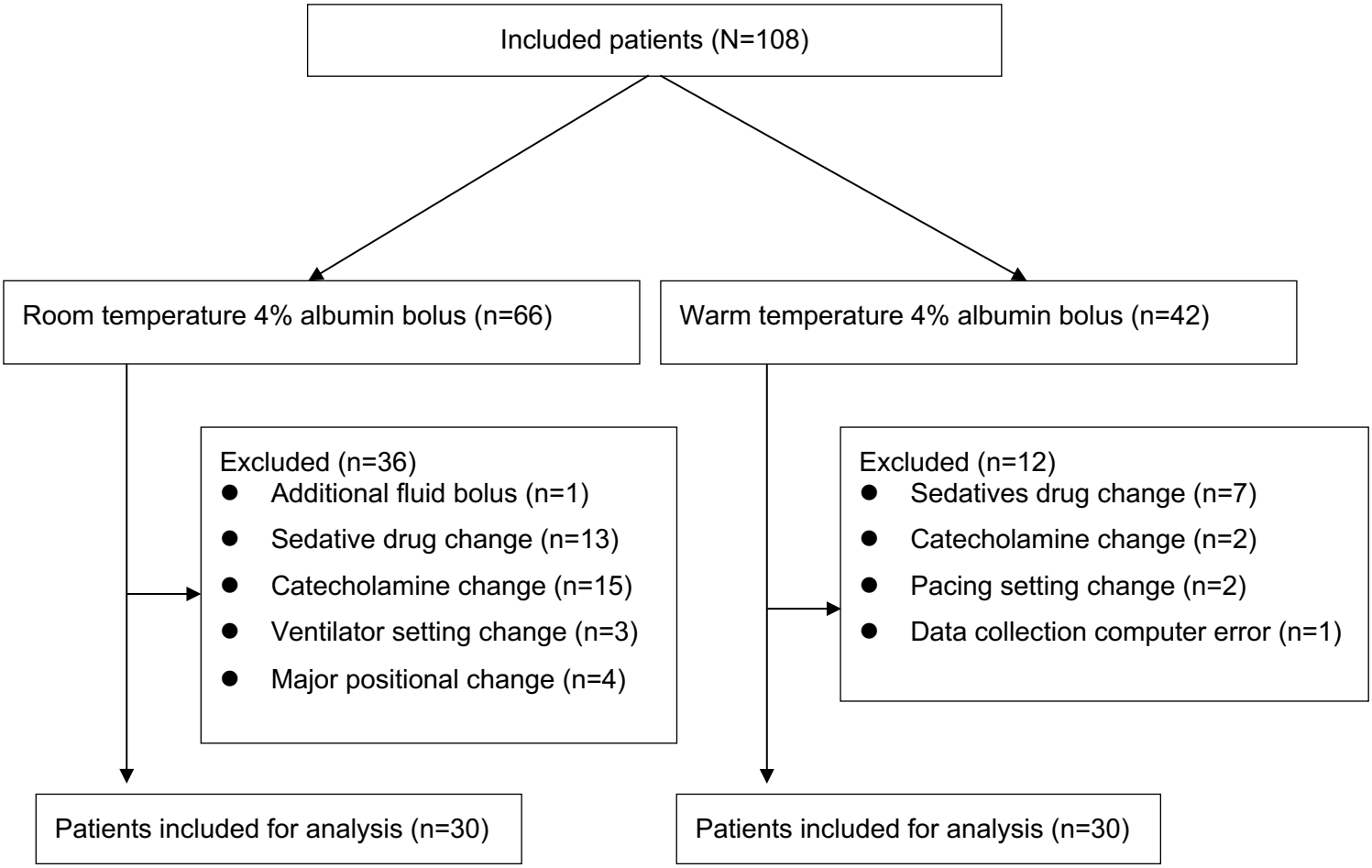
Supplementary Appendix Item 1. Detailed exclusion criteria.

- Additional bolus: Additional fluid bolus was prescribed during the observation period.
- Catecholamine: Any change (bolus or continuous infusion) of catecholamine.
- Sedative drug: Any change (bolus or continuous infusion) of sedative drugs.
- Muscle relaxant: Any dose of muscle relaxant infusion.
- Pacing: Any change of pacing setting.
- Ventilation setting: Any change of ventilator parameters or ventilation setting except for FiO_2 setting.
- Other: Care givers' intervention that may affect patients' homodynamic parameters, for example, tracheal suctioning, patients' movement or movement of transducers.

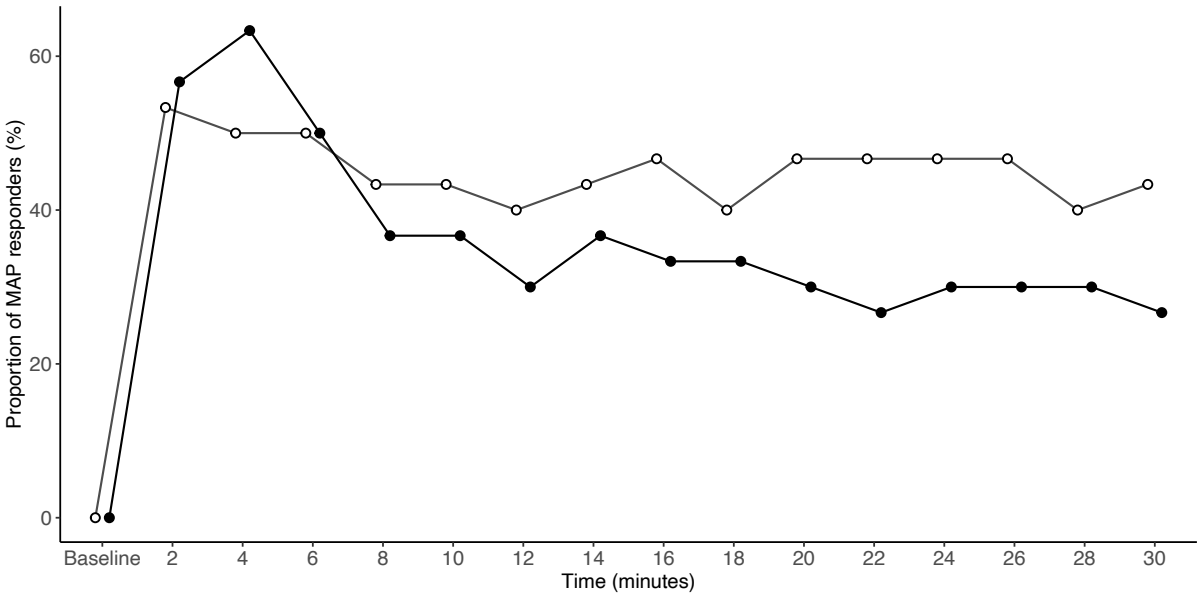
Supplementary Appendix Item 2. Minor cofounders in the study patients.

- Patients awoke during the study periods, but kept stable sedative status without any change of sedative drugs.
- When patients awoke, bedside nurse talked to patients without touching.

Supplementary Figure 1. Flow chart of study inclusion process

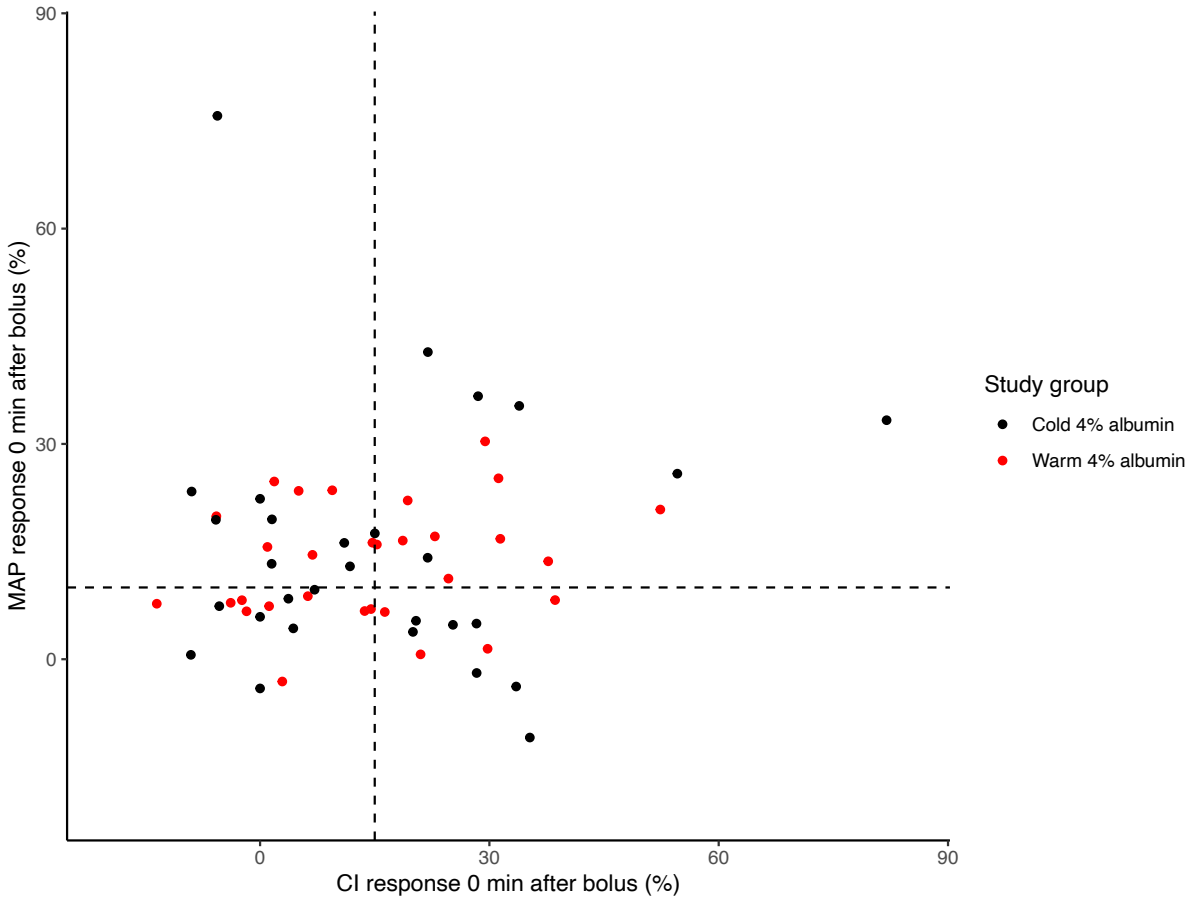


Supplementary Figure 2



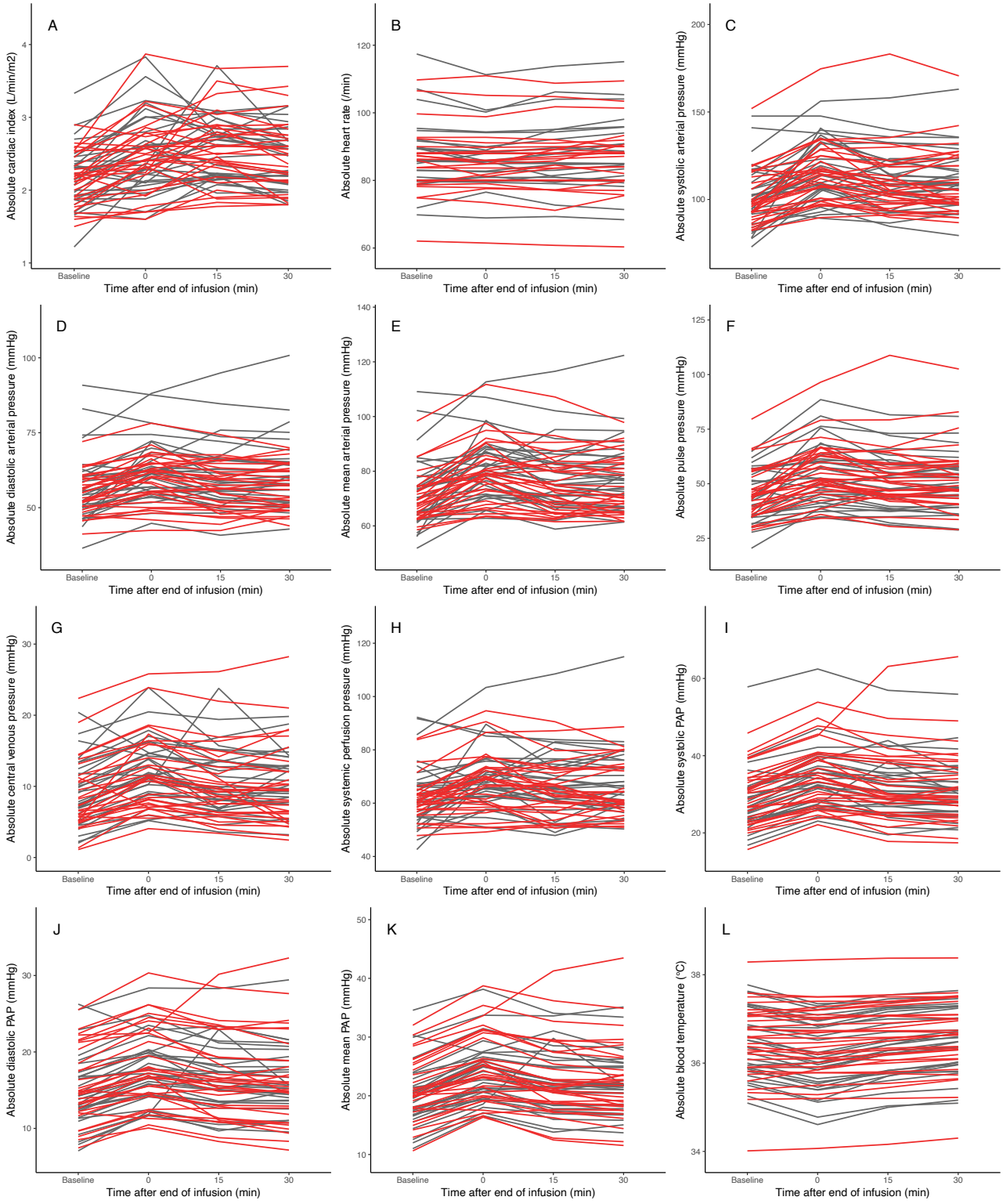
Proportion of MAP responders over time according to cold FBT (white) and warm FBT (black).

Supplementary Figure 3. Scatter plot for %CI response and %MAP response 0 minute after 4% albumin bolus.



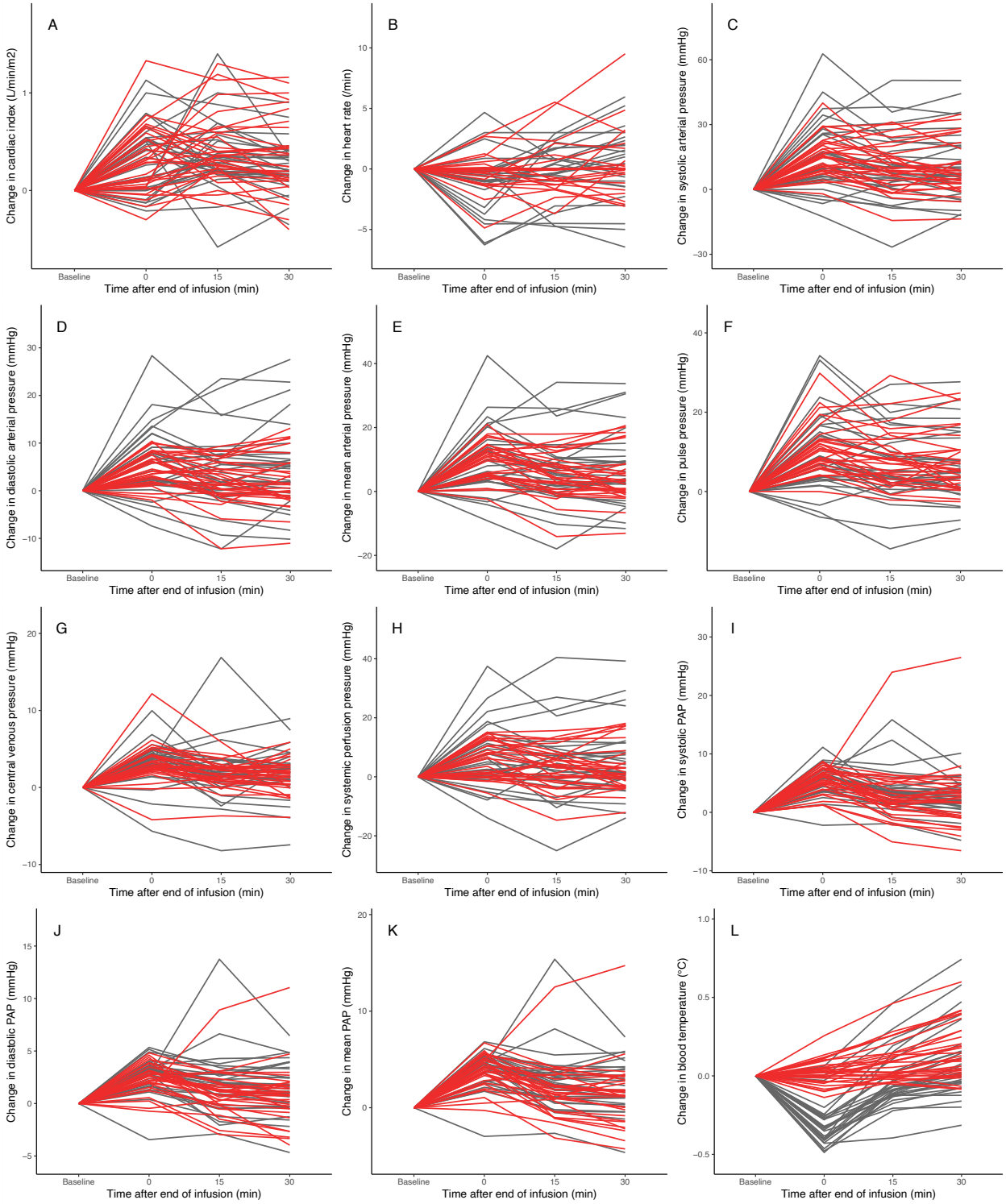
Vertical line shows cardiac index (CI) response (15%) and horizontal line shows mean arterial pressure (MAP) response (10%).

Supplementary Figure 4. Individual hemodynamics and temperature measurements



Data shown are the absolute value for each patient, at each time point for cardiac index (A), heart rate (B), systolic arterial pressure (C), diastolic arterial pressure (D), mean arterial pressure (E), pulse pressure (F), central venous pressure (G), systemic perfusion pressure (H), systolic pulmonary arterial pressure (I), diastolic pulmonary arterial pressure (J), mean pulmonary arterial pressure (K), and body temperature (L). Patients in the room temperature albumin group are represented by a grey line, those in the warm albumin group are represented by a red line.

Supplementary Figure 5. Relative change from baseline of individual hemodynamics and temperature measurements



Data shown is the relative value for each patient, at each time point, for cardiac index (A), heart rate (B), systolic arterial pressure (C), diastolic arterial pressure (D), mean arterial pressure (E), pulse pressure (F), central venous pressure (G), systemic perfusion pressure (H), systolic pulmonary arterial pressure (I), diastolic pulmonary arterial pressure (J), mean pulmonary arterial pressure (K), and body temperature (L). Patients in the room temperature albumin group are represented by a grey line, those in the warm albumin are represented by a red line.

Supplementary Table 1. Characteristics of study patients at the time of fluid bolus

	All patients N = 60	Cold 4% albumin N = 30	Warm 4% albumin N = 30	P
Mechanical ventilation mode				0.42
SIMV	53 (88%)	28 (93%)	25 (83%)	-
PSV	7 (12%)	2 (7%)	5 (17%)	-
Mechanical ventilation settings				
Tidal volume (mL/kg PBW)[§]	7.3 [6.6; 8.1]	7.3 [6.6; 8.3]	7.2 [6.6; 8.0]	>0.99
PIP (cm H_2O)	18 [16; 21]	18 [16; 22]	19 [16; 20]	0.77
PEEP (cm H_2O)	5 [5; 5]	5 [5; 5]	5 [5; 5]	0.97
FiO_2	0.30 [0.25; 0.40]	0.32 [0.30; 0.40]	0.30 [0.22; 0.38]	0.29
PaO_2/FiO_2 ratio	330 [263; 380]	323 [256; 379]	338 [274; 380]	0.64
SpO_2 (%)	99 [96; 100]	99 [97; 100]	99 [96; 100]	0.99
$EtCO_2$ (mm Hg)	36 [33; 40]	38 [33; 41]	35 [33; 39]	0.76
Body temperature control				
External body active warming	16 (27%)	11 (37%)	5 (17%)	0.14
Hemodynamic status				
Vasopressor support	16 (27%)	7 (23%)	9 (30%)	0.77
Milrinone administration	10 (17%)	7 (23%)	3 (10%)	0.30
Heart rhythm				0.60
Paced	33 (55%)	15 (50%)	18 (60%)	-
Sinus rhythm	26 (43%)	14 (47%)	12 (40%)	-
Atrial fibrillation	1 (2%)	1 (3%)	0 (0%)	-
Sedation				
Propofol	57 (95%)	29 (97%)	28 (93%)	>0.99
Propofol dose (mg/h)	100 [100; 150]	100 [100; 150]	100 [100; 150]	0.86
Opioids	2 (7%)	1 (3%)	2 (7%)	>0.99
*Biochemistry**				
pH	7.38 [7.35; 7.41]	7.37 [7.34; 7.42]	7.39 [7.37; 7.4]	0.16
PaO_2 (mm Hg)	123 [86; 234]	102 [78; 165]	173 [101; 261]	0.10
$PaCO_2$ (mm Hg)	43 [40; 45]	44 [40; 46]	42 [40; 44]	0.29
Bicarbonate (mmol/L)	25 [23; 26]	24 [23; 26]	25 [23; 27]	0.20
Lactate (mmol/L)	1.2 [0.9; 1.5]	1.2 [0.9; 1.4]	1.3 [0.9; 1.8]	0.31
Creatinine (µmol/L)	71 [60; 78]	71 [61; 76]	70 [59; 87]	0.96
Hemoglobin (g/L)	107 [92; 115]	105 [90; 115]	108 [95; 115]	0.58
Blood sugar level (mmol/L)	6.8 [6.1; 8.0]	6.8 [6.0; 7.8]	7.5 [6.2; 8.9]	0.25

administration

Data is median [interquartile range], or count (percentage).

*: measured on arterial blood gas closest to FBT. [§]: predicted body weight (ARDS Network formula). P values reflect the between-groups comparison.

$EtCO_2$: end-tidal CO_2 partial pressure; FiO_2: fraction of inspiratory oxygen ; $PaCO_2$: arterial partial pressure of CO_2; PaO_2: arterial partial pressure of oxygen; PBW: predicted body weight; PEEP: positive end-expiratory pressure; PIP: peak inspiratory pressure; PSV: pressure support ventilation; SIMV: synchronized intermittent mandatory ventilation; SpO_2: peripheral capillary oxygen saturation.

Supplementary Table 2. Hemodynamic effect of room temperature albumin fluid bolus and warm 4% albumin bolus (absolute value).

	Time after FB	All patients	Cold 4% albumin	Warm 4% albumin
		N = 60	N = 30	N = 30
Mean arterial pressure (mmHg)	Baseline	69 [63; 74]	70 [62; 77]	68 [64; 74]
	0 min	79 [73; 87]	81 [72; 88]	79 [73; 85]
	15 min	75 [68; 83]	76 [70; 83]	73 [66; 81]
	30 min	75 [67; 84]	77 [71; 85]	71 [66; 83]
Systolic arterial pressure (mmHg)	Baseline	98 [92; 109]	99 [92; 109]	97 [92; 108]
	0 min	114 [105; 128]	114 [106; 133]	115 [102; 125]
	15 min	108 [99; 120]	110 [103; 122]	105 [98; 119]
	30 min	109 [98; 123]	109 [101; 123]	102 [97; 123]
Diastolic arterial pressure (mmHg)	Baseline	55 [50; 60]	55 [50; 60]	54 [51; 59]
	0 min	61 [55; 65]	62 [56; 68]	60 [55; 65]
	15 min	58 [52; 65]	59 [54; 66]	57 [50; 62]
	30 min	59 [51; 65]	60 [54; 65]	57 [50; 63]
Pulse pressure (mmHg)	Baseline	44 [35; 54]	43 [35; 55]	44 [37; 52]
	0 min	52 [45; 64]	52 [42; 61]	54 [49; 64]
	15 min	51 [44; 59]	49 [40; 59]	51 [45; 58]
	30 min	50 [43; 58]	49 [41; 58]	50 [44; 59]
Mean PAP (mmHg)	Baseline	20 [17; 24]	20 [17; 22]	20 [18; 25]
	0 min	24 [20; 27]	24 [21; 27]	24 [20; 31]
	15 min	22 [19; 26]	22 [19; 26]	21 [19; 27]
	30 min	22 [18; 25]	22 [19; 25]	22 [18; 26]
Systolic PAP (mmHg)	Baseline	28 [23; 32]	27 [24; 32]	29 [23; 34]
	0 min	33 [28; 39]	33 [28; 37]	34 [28; 40]
	15 min	30 [26; 37]	31 [27; 35]	30 [26; 38]
	30 min	30 [26; 36]	30 [26; 35]	29 [25; 37]
Diastolic PAP (mmHg)	Baseline	14 [12; 18]	14 [12; 17]	15 [12; 20]
	0 min	17 [14; 21]	17 [15; 20]	17 [14; 22]
	15 min	16 [13; 19]	16 [14; 19]	16 [13; 19]
	30 min	16 [13; 19]	16 [14; 19]	15 [12; 19]
Central venous pressure (mmHg)	Baseline	7 [5; 12]	8 [6; 12]	7 [5; 11]
	0 min	11 [8; 15]	12 [10; 15]	11 [7; 15]
	15 min	9 [7; 14]	10 [7; 14]	9 [6; 12]
	30 min	10 [7; 13]	10 [8; 13]	9 [6; 12]
Heart rate (/min)	Baseline	86 [80; 90]	85 [80; 90]	87 [80; 88]
	0 min	86 [80; 88]	85 [80; 88]	86 [80; 88]
	15 min	86 [80; 89]	85 [80; 90]	88 [80; 89]
	30 min	88 [80; 90]	85 [80; 91]	88 [80; 90]
Cardiac index (L/min/m^2)	Baseline	2.2 [1.9; 2.4]	2.2 [1.9; 2.4]	2.2 [1.9; 2.4]
	0 min	2.4 [2.1; 2.8]	2.3 [2.1; 2.8]	2.4 [2.2; 2.7]
	15 min	2.5 [2.2; 2.8]	2.5 [2.2; 2.8]	2.6 [2.3; 2.9]
	30 min	2.5 [2.1; 2.7]	2.5 [2.1; 2.7]	2.5 [2.1; 2.8]
SVRi (dyn.s.cm^{-5}.m^{-2})	Baseline	2304 [1962; 2627]	2327 [1894; 2772]	2297 [2022; 2533]
	0 min	2151 [1884; 2587]	2314 [1875; 2563]	2132 [1901; 2631]
	15 min	1922 [1781; 2338]	1994 [1771; 2612]	1913 [1804; 2324]
	30 min	2127 [1810; 2383]	2169 [1816; 2748]	2021 [1825; 2361]

Systemic perfusion pressure (mmHg)	Baseline	61 [56; 66]	61 [55; 67]	60 [58; 64]
	0 min	68 [61; 73]	67 [63; 72]	69 [60; 73]
	15 min	65 [60; 73]	66 [60; 74]	64 [57; 71]
	30 min	64 [58; 74]	66 [59; 75]	61 [57; 74]
Blood temperature (°C)	Baseline	36.4 [35.9; 37.1]	36.4 [35.9; 37.1]	36.5 [35.9; 37.1]
	0 min	36.2 [35.8; 37.0]	36.0 [35.5; 36.8]	36.5 [35.9; 37.1]
	15 min	36.5 [35.8; 37.1]	36.4 [35.8; 37.0]	36.6 [36.1; 37.1]
	30 min	36.6 [36.0; 37.1]	36.5 [36.0; 37.2]	36.7 [36.1; 37.1]

Data as median [interquartile range].
***:** $p<0.05$: comparison between the room temperature albumin group and the warm 4% albumin group at each time point, adjusted for the repetition of measurements in a given patient.
FB: fluid bolus; PAP: pulmonary arterial pressure; SVRi: Systemic vascular resistance index.

Supplementary Table 3. Hemodynamic effect of room temperature 4% albumin bolus and warm 4% albumin bolus (relative change from baseline).

	Time after FB	All patients	Cold 4% albumin	Warm 4% albumin
		N = 60	N = 30	N = 30
Mean arterial pressure (mmHg)				
	0 min	10 [4; 14]	9 [3; 15]	11 [5; 13]
	15 min	5 [1; 10]	5 [1; 10]	4 [1; 9]
	30 min	4 [0; 9]	4 [2; 11]	3 [0; 8]
Systolic arterial pressure (mmHg)				
	0 min	15 [7; 23]	13 [5; 26]	15 [9; 21]
	15 min	9 [2; 17]	9 [2; 21]	9 [3; 15]
	30 min	7 [2; 20]	8 [0; 20]	7 [3; 13]
Diastolic arterial pressure (mmHg)				
	0 min	4 [2; 8]	5 [2; 9]	4 [2; 8]
	15 min	2 [0; 7]	3 [0; 7]	1 [-1; 6]
	30 min	2 [-1; 6]	3 [0; 8]	1 [-2; 5]
Pulse pressure (mmHg)				
	0 min	9 [6; 14]	7 [3; 16]	10 [7; 13]
	15 min	5 [2; 12]	4 [2; 12]	7 [2; 11]
	30 min	5 [1; 11]	5 [1; 12]	5 [2; 10]
Mean PAP (mmHg)				
	0 min	4 [3; 5]	5 [4; 5]	4 [3; 5]
	15 min	2 [1; 3]	2 [1; 4]	2 [1; 3]
	30 min	2 [0; 3]	2 [1; 4]	1 [-1; 3]
Systolic PAP (mmHg)				
	0 min	6 [4; 7]	6 [4; 7]	6 [4; 7]
	15 min	3 [1; 4]	3 [2; 4]	2 [1; 5]
	30 min	2 [1; 4]	3 [2; 5]	2 [-1; 4]
Diastolic PAP (mmHg)				
	0 min	3 [2; 4]	3 [2; 4]	3 [2; 3]
	15 min	1 [0; 2]	2 [1; 3]	1 [0; 2]
	30 min	1 [0; 2]	2 [0; 3]	1 [-1; 2]
Central venous pressure (mmHg)				
	0 min	3 [2; 5]	4 [3; 5]	3 [2; 4]
	15 min	2 [1; 3]	2 [1; 3]	2 [1; 3]
	30 min	2 [0; 3]	2 [1; 3]	2 [0; 3]
Heart rate (/min)				
	0 min	0 [-1; 0]	0 [-1; 0]	0 [-1; 0]
	15 min	0 [-1; 1]	0 [0; 1]	0 [-1; 0]
	30 min	0 [-1; 1]	0 [-1; 2]	0 [0; 0]
Cardiac index (L/min/m^2)				
	0 min	0.3 [0.0; 0.5]	0.3 [0.0; 0.5]	0.3 [0.0; 0.5]
	15 min	0.3 [0.2; 0.6]	0.3 [0.2; 0.5]	0.4 [0.2; 0.6]
	30 min	0.2 [0.1; 0.4]	0.2 [0.2; 0.4]	0.2 [0.1; 0.6]
SVRi (dyn.s.cm^{-5}.m^{-2})				
	0 min	-84 [-291; 124]	-59 [-290; 147]	-111 [-283; 95]
	15 min	-212 [-480; -30]	-203 [-484; 75]	-230 [-455; -85]
	30 min	-122 [-371; 124]	-86 [-246; 198]	-138 [-533; 65]
Systemic perfusion pressure (mmHg)				
	0 min	6 [0; 12]	5 [0; 11]	6 [0; 12]
	15 min	4 [-1; 8]	4 [-1; 8]	4 [-1; 7]
	30 min	2 [-2; 8]	2 [-1; 9]	1 [-3; 7]
Blood temperature (°C)				
	0 min	-0.1 [-0.3; 0.0]	-0.3 [-0.4; -0.3] *	0.0 [0.0; 0.1] *
	15 min	0.0 [-0.1; 0.1]	-0.1 [-0.1; 0.0] *	0.1 [0.0; 0.1] *
	30 min	0.1 [0.0; 0.2]	0.0 [0.0; 0.2]	0.1 [0.0; 0.3]

Data as median [interquartile range].
***:** p<0.05 between the room temperature 4% albumin group and the warm 4% albumin group at each time point, adjusted for the repetition of measurements in a given patient.
FB: fluid bolus; PAP: pulmonary arterial pressure; SVRi: Systemic vascular resistance index.

Supplementary Table 4. Dissipation of the hemodynamic response (CI and MAP)

	All patients	Cold 4% albumin	Warm 4% albumin	P
Early CI responders	N=28	N=14	N=14	
Absence of dissipation at 30 minutes*	22 (79%)	10 (71%)	12 (86%)	0.79
Effect dissipated at 15 minutes	2 (7%)	1 (7%)	1 (7%)	
Effect dissipated at 30 minutes	4 (12%)	3 (21%)	1 (7%)	
Early MAP responders	N=36	N=16	N=17	
Absence of dissipation at 30 minutes§	19 (53%)	11 (69%)	8 (47%)	0.30
Effect dissipated	14 (39%)	5 (31%)	9 (53%)	
Time to dissipation (min)	12 [9; 13]	12 [9; 14]	12 [9; 13]	

Data is median [interquartile range], or count (percentage).
*: defined as a CI within 5% of baseline CI
§: defined as a MAP within 3 mm Hg of baseline
CI: cardiac index; MAP: mean arterial pressure

Supplementary Table 5. Other clinical outcomes.

	Cold 4% albumin	Warm 4% albumin	P
Length of ICU stay (days)	1.9 [1.0, 2.7]	1.9 [1.0, 3.2]	0.94
Length of hospital stay (days)	7.9 [6.2, 11.6]	9.4 [7.3, 13.8]	0.11
In hospital mortality (%)	0 (0)	0 (0)	NA

Chapter 3: Warm vs. Cold 20% albumin fluid bolus therapy in cardiac surgery patients

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Temperature and haemodynamic effects of a 100 mL bolus of 20% albumin at room versus body temperature in cardiac surgery patients

Fumitaka Yanase, Salvatore L Cutuli, Thummaporn Naorungroj, Laurent Bitker, Alessandro Belletti, Anthony Wilson, Glenn M Eastwood and Rinaldo Bellomo

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Abstract

Objective: To study the temperature and haemodynamic effects of room versus body temperature 20% albumin fluid bolus therapy (FBT).
Design: Single-centre, prospective, before–after trial.
Setting: A tertiary intensive care unit (ICU) in Australia.
Participants: Sixty ventilated post-cardiac surgery patients.
Intervention: Room versus body temperature 100 mL 20% albumin FBT.
Main outcome measures: We recorded haemodynamic data from FBT start to 30 minutes after FBT. The cardiac index (CI) response was defined by a CI increase > 15%, and the mean arterial pressure (MAP) response was defined by a MAP increase > 10%.
Outcomes: Immediately after FBT, median blood temperature decreased by −0.1°C (interquartile range [IQR], −0.1–0.0°C) with room temperature albumin versus 0.0°C (IQR, −0.1–0.0°C) with body temperature albumin ($P < 0.001$). The CI or MAP responses were similar. There was, however, a time and study group interaction for blood temperature ($P < 0.001$) for absolute and relative changes. In addition, mean pulmonary arterial pressure (PAP) ($P = 0.002$) increased more with body temperature albumin and remained higher for most of the observation period.
Conclusion: Compared with room temperature albumin FBT, body temperature 20% albumin FBT prevents FBT-associated blood temperature fall and increases mean PAP. However, CI and MAP changes were the similar between the two groups, implying that fluid temperature has limited haemodynamic effects in these patients.

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Hypotension, low cardiac index (CI) or both are common after cardiac surgery[1,2] and fluid bolus therapy (FBT) is the usual initial treatment. The aim of FBT is to achieve optimal intravascular volume, CI and mean arterial pressure (MAP).[3,4] However, to achieve such goals, postoperative cardiac surgery patients may receive almost 2 L of crystalloid-based FBT in the first 24 hours.[3] Unfortunately, such therapy contributes to fluid overload.

The fluid overload associated with crystalloid FBT may contribute to organ dysfunction.[5-7] Therefore, the use of colloid FBT is the preferred treatment,[3,8] but regrettably starch-based colloids increase the incidence of acute kidney injury and the risk of bleeding.[9,10] Gelatin- or dextran-based solutions have similar adverse effects.[11,12] However, hyperoncotic (20%) albumin may be a more rational choice because it delivers one-fifth of the volume administered with iso-oncotic albumin solution.[13-15] An additional concern is that FBT fluid is typically given at room temperature. As post-cardiac surgery patients are often hypothermic on intensive care unit (ICU) admission, and hypothermia may contribute to postoperative coagulopathy, the delivery of "cold" FBT may be unhelpful, making fluid warming more rational.[16,17]

In keeping with the above notions, warming fluids from room to body temperature prevented decreases in body temperature and improved cardiac output in volunteers.[18,19] Thus, body temperature 20% albumin FBT may be a physiologically logical choice after cardiac surgery. However, no investigations have assessed the temperature and haemodynamic changes induced by warm versus cold 20% albumin FBT. Accordingly, we assess whether the temperature and haemodynamic changes induced by 20% body temperature albumin FBT would differ from those seen with room temperature 20% albumin FBT.

Methods

Ethics approval

This study was approved by the institutional Ethics Committee (reference number LNR/16/Austin/358, for the body temperature albumin study; and LNR/16/Austin/548, for the room temperature albumin study). The need for consent was waived by the Ethics Committee because of the observational nature of the study and the fact that 20% albumin is frequently used for FBT in the study ICU.

Study design

We conducted a sequential before–after study from July 2017 to May 2020 of patients prescribed 20% albumin FBT according to clinician preference. The first group of 30 patients received room temperature 20% albumin FBTs as previously described, and in the second group, 30 patients received body temperature 20% albumin. A pilot study of the room temperature albumin group has been previously reported.[20]

We included adult patients (aged ≥ 18 years) who were admitted to the ICU after on-pump cardiac surgery. They all had to be receiving mechanical ventilation and to have a pulmonary artery catheter in place. All patients had to be prescribed 100 mL 20% albumin FBT for a haemodynamic indication by the treating clinical team.

We excluded patients if any intervention with haemodynamic effect was necessary during the 30-minute observation period (Online Appendix, item 1). We excluded pregnant patients or those who required mechanical haemodynamic support (ie, intra-aortic balloon counterpulsation or extracorporeal membrane oxygenation).

Data collection

We have previously described the detailed data collection method.[21] In all patients, we recorded core temperature, systolic arterial pressure, diastolic arterial pressure, MAP, central venous pressure (CVP), systolic pulmonary artery pressure (PAP), diastolic PAP, mean PAP, heart rate and peripheral oxygen saturation (Spo_2) on a second-by-second basis using MediCollector logging software (MediCollector, Boston, MA, USA).

We measured CI by using the continuous or intermittent method depending on the type of pulmonary artery catheter. When patients did not have a continuous cardiac output pulmonary artery catheter, the research team performed intermittent measurements of CI at four time points: before FBT, immediately after FBT, 15 minutes after FBT, and 30 minutes after FBT.

A research team member observed study patients for the full period and recorded all interventions. When the patient unexpectedly needed other interventions that met the exclusion criteria (Online Appendix, item 1), they were removed from the study. However, when they met minor confounders (Online Appendix, item 2), recording continued and the patient was included for analysis.

Fluid bolus therapy

The intervention was the administration of 100 mL of 20% albumin (Albumex 20, CSL Behring, Melbourne, VIC, Australia). FBT was prescribed based on the clinical team's decision. In the room temperature albumin group, patients received 20% albumin stored at room temperature. In contrast, in the body temperature albumin group, during infusion,

albumin was warmed to between 37°C and 40°C by a fluid warmer (enFlow [Vital Signs, Totowa, NJ, USA] or HOTLINE [Smiths Medical ASD, Rockland, MA, USA]). After a possible risk of aluminium release from the enFlow fluid warmer was reported, we used the HOTLINE system in all patients.[22] All fluid boluses were infused rapidly using a hand pump infusion system with a compressible reservoir.

Haemodynamic response definitions

We defined the CI response (CI-R) as a CI increase greater than 15% from the baseline and the MAP response (MAP-R) as a MAP increase greater than 10%. We defined the immediate CI-R or immediate MAP-R if the CI-R or MAP-R occurred immediately after FBTs.

We also pre-defined time to dissipation of the CI effect as the time taken for a patient's CI to fall back to within 5% of baseline. We pre-defined time to dissipation of the MAP effect as the time taken for a patient's MAP to fall back to within 3 mmHg of baseline for at least 2 consecutive minutes.

The primary hypothesis was that the core temperature response would differ between body temperature and room temperature 20% albumin groups. The secondary hypothesis was that the CI and MAP response would also differ between the two groups. The exploratory hypothesis was that other haemodynamic variables might respond differently according to study group.

Sample size calculation

Based on data from our previous research, the standard deviation for temperature change by room temperature 20% albumin FBT was known to be 0.13°C after room temperature 20% albumin infusion.[20] We estimated that, with 30 patients in each group, we would have an 80% power (at a two-sided *P* value of 0.05) to detect a difference in temperature change between the two groups equivalent to 73% of the standard deviation (equivalent to a –0.1°C overall body temperature difference).

Data processing before analysis

We applied the same data processing before analysis.[21] We excluded both negative CVP values and values outside three standard deviations for all variables because there were several noisy data; for example, high CVP values during intermittent CI measurement or line flushing. Baseline haemodynamic parameters were calculated by the mean value from 3 minutes before FBT to the start of FBT. For ease of presentation, we illustrated data by 2 minutes basis by calculating its values as the mean value over 120 seconds. We analysed post-bolus data from 0 to 30 minutes after FBT with second-to-second data acquisition.

Statistical analysis

We analysed data using R software, version 3.5.2 (The R Foundation, Vienna, Austria). We reported continuous variables as median with interquartile range (IQR) and categorical variables as count with percentage. We compared all baseline characteristics using Fisher exact test for categorical variables and the Mann–Whitney U test for continuous variables. We applied the Spearman correlation test to evaluate the relationship between CI changes and MAP changes immediately after FBT. We analysed haemodynamic variables using linear mixed effects models, accounting for within subject repeated measures, and treating time as a continuous variable between the room temperature albumin group and the body temperature albumin group. When a study group effect or an interaction between time and the study group was significant, we performed post hoc analysis to examine the significance of the difference at each time point, accounting for the α inflation risk using the Tukey adjustment method. We considered a two-sided *P* value below 0.05 as statistically significant.

Results

We screened 46 patients in the room temperature albumin group and 34 patients in the body temperature albumin group (Online Appendix, supplementary figure 1) to achieve 30 patients in each group. Table 1 and the Online Appendix, supplementary table 1, show the characteristics of the study patients. Both groups were well balanced except for a small difference in haemoglobin value. In particular, core temperature was equivalent in both groups before the intervention (Table 2). All relevant haemodynamic parameters before FBT are shown in Table 2. Such parameters were also well balanced.

Temperature changes after fluid bolus therapy

There was an interaction between time and study group in mean core temperature ($P < 0.001$ for absolute value) (Figure 1) and relative change ($P < 0.001$) (Figure 2). Blood temperature in the room temperature albumin group fell, but there were no changes in the body temperature albumin FBT group.

Cardiac index changes after fluid bolus therapy

There was no time and study group interaction for the changes in CI both in absolute terms (Figure 1) and as relative changes (Figure 2). Immediately after FBT, the median CI increased in a statistically equivalent way in both groups (Online Appendix, supplementary tables 2 and 3). The number of CI responders at each time point was not statistically significant between the two groups (Table 3). Among immediate CI responders, one patient (10%) in the room temperature albumin group and two (29%) in the body temperature albumin group showed CI effect dissipation during the study period ($P = 0.15$) (Online Appendix, supplementary table 4)

Mean arterial pressure changes after fluid bolus therapy

There was no group effect or significant time and study group interaction for the changes in MAP both in absolute terms ($P = 0.22$ and $P = 0.68$) (Figure 1) and as relative changes ($P = 0.08$ and $P = 0.98$) (Figure 2). The median MAP increased by 9 mmHg (IQR, 6–13 mmHg) immediately after FBT in the room temperature albumin group and by 5 mmHg (IQR, 1–14 mmHg) in the body temperature albumin group (Online Appendix, supplementary tables 2 and 3).

The number of MAP responders at each time point was not statistically significant between the two groups (Table 3). Among immediate MAP responders, nine patients (50%) in the room temperature albumin group and four patients (33%) in the body temperature albumin group showed effect dissipation ($P = 0.47$).

Cardiac index and mean arterial pressure correlation

Immediately after the FBT, there was no correlation between changes in CI and change in MAP in the room temperature group ($P = 0.70$) or the body temperature group ($P = 0.52$) (Online Appendix, supplementary figure 2).

Additional haemodynamic changes

There was an interaction between time and study group in mean PAP ($P = 0.002$ in absolute value and relative change). Moreover, mean PAP in the body temperature albumin group returned to baseline more slowly after the initial response and from 8 to 24 minutes after FBT; mean PAP in the body temperature albumin group was significantly higher than in the room temperature albumin group (Figure 2). There were small mean increases (2 mmHg) in each group and we observed almost the same number of CVP responders in each group (Table 3 and Online Appendix, supplementary table 3).

Individual haemodynamic changes are summarised in the Online Appendix, supplementary figures 3 and 4.

Discussion

Key findings

In a cohort of postoperative cardiac surgery patients, we compared the temperature effect of room temperature versus body temperature 20% albumin 100 mL FBT as well as their differential haemodynamic effects. We observed a significantly different core temperature response both in absolute terms and in relative terms, such that FBT with body temperature 20% albumin prevented the decrease in temperature induced by room temperature 20% albumin. We observed, however, that there was no time and group interaction for changes in CI or MAP and in the number of CI responders or MAP responders. In contrast, there was an interaction for the mean PAP, with a higher mean PAP in the body temperature albumin group.

Relationship with previous studies

To our knowledge, this is the first study to report the differential temperature effects of hyperoncotic albumin FBT in any group of patients or patients after cardiac surgery. Our findings that a decrease in temperature could be prevented are aligned with those of two previous studies in volunteers.[18,19]

Hyperoncotic albumin solution has some theoretical advantages in patients after cardiac surgery because of a less positive fluid balance and fewer subsequent episodes of FBT.[15] In addition, most 20% albumin preparations are essentially chloride-free, while iso-oncotic albumin preparations may contain up to 128 mmol/L of chloride and all crystalloids contain more than 100 mmol/L. Thus, 20% albumin may attenuate chloride-induced changes in glomerular filtration rate.[14] A controlled trial showed that the supplementary administration of 20% albumin in patients undergoing off-pump coronary aortic bypass graft with a baseline albumin level below 4.0 g/dL reduced the risk of postoperative acute kidney injury,[23] but our short term study could not address these issues.

In this study, there was no difference in CI response. In contrast, warm crystalloid infusion increased CI more than cold crystalloid infusion in healthy volunteers.[18] However, warm FBT increased the heart rate in volunteers which contributed to the increased CI. In contrast, most of our patients were in a paced rhythm and unable to increase their heart rate.

Study implications

Our findings imply that, in post-cardiac surgery patients, a 100 mL bolus of body temperature 20% albumin prevents the fall in core temperature seen after room temperature FBT. However, they also imply that, when given according to

clinical indications, body temperature 20% albumin FBT achieves similar CI effects to room temperature 20% albumin FBT.

Strengths and limitations

To the best of our knowledge, our study is the first to describe the comparison of temperature and haemodynamic changes induced by room temperature or body temperature 20% albumin FBT in patients after cardiac surgery in the absence of major confounders. We recorded such detailed haemodynamic data for all variables except the CI, where measurements every 15 minutes were applied. Finally, at least one researcher observed the patient for the whole study period to exclude haemodynamic confounders.

There are several limitations to our study. This was a single-centre study but the physiological effects are unlikely to be centre-dependent. Moreover, it assessed responses in a limited number of patients, which might have affected our ability to detect additional differential effects. However, obtaining second-by-second detailed haemodynamic data in the absence of confounders is difficult. In particular, it requires physical researcher presence at the bedside, which is time-consuming but necessary, as demonstrated by the fact that 20 patients had to be excluded because of major confounders during the study period. We did not record haemodynamic changes beyond 30 minutes. However, changes in sedative drug, vasopressor or inotropic infusion are very common in these patients and would have resulted in more than 50% of study patients having to be excluded. In fact, we are not aware of any study in post-cardiac surgery patients that has assessed the haemodynamic response to fluid therapy both beyond 30 minutes and in the absence of such confounders. Of relevance, 40% of patients in the room temperature group used an external warming device during the observation period and this might have attenuated the decrease in body temperature. Nevertheless, passive warming after cardiac surgery is common and we wished to study the impact of fluid temperature in a usual care setting. In addition, a small number of patients woke up during the observation period (minor confounder), which might have affected the results. However, relatively light sedation in the ICU is common, making small episodes of awakening also relatively common.[24]

Conclusion

Body temperature 20% albumin FBT prevented the decrease in core temperature induced by room temperature 20% albumin FBT. Moreover, body temperature 20% albumin was associated with a similar CI effect as room temperature 20% albumin, and the effect of body temperature 20% albumin on mean PAP was significantly greater. These findings can inform the choice of both FBT solution and temperature in the care of post-cardiac surgery patients.

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Competing interests

None declared.

Author details

Fumitaka Yanase[1,2]
Salvatore L Cutuli[1,3]
Thummaporn Naorungroj[1,4]
Laurent Bitker[1,5]
Alessandro Belletti[1,6]
Anthony Wilson[1,7]
Glenn M Eastwood[1]
Rinaldo Bellomo[1,2,8]
1 Department of Intensive Care, Austin Hospital, Melbourne, VIC, Australia.
2 Australian and New Zealand Intensive Care Research Centre, Melbourne, VIC, Australia.
3 School of Public Health and Preventive Medicine, Monash University, Melbourne, VIC, Australia.
4 Dipartimento di Scienze dell'emergenza, anestesiologiche e della Rianimazione, Rome, Italy.
5 Fondazione Policlinico Universitario A Gemelli IRCCS, Rome, Italy.
6 Department of Intensive Care, Siriraj Hospital, Mahidol University, Bangkok, Thailand.
7 Service de Médecine Intensive et Réanimation, Hôpital de la Croix-Rousse, Hospices Civils de Lyon, Lyon, France.
8 Department of Anaesthesia and Intensive Care, IRCCS San Raffaele Scientific Institute, Milan, Italy.
9 Adult Critical Care, Manchester University Hospitals NHS Foundation Trust, Manchester, United Kingdom.
10 Centre for Integrated Critical Care, School of Medicine, University of Melbourne, Melbourne, VIC, Australia.
***Correspondence*:** rinaldo.bellomo@austin.org.au

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[Insert boxes]

[Table 1]

Table 1. Baseline characteristics

	All patients	Room temperature 20% albumin	Body temperature 20% albumin	*P**
Total number of patients	60	30	30	
Age (years); median (IQR)	70 (63–77)	70 (65–78)	72 (59–77)	0.62
Sex (male)	48 (80%)	23 (77%)	25 (83%)	0.75
Body mass index (kg/m^2), median (IQR)	27.8 (25.1–30.5)	28.1 (25.1–30.7)	27.7 (25.2–30.1)	0.76
Comorbidities				
Atrial fibrillation	7 (12%)	5 (17%)	2 (7%)	0.24
COPD	1 (2%)	1 (3%)	0 (0%)	0.48
Chronic kidney disease	7 (12%)	3 (10%)	4 (13%)	> 0.99
Diabetes mellitus	17 (28%)	9 (30%)	8 (27%)	> 0.99
Hypertension	42 (70%)	20 (67%)	22 (73%)	> 0.99
Ischaemic heart disease	44 (73%)	20 (67%)	24 (80%)	0.75
APACHE III score, median (IQR)	42 (35–48)	41 (34–45)	45 (37–50)	0.17
EuroSCORE, median (IQR)	5 (4–7)	5 (4–7)	5 (2–7)	0.37
Type of surgery				0.23
On-pump CABG	33 (55%)	13 (43%)	20 (67%)	
Valve	17 (28%)	11 (37%)	6 (20%)	
CABG + valve	7 (12%)	5 (17%)	2 (7%)	
Other	3 (5%)	1 (3%)	2 (7%)	
Urgency of surgery				0.14
Elective	44 (73%)	19 (63%)	25 (83%)	
Non-elective	16 (27%)	11 (37%)	5 (17%)	
CPB duration (min), median (IQR)	114 (91–146)	118 (86–144)	112 (92–148)	0.92
Aorta clamp duration (min), median (IQR)	88 (71–122)	88 (70–120)	88 (74–122)	0.80
Post-CPB TOE assessment				
Left ventricular dysfunction	11 (18%)	8 (30%)	3 (11%)	0.10
Right ventricular dysfunction	5 (8%)	2 (7%)	3 (11%)	> 0.99

APACHE = Acute Physiology and Chronic Health Evaluation; CABG = coronary aortic bypass graft; COPD = chronic obstructive pulmonary disease; CPB = cardiopulmonary bypass; EuroSCORE = European System for Cardiac Operative Risk Evaluation; IQR = interquartile range; TOE = transoesophageal echocardiography. * *P* values reflect the between-groups comparison.

[Table 2]

Table 2. Baseline haemodynamics

	All patients	Room temperature 20% albumin	Body temperature 20% albumin	*P**
Total number of patients	60	30	30	
Blood temperature (°C)	36.1 (35.6–37.0)	36.2 (35.4–37.0)	36.1 (35.9–36.9)	0.77
Systolic arterial pressure (mmHg)	100 (89–110)	98 (89–111)	101 (89–108)	0.95
Diastolic arterial pressure (mmHg)	53 (48–61)	52 (48–58)	55 (49–61)	0.45
Pulse pressure (mmHg)	47 (36–53)	47 (36–54)	46 (37–51)	0.50
Mean PAP (mmHg)	19 (17–23)	19 (16–23)	19 (17–23)	0.87
Systolic PAP (mmHg)	28 (23–32)	26 (22–31)	28 (25–32)	0.53
Diastolic PAP (mmHg)	14 (12–17)	14 (11–18)	14 (12–17)	0.95
Central venous pressure (mmHg)	8 (6–10)	8 (6–10)	8 (6–10)	0.77
Heart rate (beats/min)	88 (80–90)	88 (80–90)	86 (79–88)	0.36
Cardiac index (L/min/m^2)	2.1 (1.7–2.6)	2.2 (1.8–2.8)	2.0 (1.7–2.5)	0.30
Stroke volume index (mL/m^2)	26 (20–32)	27 (20–33)	26 (21–30)	0.71
SVRi (dyn*s/cm^5*m^2)	2198 (1783–2951)	2099 (1737–2702)	2507 (1818–3020)	0.30
Systemic perfusion pressure (mmHg)	61 (55–68)	60 (56–65)	63 (55–69)	0.60

CI = cardiac index; PAP = pulmonary arterial pressure; SVRi = Systemic Vascular Resistance Index. Data are presented as median (interquartile range). * *P* values reflect the between-groups comparison.

[Table 3]

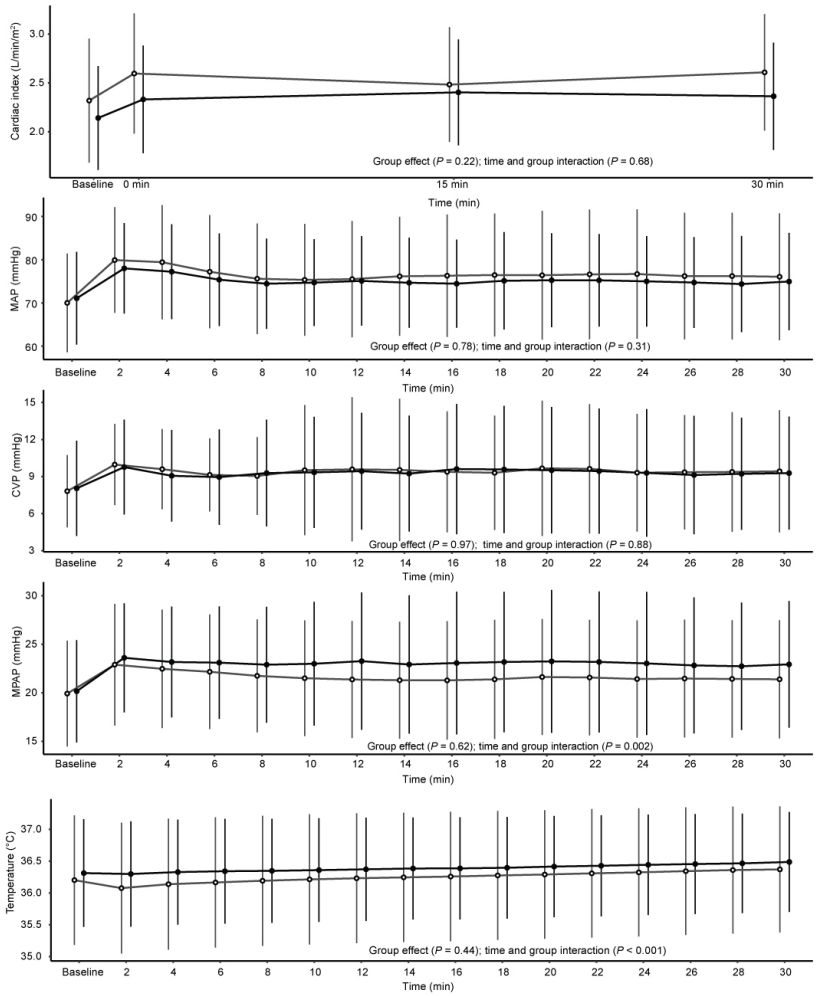
Table 3. Fluid bolus characteristics and hemodynamic response

	All patients	Room temperature 20% albumin	Body temperature 20% albumin	*P**
Total number of patients	60	30	30	
Time from ICU admission to FBT (hour), median (IQR)	1.4 (0.9–2.5)	1.1 (0.9–2.7)	1.6 (0.9–2.4)	0.74
Fluid bolus indication				0.35
Tachycardia	1 (2%)	1 (3%)	0 (0%)	
Low cardiac output	15 (25%)	9 (30%)	6 (20%)	
Low filling pressures	6 (10%)	3 (10%)	3 (10%)	
Hypotension	36 (60%)	15 (50%)	21 (70%)	
Other	2 (3%)	2 (7%)	0 (0%)	
Duration of fluid bolus infusion (min), median (IQR)	3.2 (2.5–4.9)	3.4 (2.5–7.6)	3.0 (2.5–4.0)	0.20
CI response				
At end of bolus administration	17 (28%)	10 (33%)	7 (23%)	0.57
At 15 min of bolus administration	18 (38%)†	7 (39%)†	11 (37%)	> 0.99
At 30 min of bolus administration	25 (42%)	15 (50%)	10 (33%)	0.30
MAP response				
At end of bolus administration	30 (50%)	18 (60%)	12 (40%)	0.20
At 15 min of bolus administration†	19 (32%)	13 (43%)	6 (20%)	0.10
At 30 min of bolus administration	20 (33%)	14 (47%)	6 (20%)	0.054
CVP response‡	20 (33%)	9 (30%)	11 (37%)	0.79
Confounding event§				
Minor event	5 (8%)	1 (3%)	4 (13%)	0.35

CI = cardiac index; CVP = central venous pressure; FBT = fluid bolus therapy; ICU = intensive care unit; MAP = mean arterial pressure.
* *P* values reflect the between-groups comparison. † Twelve patients in the room temperature albumin group did not have the CI measured at 15 minutes after FBT. ‡ Defined as +2 mmHg increase in CVP from baseline value, at the end of the bolus. § Refer to the Online Appendix, item 2, for definitions.

[Figure 1]

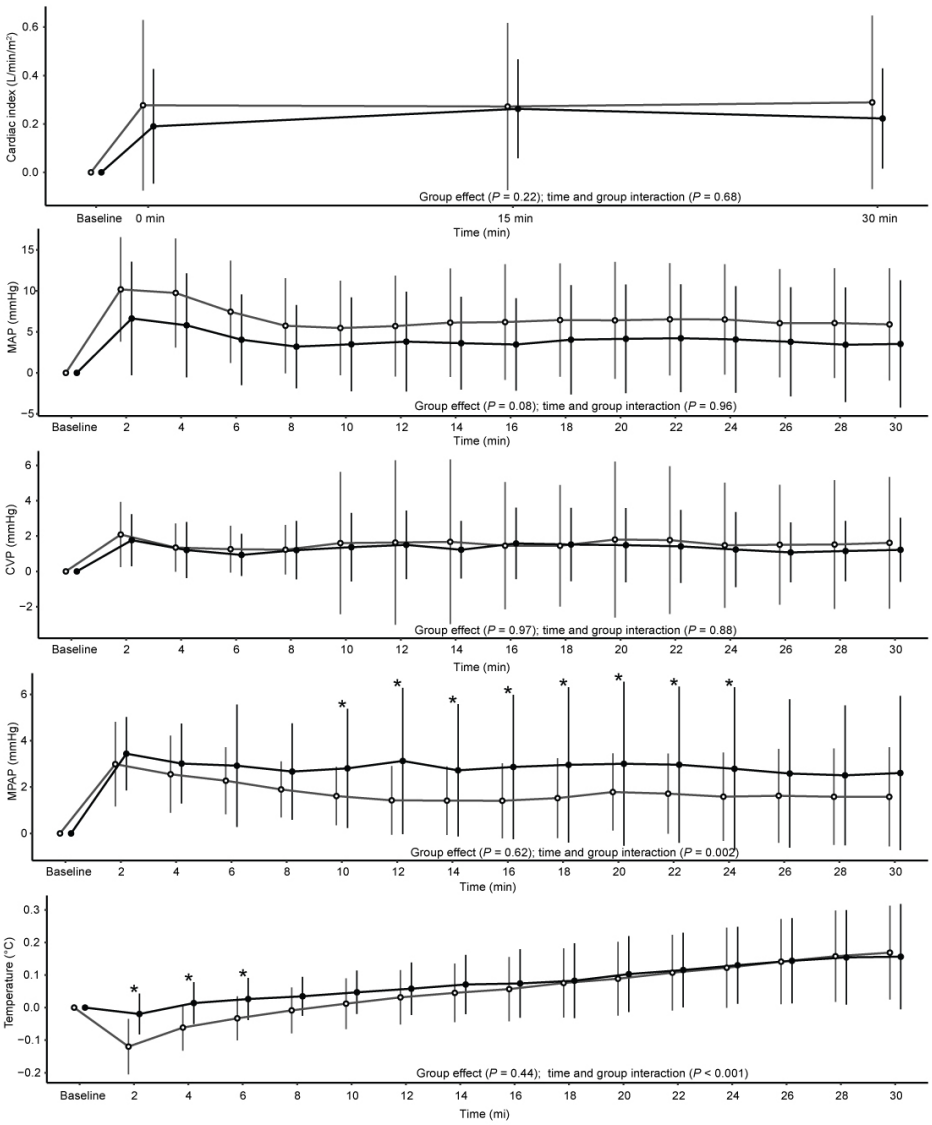
Figure 1. Haemodynamic response (absolute values) to a room temperature 20% albumin fluid bolus (white) versus a body temperature 20% albumin fluid bolus (black)



MPAP = mean pulmonary arterial pressure. Data are shown as mean with standard deviation. * Significant difference ($P < 0.05$) between the two groups.

[Figure 2]

Figure 2. Haemodynamic response (absolute change from baseline) to a room temperature 20% albumin fluid bolus (white) versus a body temperature 20% albumin fluid bolus (black)



MPAP = mean pulmonary arterial pressure. Data are shown as mean with standard deviation. * Significant difference ($P < 0.05$) between the two groups.

Appendix

This appendix was part of the submitted manuscript and has been peer reviewed. It is posted as supplied by the authors.

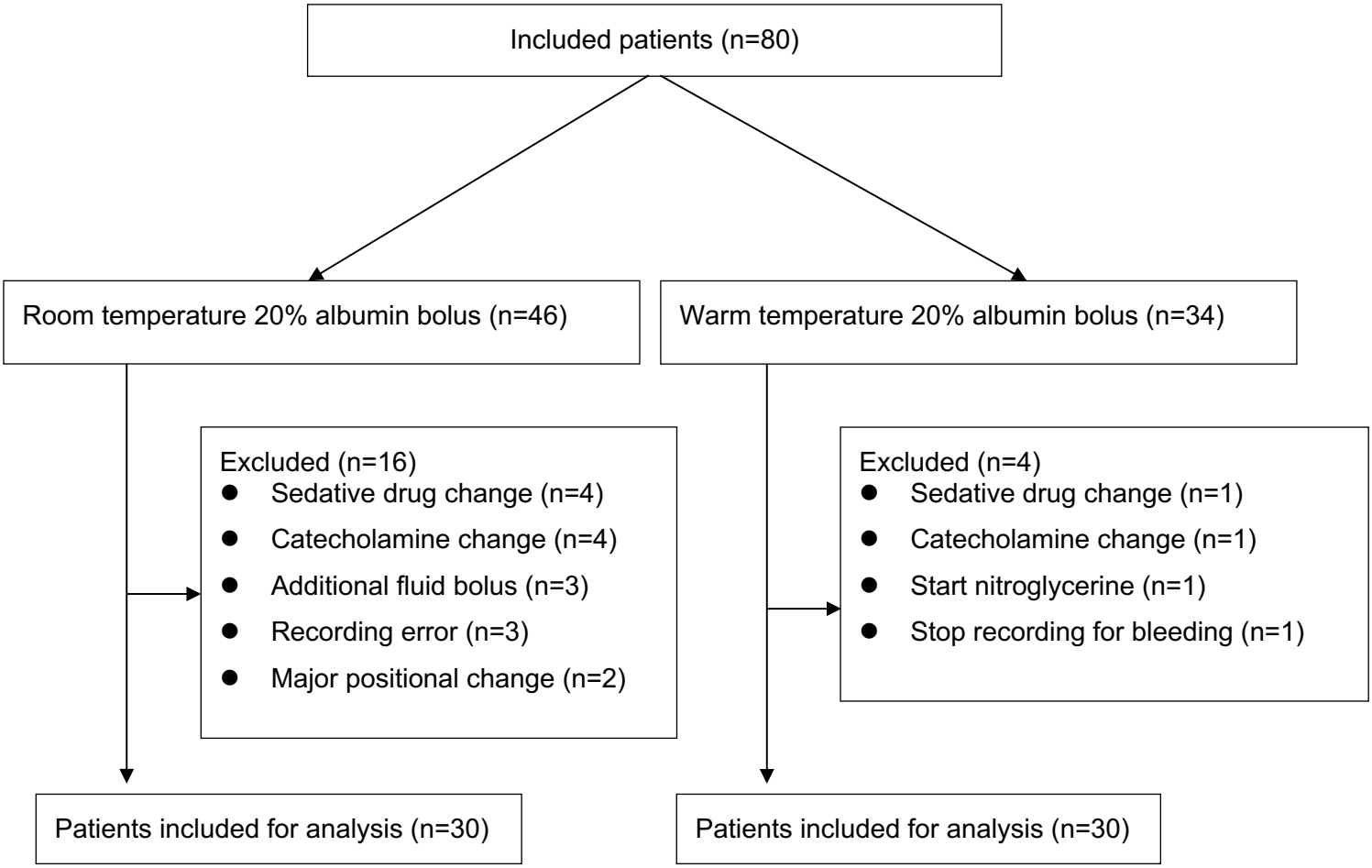
Supplementary Appendix Item 1. Detailed exclusion criteria.

- Additional bolus: Additional fluid bolus was prescribed during the observation period.
- Catecholamine: Any change (bolus or continuous infusion) of catecholamine.
- Sedative drug: Any change (bolus or continuous infusion) of sedative drugs.
- Muscle relaxant: Any dose of muscle relaxant infusion.
- Pacing: Any change of pacing setting.
- Ventilation setting: Any change of ventilator parameters or ventilation setting except for FiO_2.
- Other: Care givers' intervention that may affect patients' haemodynamic parameters, for example, tracheal suctioning, patients' movement or movement of transducers.

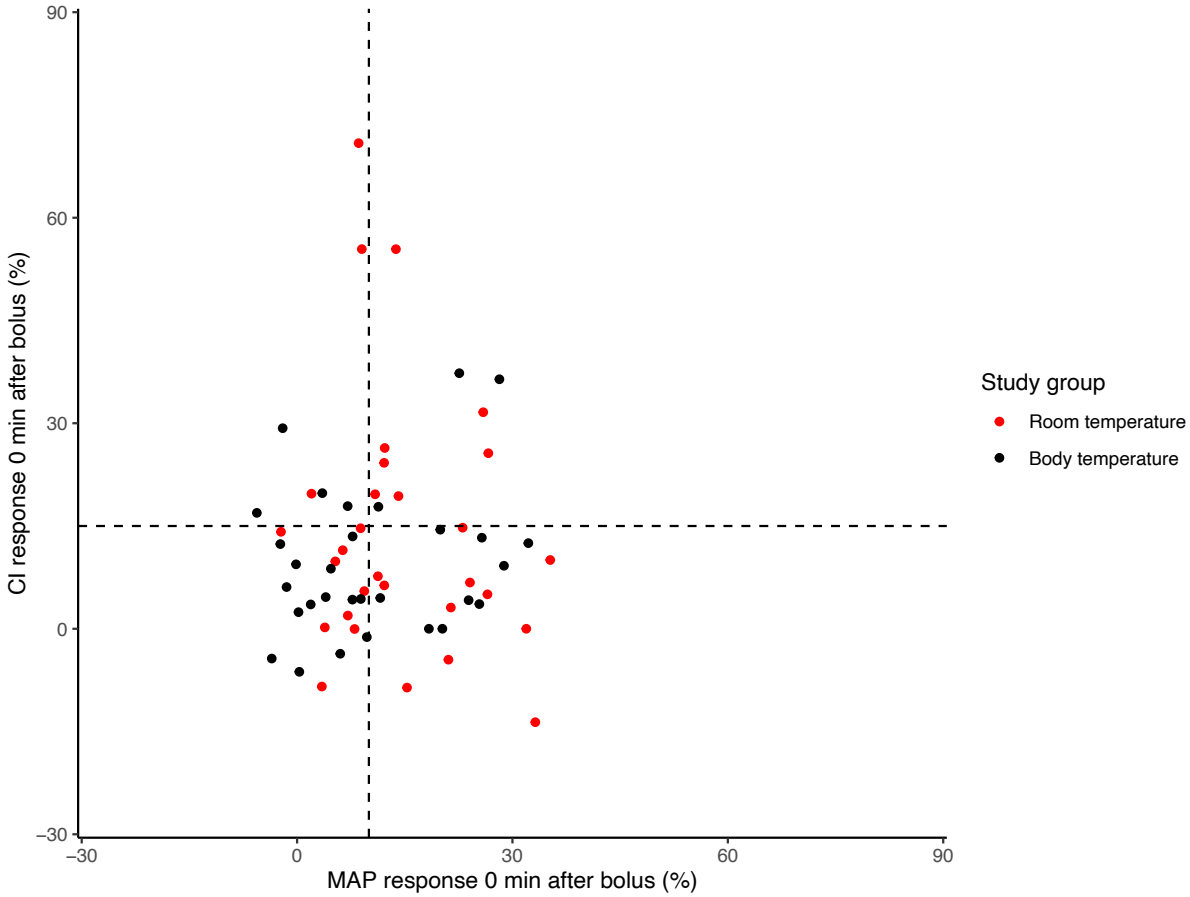
Supplementary Appendix Item 2. Minor confounders in the study patients.

- Patients awoke during the study periods, but kept stable sedative status without any change of sedative drugs.
- When patients awoke, bedside nurse talked to patients without touching.

Supplementary Figure 1. Flow chart of study inclusion process

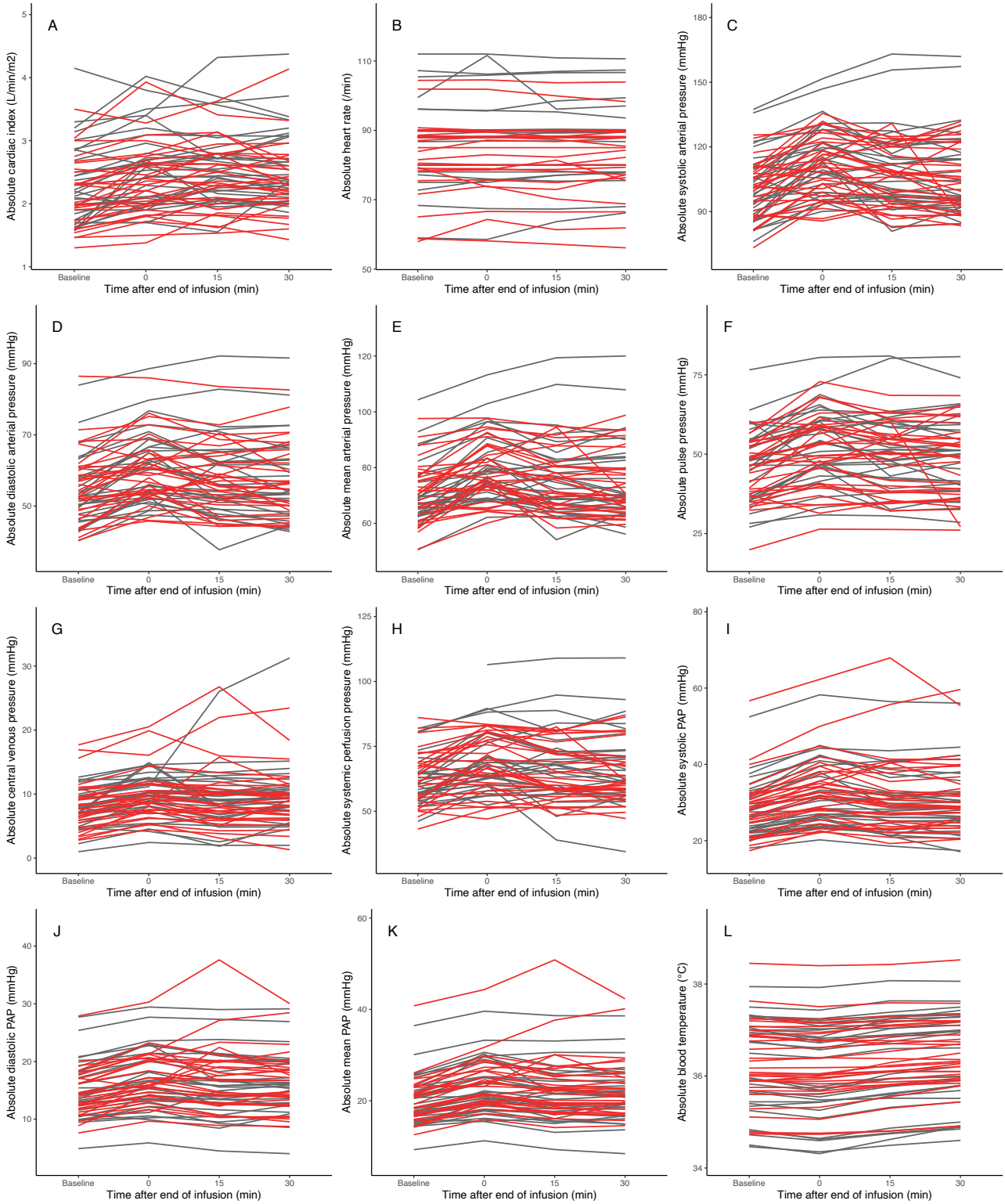


Supplementary Figure 2. CI response and MAP response 0 minute after 20% albumin bolus.



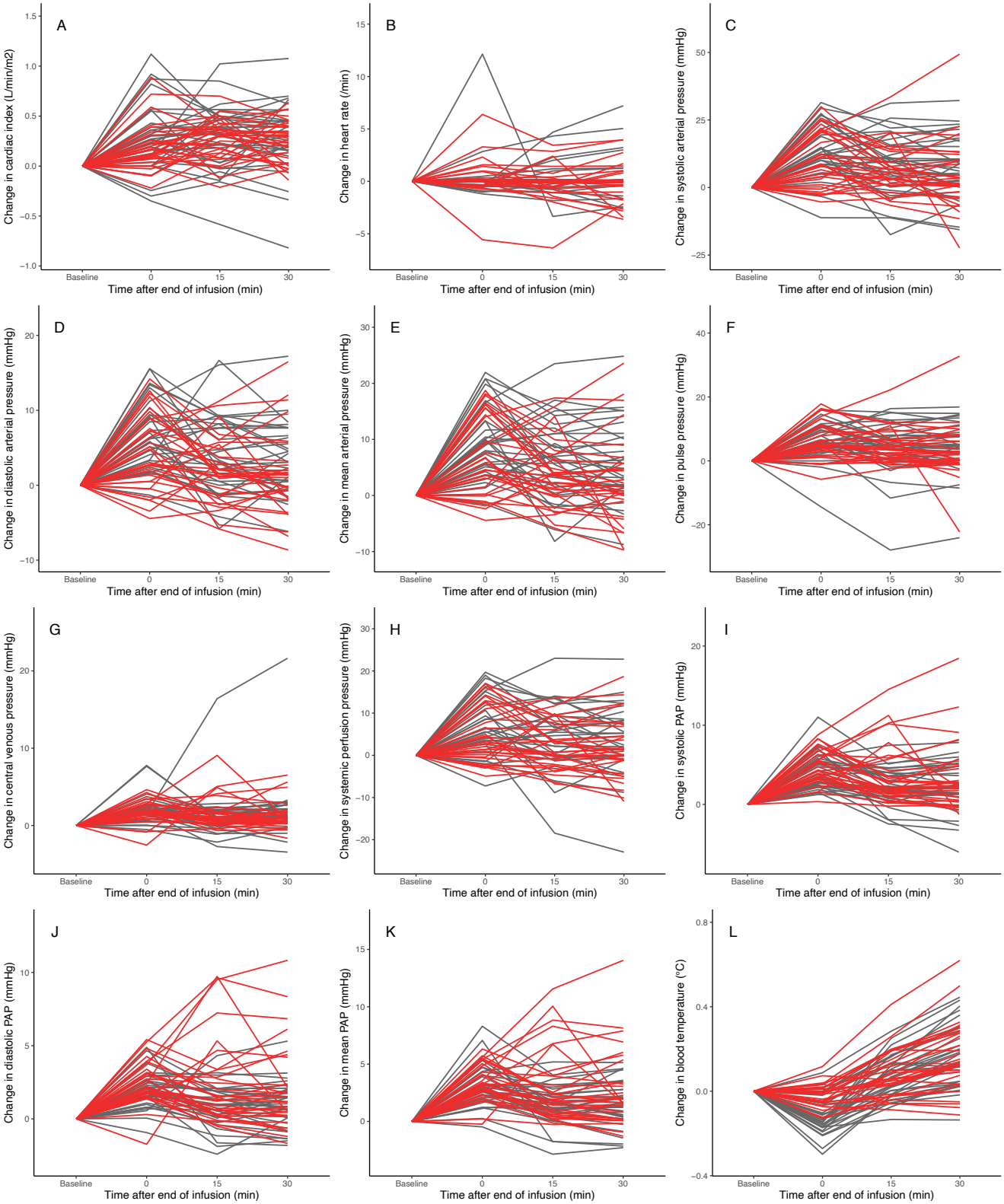
Vertical line shows mean arterial pressure (MAP) response (10%) and horizontal line shows cardiac index (CI) response (15%).

Supplementary Figure 3. Individual haemodynamics and temperature changes (absolute value).



Patients in the room temperature albumin group are represented by a grey line and those in the body temperature albumin group are represented by a red line.

Supplementary Figure 4. Individual haemodynamics and temperature changes (relative change).



Patients in the room temperature albumin group are represented by a grey line and those in the body temperature albumin are represented by a red line.

Supplementary Table 1. Characteristics of study patients at the time of fluid bolus

	All patients N = 60	**Room temperature 20% albumin** N = 30	**Body temperature 20% albumin** N = 30	***P***
Mechanical ventilation mode				0.67
SIMV	54 (90%)	28 (93%)	26 (87%)	-
PSV	6 (10%)	2 (7%)	4 (13%)	-
Mechanical ventilation settings				
Tidal volume (mL/kg PBW)[§]	7.3 [6.4; 8.5]	7.7 [6.9; 8.7]	6.9 [6.2; 7.6]	0.061
PIP (cm H_2O)	19 [17; 21]	19 [17; 21]	18 [16; 21]	0.54
PEEP (cm H_2O)	5 [5; 5]	5 [5; 5]	5 [5; 5]	0.33
FiO_2	0.3 [0.3; 0.5]	0.3 [0.3; 0.5]	0.3 [0.25; 0.4]	0.23
PaO_2/FiO_2 ratio	311 [250; 367]	309 [251; 378]	312 [250; 348]	0.68
SpO_2 (%)	99 [97; 100]	99 [97; 100]	99 [98; 100]	0.61
$EtCO_2$ (mm Hg)	35 [31; 39]	35 [32; 37]	35 [31; 39]	0.75
Body temperature control				
External body active warming	19 (32%)	12 (40%)	7 (23%)	0.17
Haemodynamic status				
Vasopressor support	15 (25%)	8 (27%)	7 (23%)	>0.99
Milrinone administration	6 (10%)	3 (10%)	3 (10%)	>0.99
Heart rhythm				0.44
Paced	33 (55%)	18 (60%)	15 (50%)	-
Sinus rhythm	26 (43%)	11 (37%)	15 (50%)	-
Atrial fibrillation	1 (2%)	1 (3%)	0 (0%)	-
Sedation				
Propofol	54 (90%)	28 (93%)	26 (87%)	0.67
Propofol dose (mg/h)	100 [80; 150]	100 [80; 150]	100 [100; 150]	0.39
Opioids	4 (7%)	0 (0%)	4 (13%)	0.11
*Biochemistry**				
pH	7.4 [7.36; 7.42]	7.41 [7.37; 7.43]	7.39 [7.36; 7.41]	0.14
PaO_2 (mm Hg)	126 [87; 200]	125 [82; 206]	127 [99; 193]	0.61
$PaCO_2$ (mm Hg)	42 [39; 45]	42 [41; 45]	41 [39; 46]	0.80
Bicarbonate (mmol/L)	24 [23; 26]	26 [23; 27]	24 [23; 26]	0.12
Lactate (mmol/L)	1.1 [0.9; 1.5]	1.1 [0.9; 1.5]	1.1 [0.9; 1.6]	0.66
Creatinine (µmol/L)	78 [65; 102]	72 [64; 104]	79 [66; 100]	0.67
Haemoglobin (g/L)	100 [90; 108]	96 [89; 104]	105 [96; 112]	0.017
Blood sugar level (mmol/L)	7.2 [6.5; 8.4]	7.2 [6.3; 8]	7.3 [6.5; 8.8]	0.27

administration

Data is median [interquartile range], or count (percentage).

*: measured on arterial blood gas closest to FBT. [§]: predicted body weight (ARDS Network formula). P values reflect the between-groups comparison.

$EtCO_2$: end-tidal CO_2 partial pressure; FiO_2: fraction of inspiratory oxygen ; $PaCO_2$: arterial partial pressure of CO_2; PaO_2: arterial partial pressure of oxygen; PBW: predicted body weight; PEEP: positive end-expiratory pressure; PIP: peak inspiratory pressure; PSV: pressure support ventilation; SIMV: synchronized intermittent mandatory ventilation; SpO_2: peripheral capillary oxygen saturation.

Supplementary Table 2. Haemodynamic effect of room temperature 20% albumin fluid bolus and body temperature 20% albumin bolus (absolute value).

	Time after FB	All patients	Room temperature 20% albumin	Body temperature 20% albumin
		N = 60	N = 30	N = 30
Mean arterial pressure (mmHg)	Baseline	69 [63; 76]	67 [63; 74]	70 [64; 77]
	0 min	76 [71; 86]	77 [70; 85]	76 [72; 87]
	15 min	74 [67; 82]	74 [67; 82]	73 [67; 81]
	30 min	70 [65; 83]	70 [66; 84]	70 [65; 80]
Systolic arterial pressure (mmHg)	Baseline	100 [89; 110]	98 [89; 111]	101 [89; 108]
	0 min	113 [99; 122]	113 [100; 127]	111 [99; 121]
	15 min	107 [96; 120]	107 [97; 119]	108 [94; 119]
	30 min	104 [93; 120]	104 [95; 121]	103 [92; 117]
Diastolic arterial pressure (mmHg)	Baseline	53 [48; 61]	52 [48; 58]	55 [49; 61]
	0 min	60 [53; 66]	60 [53; 67]	59 [54; 64]
	15 min	56 [51; 64]	56 [51; 64]	56 [52; 64]
	30 min	55 [50; 65]	54 [49; 65]	55 [50; 64]
Pulse pressure (mmHg)	Baseline	47 [36; 53]	47 [36; 54]	46 [37; 51]
	0 min	53 [45; 59]	54 [46; 61]	50 [41; 59]
	15 min	51 [40; 58]	51 [43; 60]	51 [38; 56]
	30 min	50 [39; 57]	51 [41; 59]	49 [38; 55]
Mean PAP (mmHg)	Baseline	19 [17; 23]	19 [16; 23]	19 [17; 23]
	0 min	22 [19; 27]	21 [18; 28]	22 [20; 27]
	15 min	22 [18; 25]	21 [17; 25]	22 [18; 26]
	30 min	21 [18; 25]	21 [18; 24]	21 [18; 25]
Systolic PAP (mmHg)	Baseline	28 [23; 32]	26 [22; 31]	28 [25; 32]
	0 min	31 [26; 38]	29 [26; 38]	33 [28; 37]
	15 min	29 [25; 36]	28 [23; 33]	30 [26; 37]
	30 min	29 [25; 35]	28 [25; 33]	29 [26; 36]
Diastolic PAP (mmHg)	Baseline	14 [12; 17]	14 [11; 18]	14 [12; 17]
	0 min	16 [14; 20]	16 [13; 20]	16 [14; 20]
	15 min	15 [13; 20]	15 [13; 18]	17 [13; 20]
	30 min	15 [12; 19]	15 [12; 19]	17 [13; 19]
Central venous pressure (mmHg)	Baseline	8 [6; 10]	8 [6; 10]	8 [6; 10]
	0 min	10 [8; 12]	10 [8; 12]	9 [7; 11]
	15 min	8 [6; 11]	9 [7; 10]	8 [6; 11]
	30 min	9 [7; 11]	10 [8; 11]	9 [7; 11]
Heart rate (/min)	Baseline	88 [80; 90]	88 [80; 90]	86 [79; 88]
	0 min	88 [80; 90]	88 [80; 90]	88 [79; 88]
	15 min	88 [80; 90]	88 [80; 90]	87 [79; 88]
	30 min	88 [80; 90]	88 [80; 90]	87 [78; 88]
Cardiac index (L/min/m^2)	Baseline	2.1 [1.7; 2.6]	2.2 [1.8; 2.8]	2.0 [1.7; 2.5]
	0 min	2.4 [2.0; 2.7]	2.6 [2.1; 2.9]	2.3 [2.0; 2.6]
	15 min	2.4 [2.0; 2.7]	2.4 [2.1; 2.7]	2.3 [1.9; 2.8]
	30 min	2.4 [2.1; 2.7]	2.4 [2.2; 3.1]	2.3 [2.0; 2.7]
SVRi (dyn.s.cm^{-5}.m^{-2})	Baseline	2198 [1783; 2951]	2099 [1737; 2702]	2507 [1818; 3020]
	0 min	2321 [1791; 2876]	2128 [1847; 2842]	2519 [1772; 2998]
	15 min	2256 [1816; 2674]	2254 [1954; 2572]	2276 [1616; 2748]
	30 min	2208 [1722; 2557]	2208 [1726; 2562]	2197 [1760; 2540]

Systemic perfusion pressure (mmHg)	Baseline	61 [55; 68]	60 [56; 65]	63 [55; 69]
	0 min	68 [60; 78]	68 [63; 76]	68 [60; 78]
	15 min	65 [58; 73]	66 [58; 73]	62 [58; 73]
	30 min	62 [57; 71]	63 [57; 73]	61 [57; 71]
Blood temperature (°C)	Baseline	36.1 [35.6; 37.0]	36.2 [35.4; 37.0]	36.1 [35.9; 36.9]
	0 min	36.1 [35.6; 36.9]	36.1 [35.4; 37.0]	36.1 [35.8; 36.9]
	15 min	36.3 [35.8; 37.1]	36.3 [35.6; 37.1]	36.3 [35.9; 37.0]
	30 min	36.3 [35.9; 37.2]	36.4 [35.7; 37.3]	36.3 [36.0; 37.0]

Data as median [interquartile range].
***:** $p<0.05$: comparison between the room temperature albumin group and the body temperature albumin group at each time point, adjusted for the repetition of measurements in a given patient.
FB: fluid bolus; PAP: pulmonary arterial pressure; SVRi: Systemic vascular resistance index.

Supplementary Table 3. Haemodynamic effect of room temperature 20% albumin bolus vs. body temperature 20% albumin bolus (relative change from baseline).

	Time after FB	All patients	Room temperature 20% albumin	Body temperature 20% albumin
		N = 60	N = 30	N = 30
Mean arterial pressure (mmHg)				
	0 min	8 [4; 14]	9 [6; 13]	5 [1; 14]
	15 min	4 [0; 9]	6 [2; 11]	4 [0; 6]
	30 min	3 [0; 8]	5 [2; 10]	1 [-1; 5]
Systolic arterial pressure (mmHg)				
	0 min	11 [5; 20]	12 [9; 19]	8 [2; 20]
	15 min	7 [1; 14]	10 [1; 16]	4 [1; 9]
	30 min	5 [0; 12]	8 [2; 17]	1 [-1; 10]
Diastolic arterial pressure (mmHg)				
	0 min	5 [2; 9]	6 [3; 9]	3 [0; 9]
	15 min	2 [-1; 6]	4 [1; 8]	2 [-1; 5]
	30 min	2 [-1; 6]	4 [1; 7]	1 [-2; 3]
Pulse pressure (mmHg)				
	0 min	6 [4; 10]	6 [5; 10]	5 [2; 10]
	15 min	4 [1; 8]	5 [2; 8]	4 [1; 6]
	30 min	3 [0; 8]	5 [1; 9]	2 [-1; 7]
Mean PAP (mmHg)				
	0 min	3 [2; 4]	3 [2; 4]	3 [3; 5]
	15 min	2 [1; 3]	2 [1; 2]*	2 [1; 4]*
	30 min	2 [0; 3]	1 [0; 3]	2 [0; 3]
Systolic PAP (mmHg)				
	0 min	4 [3; 6]	4 [2; 5]	5 [3; 6]
	15 min	2 [1; 4]	2 [1; 4]	2 [1; 5]
	30 min	2 [1; 5]	2 [1; 5]	2 [0; 5]
Diastolic PAP (mmHg)				
	0 min	2 [2; 3]	2 [1; 3]	2 [2; 3]
	15 min	1 [0; 2]	1 [0; 2]*	1 [0; 3]*
	30 min	1 [0; 2]	1 [0; 2]*	1 [0; 2]*
Central venous pressure (mmHg)				
	0 min	2 [1; 2]	2 [2; 2]	2 [1; 3]
	15 min	1 [0; 2]	1 [0; 2]	1 [0; 2]
	30 min	1 [0; 2]	1 [0; 3]	1 [0; 1]
Heart rate (/min)				
	0 min	0 [0; 0]	0 [0; 0]	0 [0; 0]
	15 min	0 [0; 0]	0 [0; 0]	0 [-1; 0]
	30 min	0 [0; 0]	0 [0; 0]	0 [-1; 1]
Cardiac index (L/min/m^2)				
	0 min	0.2 [0.1; 0.4]	0.3 [0.1; 0.4]	0.1 [0.1; 0.3]
	15 min	0.3 [0.1; 0.4]	0.3 [0.0; 0.5]	0.3 [0.1; 0.4]
	30 min	0.3 [0.1; 0.4]	0.3 [0.2; 0.5]	0.2 [0.0; 0.4]
SVRi (dyn.s.cm^{-5}.m^{-2})				
	0 min	-32 [-249; 171]	-47 [-212; 190]	-11 [-241; 156]
	15 min	-165 [-431; 162]	61 [-431; 376]	-217 [-419; -71]
	30 min	-125 [-469; 70]	-90 [-483; 120]	-133 [-431; -22]
Systemic perfusion pressure (mmHg)				
	0 min	5 [1; 12]	6 [3; 11]	3 [0; 12]
	15 min	3 [-1; 8]	6 [0; 10]	1 [-2; 5]
	30 min	2 [-1; 8]	5 [0; 8]	1 [-4; 5]
Blood temperature (°C)				
	0 min	-0.1 [-0.1; 0.0]	-0.1 [-0.2; -0.1]*	0.0 [-0.1; 0.0]*
	15 min	0.1 [0.0; 0.1]	0.1 [0.0; 0.1]	0.1 [0.0; 0.1]
	30 min	0.1 [0.0; 0.3]	0.1 [0.0; 0.3]	0.1 [0.0; 0.3]

Data as median [interquartile range]. *p<0.05 between the room temperature vs. body temperature albumin group at each time point, adjusted for the repeated measures in a given patient.
FB: fluid bolus; PAP: pulmonary arterial pressure; SVRi: Systemic vascular resistance index.

Supplementary Table 4. Dissipation of the haemodynamic response (CI and MAP)

	All patients	Room temperature 20% albumin	Body temperature 20% albumin	P
Early CI responders	N=17	N=10	N=7	
Absence of dissipation at 30 minutes*	14 (82%)	9 (90%)	5 (71%)	0.15
Effect dissipated at 15 minutes§	1 (6%)	1 (10%)	0 (0%)	
Effect dissipated at 30 minutes	2 (12%)	0 (0%)	2 (29%)	
Early MAP responders	N=30	N=18	N=12	
Absence of dissipation at 30 minutes†	17 (57%)	9 (50%)	8 (67%)	0.47
Effect dissipated	13 (43%)	9 (50%)	4 (33%)	
Time to dissipation (min)	6 [5; 11]	5 [3; 6]	9 [7; 12]	

Data as median [interquartile range], or count (percentage).
*: defined as a CI within 5% of baseline CI
§: twelve patients in the room temperature albumin patients were not measured CI at 15 minutes after FBT
†: defined as a MAP within 3 mm Hg of baseline
CI: cardiac index; MAP: mean arterial pressure

Chapter 4: Rapid vs. Slow 4% albumin fluid bolus therapy in cardiac surgery patients

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Rapid 500 mL albumin bolus versus rapid 200 mL bolus followed by slower continuous infusion in post-cardiac surgery patients: a pilot before-and-after study

Fumitaka Yanase, Thummaporn Naorungroj, Salvatore L Cutuli, Glenn M Eastwood and Rinaldo Bellomo

Abstract

Objective: To evaluate the haemodynamic effects of rapid fluid bolus therapy (FBT) (500 mL of 4% albumin over several minutes) versus combined FBT (rapid 200 mL FBT followed by a 300 mL infusion over 30 minutes).
Design: Single centre, prospective, before-and-after trial.
Setting: A tertiary intensive care unit in Australia.
Participants: Fifty mechanically ventilated post-cardiac surgery patients.
Interventions: Rapid 4% albumin FBT versus combined FBT.
Main outcome measures: We recorded haemodynamic parameters from before FBT to 30 minutes after FBT. A mean arterial pressure (MAP) response was defined by a MAP increase > 10%, and a cardiac index (CI) response was defined by a CI increase > 15%.
Results: Immediately after rapid FBT versus combined FBT, there was a CI response in 13 patients (52%) compared with five patients (20%) respectively ($P = 0.038$), and a MAP response in 11 patients (44%) in each group. However, from FBT administration to 30 minutes, there was a time and group interaction such that MAP was higher in the rapid FBT group ($P = 0.003$), as was the case for central venous pressure ($P = 0.002$) and mean pulmonary artery pressure ($P < 0.001$). Body temperature fell immediately and was lower with rapid FBT but became warmer than with combined FBT later ($P < 0.001$). At 30 minutes, a MAP response was seen in ten patients (40%) compared with nine patients (36%) ($P < 0.99$) and a CI response was present in eight patients (32%) compared with 11 patients (44%) ($P = 0.56$) in the rapid versus combined FBT groups respectively.
Conclusion: Rapid FBT was superior to combined FBT in terms of mean MAP levels and immediate CI response. However, the number of MAP responders or CI responders was similar at 30 minutes.

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A low mean arterial pressure (MAP) or cardiac index (CI) is common after cardiac surgery[1,2] and typically triggers treatment with fluid bolus therapy (FBT).[3,4] However, insufficient FBT may fail to correct such instability, while excessive FBT may contribute to pulmonary congestion. Therefore, defining the optimal approach to FBT after cardiac surgery is clinically relevant.[5,6]

During haemodynamic instability, FBT is often administered rapidly. However, it may be better to administer the same amount of fluid as a smaller very rapid (3–4 minutes) bolus followed by a continuous infusion of the remaining fluid. This is because rapid FBT increases plasma atrial natriuretic peptide levels, an effect associated with glycocalyx injury, greater fluid extravasation, and intestinal oedema.[7] In contrast, a slow infusion leads to long-lasting plasma volume expanding effects.[8,9]

Slower 4% albumin infusion could achieve more sustained haemodynamic changes after cardiac surgery, but it may fail to correct hypotension or low cardiac output rapidly enough. Logically, a small but very rapid fluid bolus followed by continuous infusion may combine the advantage of a rapid response with those associated with a sustained effect. Despite these physiological considerations and the findings of an international survey that about 30% of intensive care unit (ICU) clinicians define FBT as fluid given in less than 10 minutes while 50% define it as given over 30 minutes,[10] no investigation has compared these two approaches to FBT in post-cardiac surgery patients.

Accordingly, we conducted a prospective before-and-after study of the haemodynamic effect of a rapid complete bolus of 4% albumin compared with a rapid small bolus of 4% albumin followed by a slower continuous infusion over 30 minutes (combined FBT) in post-cardiac surgery patients. We hypothesised that the combined FBT approach would achieve greater MAP effects than rapid FBT during a 30-minute post-FBT observation period.

Methods

We prospectively obtained institutional ethics approval for this study (Reference No. LNR/16/Austin/358). Individual consent was waived, as giving FBT within 30 minutes is common practice in Australia and New Zealand and in our ICU.[10]

Study design

Within an overarching quality improvement program evaluating FBT after cardiac surgery, this single-centre prospective before-and-after study was conducted in a university-affiliated tertiary teaching hospital in Melbourne, Australia, between July 2017 and January 2020.

As with a previous study, we included mechanically ventilated adult patients (aged 18 years or older) admitted to the ICU after on-pump cardiac surgery. All patients had a pulmonary artery catheter in situ and, at the discretion of their treating doctors, were prescribed 500 mL 4% albumin for haemodynamic instability (Albumex 4%, CSL Behring, Melbourne, Australia) within the first 12 hours of ICU admission.

We excluded pregnant women and patients receiving intra-aortic balloon counterpulsation or extracorporeal membrane oxygenation. Patients were also excluded if any major confounding interventions, which could affect haemodynamic parameters, became necessary during the study period (Online Appendix, supplementary appendix 1).

Fluid bolus therapy

The clinicians prescribed 4% albumin FBT based on their clinical judgements. As a part of their FBT quality improvement program, they introduced small bolus FBT followed by continuous infusion (combined FBT). The albumin was stored and given at room temperature. We allocated the first 25 patients to the rapid FBT group and the second 25 patients to the combined FBT group. In the rapid FBT group, 500 mL of albumin was infused rapidly (within 15 minutes) through a central line using a hand pump as previously reported.[11] In the combined FBT group, a small 4% albumin bolus (200 mL) was given rapidly (within 10 minutes) through a central line, and a continuous infusion (300 mL albumin) was given over 30 minutes (Online Appendix, supplementary figure 1).

Data collection

We collected haemodynamic data, including systolic arterial pressure, diastolic arterial pressure, MAP, central venous pressure (CVP), systolic pulmonary arterial pressure (PAP), diastolic PAP, mean PAP, heart rate, CI and peripheral oxygen saturation (SpO_2) on a second-by-second basis using the MediCollector logging software (MediCollector, Boston, MA, USA). We measured CI continuously or intermittently according to the pulmonary artery catheter using the thermodilution technique.

In the rapid FBT group, CI was measured at four time points: before FBT, immediately (0 minutes) after FBT, 15 minutes after FBT, and 30 minutes after FBT. In the combined FBT group, CI was measured immediately after the small fluid bolus, 15 minutes after small fluid bolus, and 30 minutes after FBT. We recorded baseline haemodynamic parameters for a minimum of 3 minutes before the large FBT or the small FBT. Ventilator setting and all drug infusions (catecholamine and sedative drugs) at the time of inclusion were recorded and remained unchanged during the study period.

Finally, patients admitted to the ICU only during business hours were recruited because at least one trained researcher was needed to observe study patients and record all interventions, including minor confounders during the study periods. When patients needed unexpected interventions that met the exclusion criteria (Online Appendix, supplementary appendix 1), we stopped data collection and excluded such patients. However, when there were minor confounders (Online Appendix, supplementary appendix 2), we continued the recording and the data were included for analysis.

Haemodynamic response definitions

We defined MAP response (MAP-R) as a MAP increase greater than 10% above the baseline and an immediate MAP-R as an increase immediately after the administration of the large bolus or the small FBT. Similarly, we defined a CI response (CI-R) as a CI increase greater than 15% at the same time points. We defined FBT effect dissipation as the time when a patient's MAP was within 3 mmHg of baseline for at least 2 consecutive minutes (MAP dissipation) or a patient's CI was within 5% of baseline (CI dissipation).

Data processing

Negative value of CVP and values outside three standard deviations (SDs) in all variables were excluded as data noise (eg, high CVP when flushing normal saline to measure CI). Baseline haemodynamic parameters were calculated as the mean value from 3 minutes before large FBT or small FBT. Except for CI, which was measured intermittently, we calculated the mean value over 120 seconds from 0 minutes after large FBT or small FBT to 30 minutes after large FBT or small FBT to present 2-minutely data.

Power calculation

The SDs of MAP were estimated at 10 mmHg.[11] We estimated that 25 patients in each group would have an 80% power (two-sided $P = 0.05$) to detect differences between the two groups equivalent to 80% of the SD (equivalent to 8.0 mmHg overall MAP difference), which would be clinically important.

Statistical analysis

We performed our analyses using the R software, version 3.5.2 (The R Foundation for Statistical Computing, Vienna, Austria). We considered two-sided $P < 0.05$ as significant.

We reported categorical data as count (percentage) and continuous data as mean (SD) for haemodynamic variables and median (interquartile range [IQR]) for other variables. We compared all baseline characteristics between the two groups, using Fisher exact test for categorical variables, Student *t* test for haemodynamic variables, and Mann–Whitney U test for other non-parametric continuous variables. We compared haemodynamic variables collected over the observation period (either absolute values or relative changes from baseline) between the two groups using linear mixed effects modelling, accounting for within-subject repeated measures and treating time as a continuous variable. When a study group effect or an interaction between time and the study group was significant, we performed post hoc analysis to examine the significance of the difference at each time point, accounting for the alpha inflation risk using the Tukey adjustment method. We applied Spearman correlation to evaluate the relationship between CI change and MAP change immediately after FBT and 30 minutes after FBT.

Results

Patients' characteristics

Of 63 eligible patients, 33 received a 4% albumin rapid FBT and 30 received combined FBT. Of these, 25 patients in each group did not have major confounders and were included in the analysis (Online Appendix, supplementary figure 2).

The patients' characteristics are described in Table 1 and in the Online Appendix, supplementary table 1. The median European System for Cardiac Operative Risk Evaluation (EuroSCORE) was similar. Norepinephrine infusion was present in four patients in each group. Finally, half of the patients were in a paced rhythm. There was no difference in baseline haemodynamic parameters (Table 2).

Fluid bolus therapy description

The FBTs were infused over a median time of 6.2 minutes (IQR, 4.4–10.8 minutes) in the rapid FBT group compared with 1.9 minutes (IQR, 1.7–2.3 minutes) in the combined FBT group ($P < 0.001$) (Table 3).

Mean arterial pressure changes

We observed a significant interaction between time and study group. The rapid FBT group achieved a higher immediate MAP increase, which was sustained at higher levels for the full 30 minutes compared with combined FBT ($P = 0.003$ for absolute value and $P = 0.003$ for relative change from baseline) (Figure 1 and Figure 2).

Moreover, mean MAP increased by 9 mmHg (SD, 11 mmHg) immediately after FBT and by 7 mmHg (SD, 12 mmHg) at 30 minutes in the rapid FBT group. It also increased by 7 mmHg (SD, 7 mmHg) immediately after FBT and by 4 mmHg (SD, 9 mmHg) at 30 minutes in the combined FBT group (Online Appendix, supplementary tables 2 and 3).

Eleven patients (44%) in both groups experienced an immediate MAP-R (Table 3), with a similar proportion in the two groups, which remained statistically equivalent at 15 and 30 minutes.

Cardiac index changes

The study group effect or the interaction between time and study group was not significant in both absolute value and relative change from baseline (Figure 1 and Figure 2). Immediately after intervention, mean CI increased by 0.3 L/min/m^2 (SD, 0.4 L/min/m^2) in the rapid FBT group compared with 0.1 L/min/m^2 (SD, 0.3 L/min/m^2) in the combined FBT group. At 30 minutes, the mean CI was 0.3 L/min/m^2 (SD, 0.3 L/min/m^2), greater than the baseline in the rapid FBT group compared with 0.3 L/min/m^2 (SD, 0.3 L/min/m^2) in the combined FBT group (Online Appendix, supplementary tables 2 and 3).

Overall, 13 patients (52%) had an immediate CI-R in the rapid FBT group compared with five patients (20%) in the combined FBT group ($P = 0.038$) (Table 3). Among immediate CI-responders, three patients (23%) experienced CI effect dissipation in the rapid FBT group compared with one patient (20%) in the combined FBT group (Online Appendix, supplementary table 4). After 30 minutes, eight patients (32%) had achieved a CI-R in the rapid FBT group compared with 11 patients (44%) in the combined FBT group ($P = 0.56$).

Other haemodynamic effects

There were time and study group interactions for absolute changes and relative changes in CVP ($P = 0.002$) and mean PAP ($P < 0.001$) (Figure 1 and Figure 2). Therefore, mean CVP and mean PAP increased by 4 mmHg (SD, 3 mmHg)

and 4 mmHg (SD, 2 mmHg) immediately after rapid FBT and by 2 mmHg (SD, 3 mmHg) and 2 mmHg (SD, 1 mmHg) immediately after combined FBT ($P = 0.023$ and $P < 0.001$ respectively) (Online Appendix, supplementary tables 2 and 3).

There was also time and study group interaction for both absolute changes and relative changes in blood temperature ($P < 0.001$) (Figure 1 and Figure 2). The mean blood temperature dropped significantly more in the rapid FBT group immediately after FBT (mean, –0.3°C [SD, 0.1°C] *v* –0.2°C [SD, 0.1°C]; $P = 0.007$). However, it rebounded rapidly and was significantly higher in the rapid FBT group 30 minutes after FBT (mean, 0.1°C [SD, 0.3°C] *v* –0.1°C [SD, 0.2°C]; $P < 0.001$) (Figure 1 and Figure 2). The individual haemodynamic and physiological changes are presented in the Online Appendix, supplementary figures 5 and 6.

Relationship between mean arterial pressure change and cardiac index and oxygenation changes

There was no significant correlation between percentage immediate MAP change and percentage immediate CI change from baseline when analysing both groups together ($P = 0.56$) (Online Appendix, supplementary figure 3). In contrast, there was a correlation between percentage MAP and percentage CI change at 30 minutes ($P = 0.001$; $\rho = 0.47$) (Online Appendix, supplementary figure 4), which was stronger after combined FBT ($\rho = 0.56$) than the rapid group ($\rho = 0.38$).

The arterial partial pressure of oxygen to traction of inspired oxygen (PaO_2:FiO_2) ratio [Author, OK?] after each treatment were not different (median, 312 mmHg [IQR, 273–370 mmHg] in the rapid FBT group *v* 333 mmHg [IQR, 290–390 mmHg] in the combined FBT group; $P = 0.37$).

Discussion

Key findings

We compared the effect of the rapid FBT with that of the combined FBT in post-cardiac surgery patients. We found a greater number of immediate CI responders in the rapid FBT group but an equal number of immediate MAP responders. However, there were clear time and study group interactions for MAP, CVP, mean PAP, and blood temperature, such that the rapid FBT group showed a greater initial response followed by a slow decline for MAP, CVP and mean PAP for most of the observation period, while temperature immediately fell and then recovered to a higher value. Nevertheless, at the end of the 30 minutes, the number of MAP and CI responders was the same between the two groups. Finally, there was a correlation between MAP changes and CI changes at 30 minutes which was stronger after combined FBT.

Relationship to previous studies

To our knowledge, this is the first study to compare the haemodynamic changes induced by rapid FBT with those by combined FBT. Previous studies have only reported the haemodynamic changes induced by rapid FBT alone and did not report the effect of combined FBT.[12]

In animal models, slower fluid infusion appears to produce less atrial natriuretic peptide release, less glycocalyx injury, and a more sustained intravascular volume effect.[8,9] Few studies, however, have reported the effect of different fluid infusion rates in humans. In patients after abdominal surgery, one study compared different albumin infusion rates (10 mL/kg 5% albumin over 30 minutes *v* 180 minutes) and concluded that both groups achieved similar haemodynamic changes, similar plasma volume expansion, and similar endothelial injury biomarker levels.[13] However, 5% albumin was given over 30 minutes even in the rapid infusion group, a value at the slowest limit of the FBT concept, while the infusion rate in the slow infusion group was well outside the fluid bolus concept.[10]

In keeping with previous studies,[14,15] there was no correlation between percentage CI change and percentage MAP change immediately after FBT. However, at 30 minutes, such a correlation was present and strongest with combined FBT. A previous study also reported a MAP and stroke volume correlation 30 minutes after FBT in patients after cardiac surgery.[16] It is possible that continued extended infusion allows for a more reliable and extended effect on both parameters over time, thus facilitating greater correlation.

Study implications

Our findings imply that, in patients after cardiac surgery, a rapid 500 mL 4% albumin FBT delivers more immediate CI responders and a greater overall increase in MAP, CVP and mean PAP over the following 30 minutes than the combined FBT approach. This observation logically implies that, for the same total amount of fluid, rapid FBT delivers more favourable and pronounced haemodynamic effects than the same amount administered over 30 minutes. Therefore, FBT over a few minutes is a better resuscitation strategy than FBT over 30 minutes, which is a clinically relevant finding.

Strengths and limitations

Our study is the first to compare the haemodynamic changes induced by a rapid 500 mL bolus of 4% albumin with a small 4% albumin bolus followed by a continuous infusion in post-cardiac surgery patients. We recorded secondly haemodynamic data and excluded haemodynamic confounders, with one research staff monitoring the whole data collection. Moreover, iso-oncotic albumin FBT after cardiac surgery is common clinical practice worldwide.[3,17] Finally,

our findings clearly indicate the superiority of giving FBT over a few minutes rather than over 30 minutes, a clinically relevant observation.

There are some limitations to our study. Because of the nature of the two different fluid infusion protocols, we had to assess the immediate MAP and CI response when different volumes of 4% albumin were given (500 mL *v* 200 mL) as well as the overall response and the final (at 30 minutes) response, making the comparison complex. But the graphic displays provide clinicians with a clear sense of the haemodynamic events over time. This was a single-centre study including a small number of patients and we could not find a significant difference of MAP at specific time points. However, we identified clear and clinically relevant overall interactions implying adequate power. We only recorded haemodynamic changes over 30 minutes, given that recording haemodynamic parameters without major confounders, such as sedative drug changes, beyond 30 minutes is difficult. Moreover, no previous research has reported the time course of fluid responsiveness beyond 20 minutes after FBT.[18] We did not assess for pulmonary oedema by lung ultrasonography or other test after the FBT. However, the PaO_2:FiO_2 ratio after each treatment was not different between the two groups. We continuously recorded CPV, and did not assess CVP at the end-expiration or at the C wave, but our approach minimised an observer bias. Finally, FBT over 30 minutes might not be a widely accepted approach by some clinicians. However, fluid infusion over 30 minutes is a common practice and was found to be an accepted time frame for FBT in half of the over 3000 survey responses among ICU clinicians.[10]

Conclusion

In conclusion, although the number of MAP responders or CI responders did not change between the two groups at 30 minutes, a rapid FBT of 500 mL 4% albumin achieved a greater number of immediate CI responders, and higher overall MAP, CVP and mean PAP values than a slower combined FBT. Taken together, these findings imply that rapid FBT is haemodynamically superior to combined FBT.

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Competing interests

No relevant disclosures.

Author details

Fumitaka Yanase[1,2]
Thummaporn Naorungroj[1,3]
Salvatore L Cutuli[1,4]
Glenn M Eastwood[1]
Rinaldo Bellomo[1,2,5]

1 Department of Intensive Care, Austin Hospital, Melbourne, Victoria, Australia.
2 Australian and New Zealand Intensive Care Research Centre (ANZIC-RC), School of Public Health and Preventive Medicine, Monash University, Melbourne, Victoria, Australia.
3 Department of Intensive Care, Siriraj Hospital, Mahidol University, Bangkok, Thailand.
4 Dipartimento di Scienze dell'emergenza, anestesiologiche e della Rianimazione, Fondazione Policlinico Universitario A Gemelli IRCCS, Rome, Italy.
5 Centre for Integrated Critical Care, School of Medicine, University of Melbourne, Melbourne, Victoria, Australia.

***Correspondence*:** rinaldo.bellomo@austin.org.au

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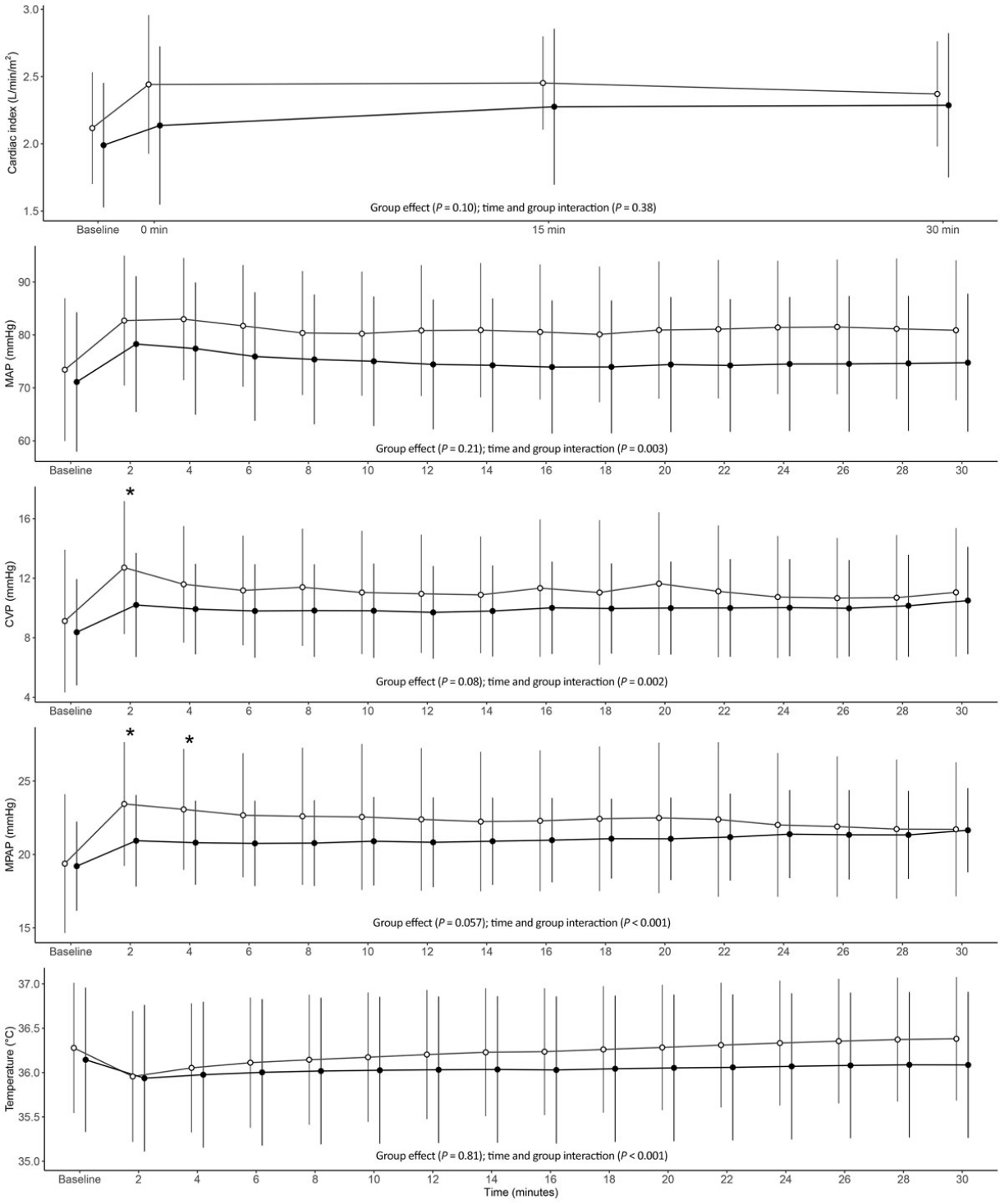
[Insert Boxes]
[Figure 1; CCR_Yanase514_Sept2021_gr1]

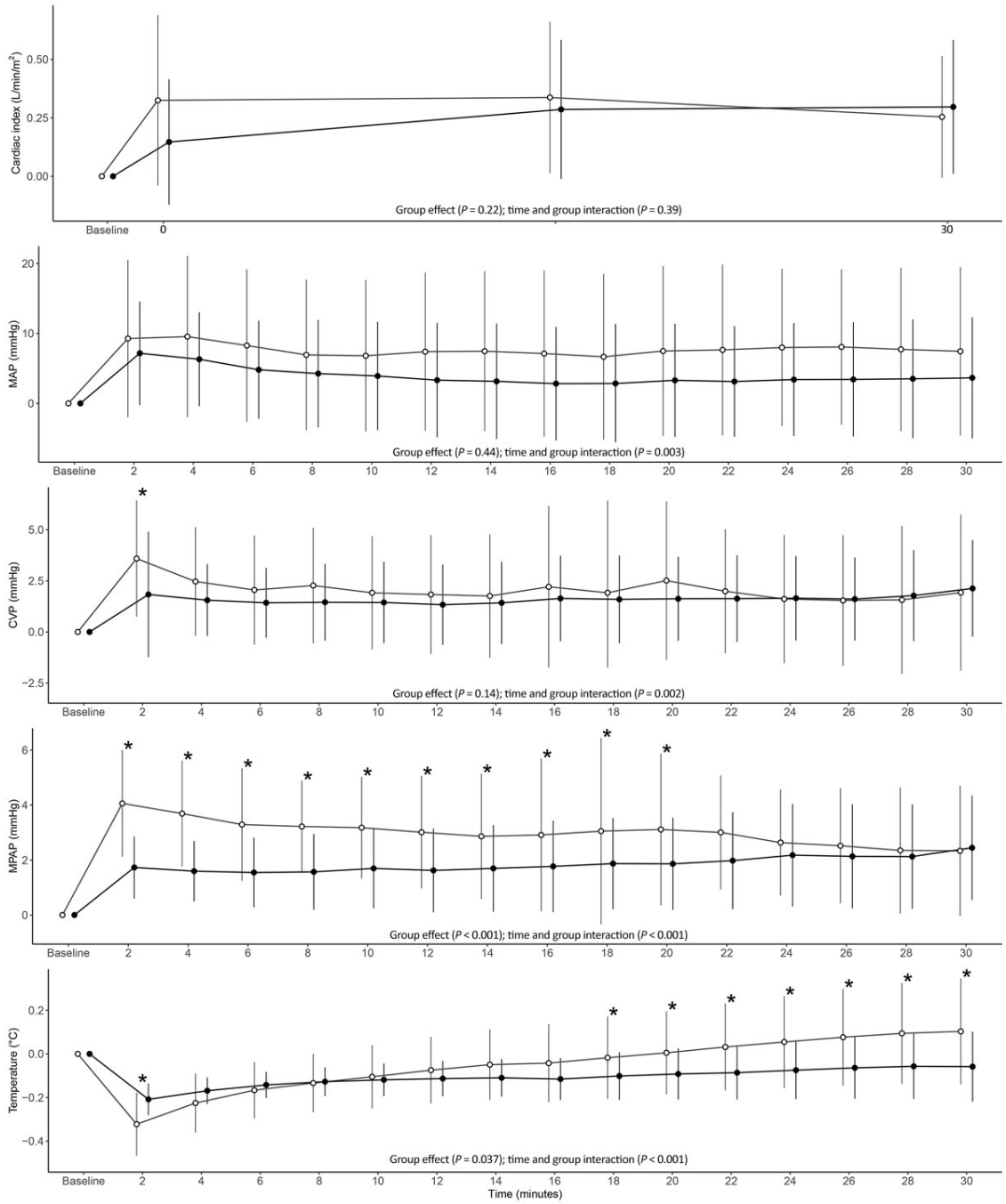
Figure 1. Comparison of the haemodynamic response (absolute value) to a 500 mL 4% albumin rapid fluid bolus therapy (FBT; white) and to a combined FBT (black)



Data are shown as mean with standard deviation. The asterisk represents a significant difference ($P < 0.05$) at this time point between the two groups adjusted for repeated measurements in a given individual.

[Figure 2; CCR_Yanase514_Sept2021_gr2]

Figure 2. Comparison of the haemodynamic response (as absolute change from baseline) to a 500 mL 4% albumin rapid fluid bolus therapy (FBT; white) and to a combined FBT (black)



Data are shown as mean with standard deviation. The asterisk represents a significant difference ($P < 0.05$) at this time point between the two groups adjusted for repeated measurements in a given individual.

[Table 1]

Table 1. Baseline characteristics of study patients

	All patients	Rapid FBT group	Combined FBT group	*P**
Total number of patients	50	25	25	
Age (years), median (IQR)	68 (62–74)	66 (62–70)	70 (62–75)	0.31
Sex, male	41 (82%)	20 (80%)	21 (84%)	> 0.99
BMI (kg/m^2), median (IQR)	27.4 (25.9–30.8)	27.4 (25.9–31.9)	28.1 (25.9–30.1)	0.76
Comorbidities				
Atrial fibrillation	7 (14%)	3 (12%)	4 (16%)	> 0.99
COPD	4 (8%)	2 (8%)	2 (8%)	> 0.99
Chronic kidney disease	3 (6%)	2 (8%)	1 (4%)	> 0.99
Diabetes mellitus	16 (32%)	7 (28%)	9 (36%)	0.76
Hypertension	31 (62%)	16 (64%)	15 (60%)	> 0.99
Ischemic heart disease	35 (70%)	21 (84%)	17 (68%)	0.16
EuroSCORE, median (IQR)	5 (3–6)	3 (2–6)	5 (4–6)	0.19
Type of surgery				0.20
On-pump CABG	29 (58%)	18 (72%)	11 (44%)	
Valve	11 (22%)	4 (16%)	7 (28%)	
CABG + valve	9 (18%)	3 (12%)	6 (24%)	
Other	1 (2%)	0	1 (4%)	
CPB duration (min), median (IQR)	118 (100–157)	113 (104–149)	120 (95–160)	0.93
Aorta clamp duration (min), median (IQR)	93 (74–118)	93 (84–118)	93 (70–116)	0.69
Post-CPB TOE assessment				
Left ventricular dysfunction	7 (14%)	5 (20%)	2 (8%)	0.23
Right ventricular dysfunction	1 (2%)	1 (4%)	0	> 0.99

BMI = body mass index; CABG = coronary aortic bypass graft; COPD = chronic obstructive pulmonary disease; CPB = cardiopulmonary bypass; EuroSCORE = European System for Cardiac Operative Risk Evaluation; FBT = fluid bolus therapy; IQR = interquartile range; na = not applicable; TOE = transoesophageal echocardiography. * *P* values reflect the between-groups comparison.

[Table 2]

Table 2. Baseline haemodynamics

	All patients	Rapid FBT group	Combined FBT group	*P**
Total number of patients	50	25	25	
Arterial pressure (mmHg), mean (SD)	72 (13)	73 (13)	71 (13)	0.54
Systolic arterial pressure (mmHg), mean (SD)	102 (17)	102 (19)	102 (15)	0.94
Diastolic arterial pressure, (mmHg), mean (SD)	58 (11)	59 (12)	56 (11)	0.52
Pulse pressure (mmHg), mean (SD)	44 (11)	43 (11)	45 (10)	0.63
PAP (mmHg), mean (SD)	19 (4)	19 (5)	19 (3)	0.88
Systolic PAP (mmHg), mean (SD)	27 (5)	27 (6)	27 (4)	0.85
Diastolic PAP (mmHg), mean (SD)	14 (4)	15 (5)	14 (3)	0.87
Central venous pressure (mmHg), mean (SD)	9 (4)	9 (5)	8 (4)	0.53
Heart rate (beats/min), mean (SD)	86 (8)	87 (9)	84 (7)	0.19
Cardiac index (L/min/m^2), mean (SD)	2.1 (0.4)	2.1 (0.4)	2.0 (0.5)	0.31
Stroke volume index (mL/m), mean (SD)	24 (5)	24 (4)	24 (6)	0.79
SVRi (dyn*s/cm^5*m^2), mean (SD)	2586 (717)	2528 (696)	2643 (746)	0.58
Systemic perfusion pressure (mmHg), mean (SD)	64 (12)	64 (12)	63 (12)	0.63
Blood temperature (°C), mean (SD)	36.2 (0.8)	36.3 (0.7)	36.2 (0.8)	0.55

CI = cardiac index; FBT = fluid bolus therapy; PAP = pulmonary arterial pressure; SD = standard deviation; SVRi = systemic vascular resistance index. * *P* values reflect the between-groups comparison.

[Table 3]

Table 3. Fluid bolus characteristics and haemodynamic response

	All patients	Rapid FBT group	Combined FBT group	*P**
Total number of patients	50	25	25	
Time from ICU admission to FBT (h), median (IQR)	1.3 (0.8–2.5)	1.5 (0.8–2.2)	1.3 (0.8–3.1)	0.79
Fluid bolus indication				0.69
Low cardiac output	15 (30%)	7 (28%)	8 (32%)	
Low filling pressures	4 (8%)	3 (12%)	1 (4%)	
Hypotension	31 (62%)	15 (60%)	16 (64%)	
Duration of fluid bolus infusion (min), median (IQR)	4.0 (1.9–6.2)	6.2 (4.4–10.8)	1.9 (1.7–2.3)	< 0.001
Fluid bolus speed (mL/min), median (IQR)	105 (62–118)	81 (46–114)	107 (88–120)	0.054
MAP response†				
At end of bolus administration	22 (44%)	11 (44%)	11 (44%)	> 0.99
At 15 min of bolus administration	17 (34%)	10 (40%)	7 (28%)	0.55
At 30 min of bolus administration	19 (38%)	10 (40%)	9 (36%)	> 0.99
CI response‡				
At end of bolus administration	18 (36%)	13 (52%)	5 (20%)	0.038
At 15 min of bolus administration	21 (42%)	10 (40%)	11 (44%)	>0.99
At 30 min of bolus administration	19 (38%)	8 (32%)	11 (44%)	0.56
CVP increase§	29 (58%)	22 (88%)	7 (28%)	< 0.001
Perfusion pressure response¶	23 (46%)	11 (44%)	12 (48%)	> 0.99
Occurrence of a confounding event**				
Minor event	5 (10%)	4 (16%)	1 (4%)	0.35

CI = cardiac index; CVP = central venous pressure; FBT = fluid bolus therapy; ICU = intensive care unit; IQR = interquartile range; MAP = mean arterial pressure. * *P* values reflect the between-groups comparison. † Defined as an increase > 10% of baseline value. ‡ Defined as an increase > 15% of baseline value. § Defined as +2 mmHg increase in CVP from baseline value, at the end of the bolus. ¶ Defined as 5% mmHg increase in perfusion pressure from baseline value, at the end of the bolus. ** The definitions are provided in the Online Appendix, supplementary appendix 2.

Online Appendix

This appendix was part of the submitted manuscript and has been peer reviewed.
It is posted as supplied by the authors.

Appendix to: Yanase F, Naorungroj T, Cutuli SL, et al. Rapid 500 mL albumin bolus versus rapid 200 mL bolus followed by slower continuous infusion in post-cardiac surgery patients: a pilot before-and-after study. *Crit Care Resusc* 2021; 23: xxx-xxx.

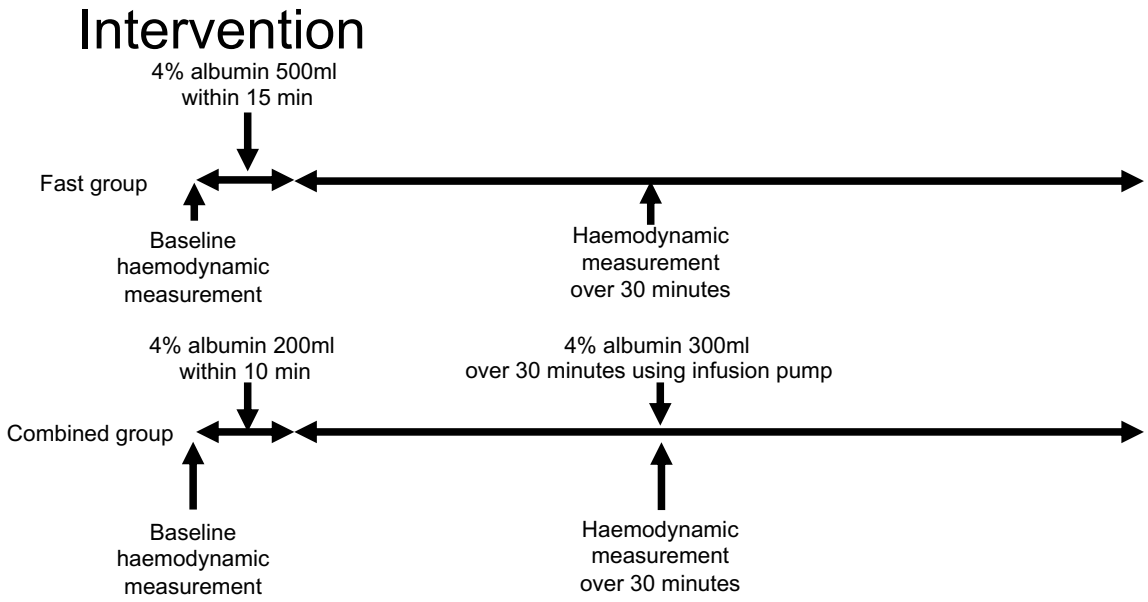
Supplementary Appendix 1. Detailed exclusion criteria.

- Additional bolus: Additional fluid bolus was prescribed during the observation period.
- Catecholamine: Any change (bolus or continuous infusion) of catecholamine.
- Sedative drug: Any change (bolus or continuous infusion) of sedative drugs.
- Muscle relaxant: Any dose of muscle relaxant infusion.
- Pacing: Any change of pacing setting.
- Ventilation setting: Any change of ventilator parameters or ventilation setting except for FiO_2 setting.
- Other: Care givers' intervention that may affect patients' haemodynamic parameters, for example, tracheal suctioning, patients' movement or movement of transducers.

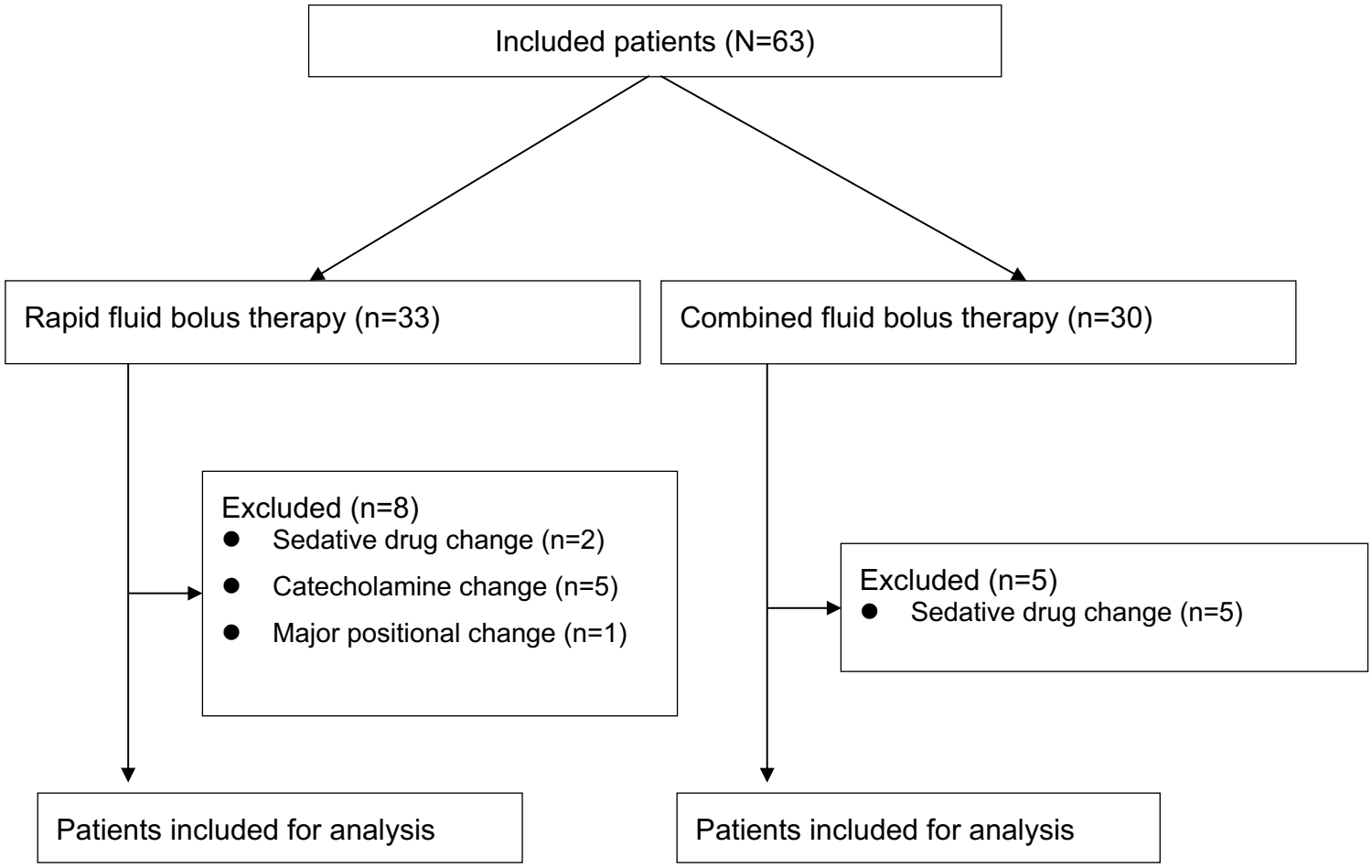
Supplementary Appendix 2. Minor confounders in the study patients.

- Patients awoke during the study periods, but kept stable without any change of sedative drug use.
- When patients awoke, bedside nurse talked to patients without touching.

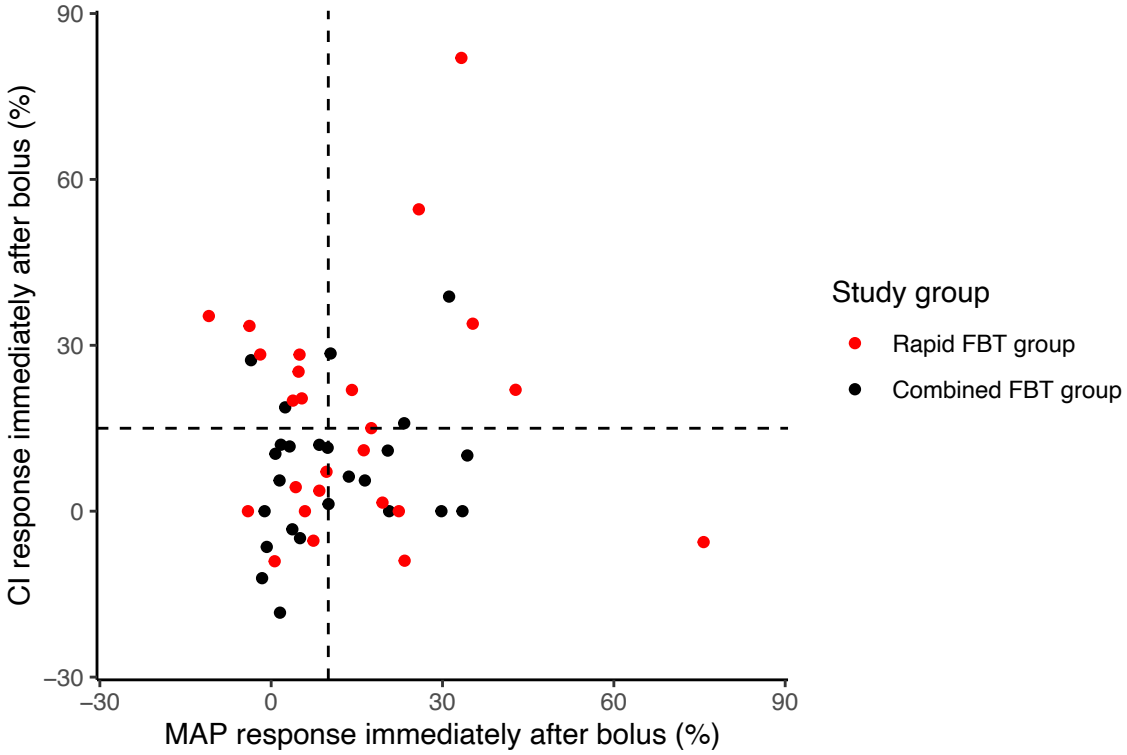
Supplementary Figure 1. Intervention protocol.



Supplementary Figure 2. Flow chart of study inclusion process

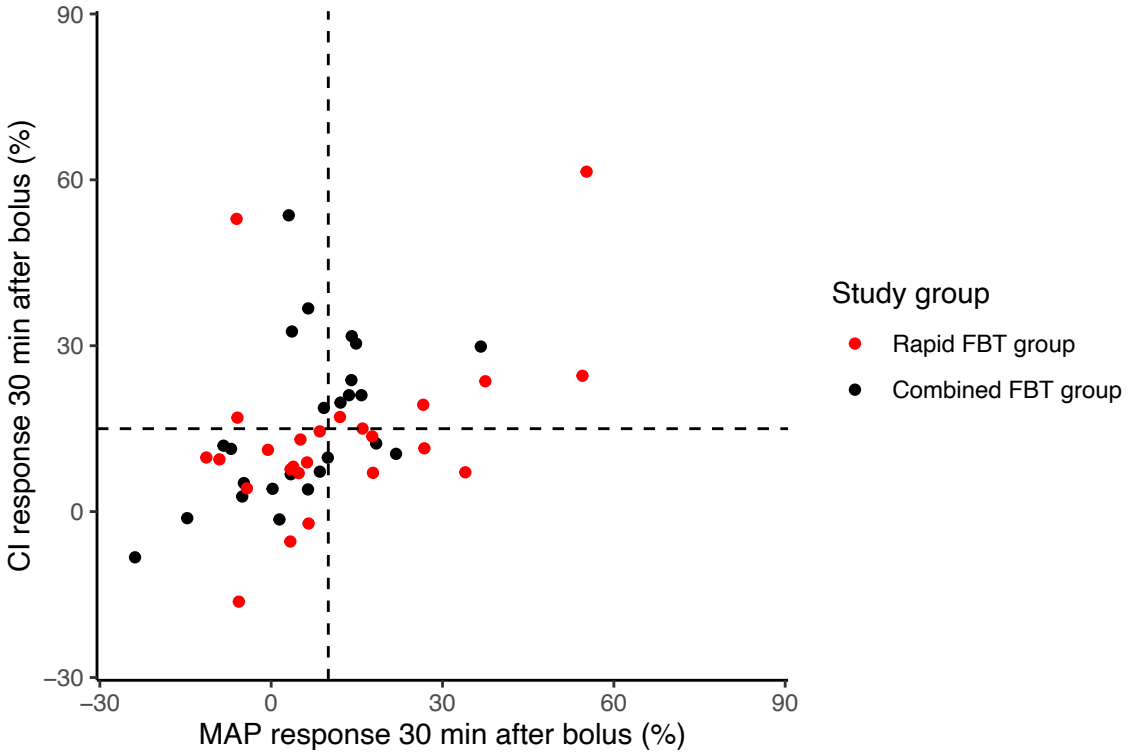


Supplementary Figure 3. Scatter plot for %MAP response and %CI response immediately after 4% albumin large bolus (500 ml) bolus and small (200 ml) bolus.



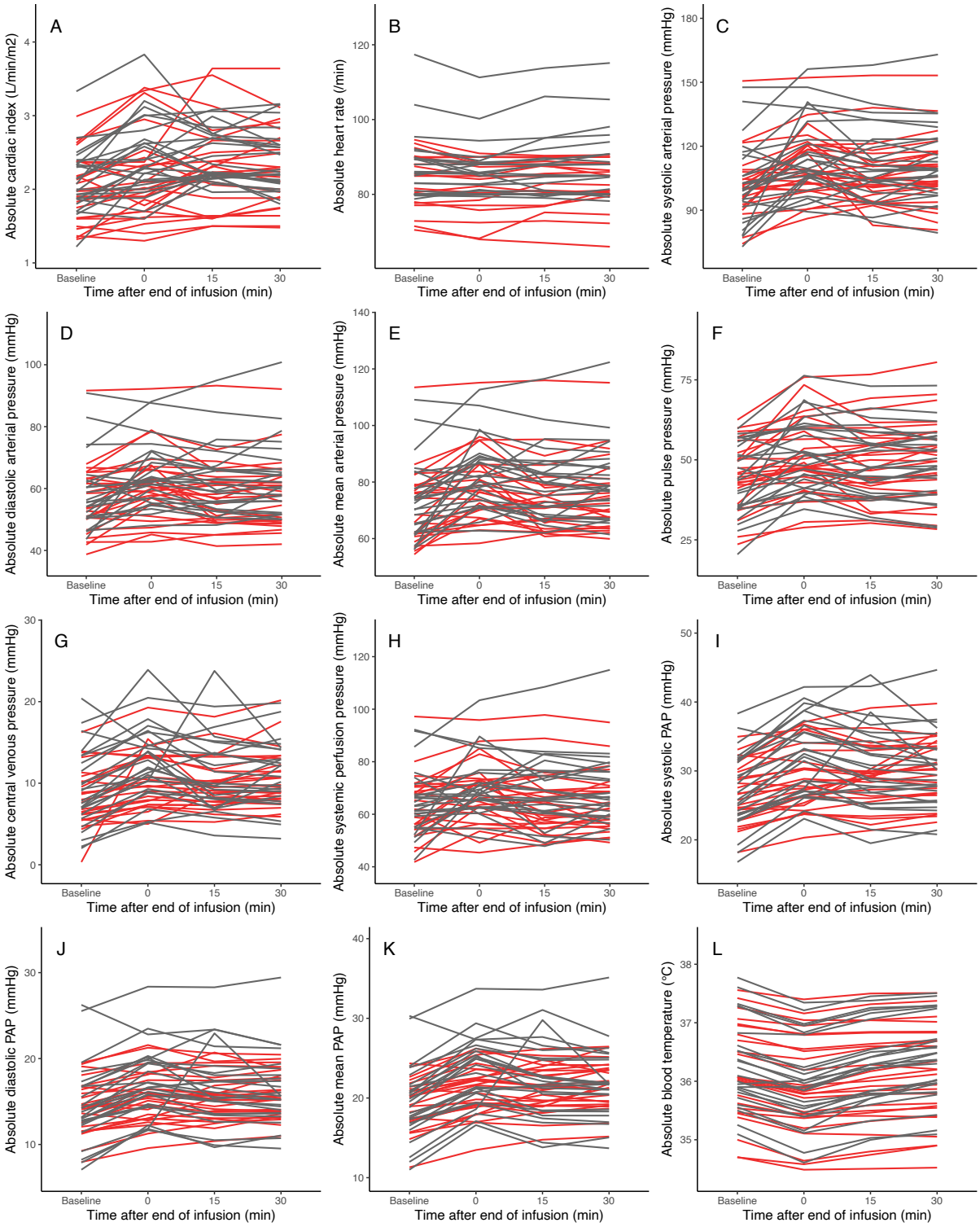
Vertical line shows mean arterial pressure (MAP) response (10%) and horizontal line shows cardiac index (CI) response (15%). Over all rho was 0.09.

Supplementary Figure 4. Scatter plot for %MAP response and %CI response 30 minutes after 4% albumin rapid FBT vs 30 minutes after small bolus (end of 500 ml infusion).



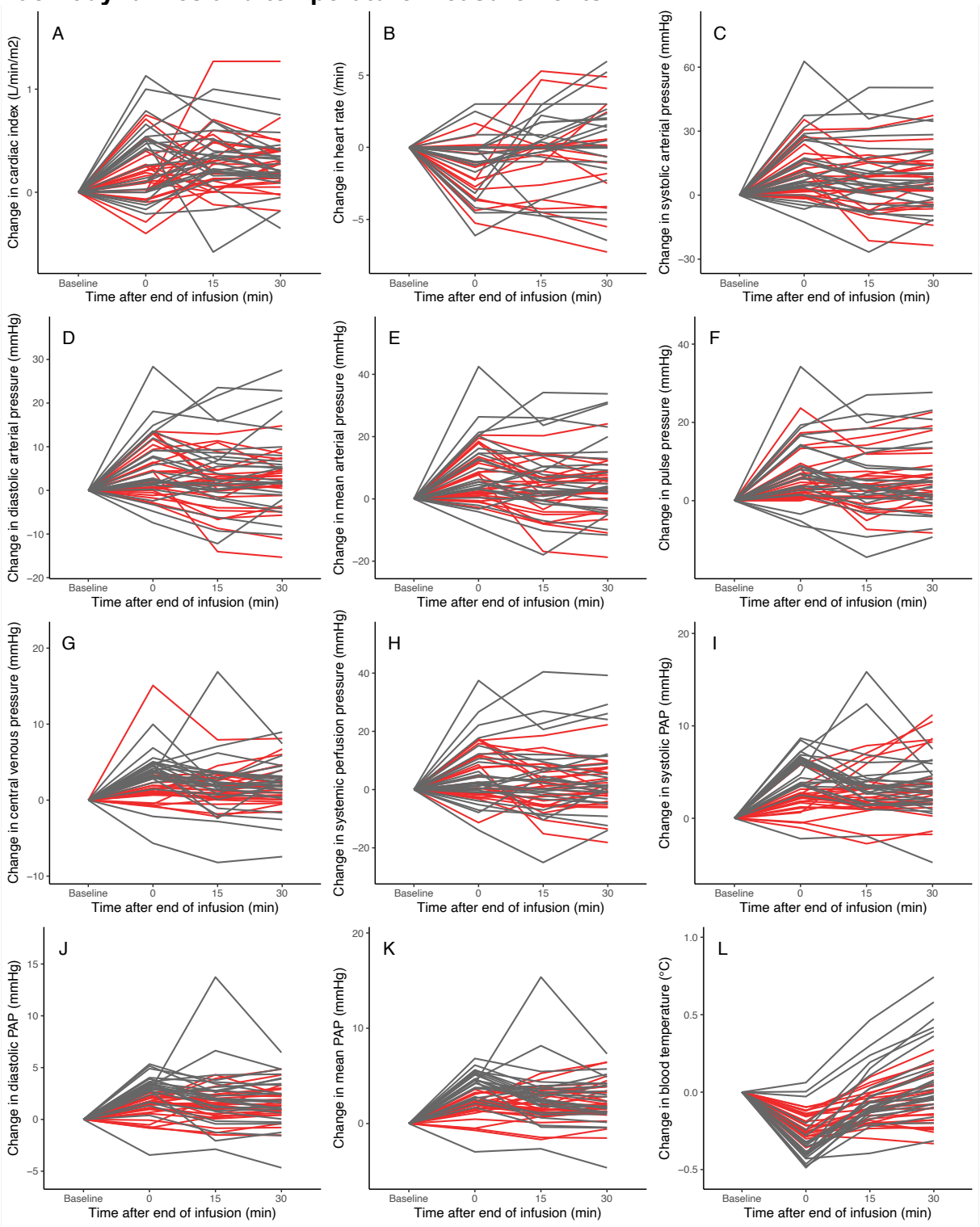
Vertical line shows mean arterial pressure (MAP) response (10%) and horizontal line shows cardiac index (CI) response (15%). Overall rho was 0.47. Rho of the rapid FBT group was 0.38 and that in the combined FBT group was 0.56.

Supplementary Figure 5. Individual haemodynamics and temperature measurements



Data shown are the absolute value for each patient, at each time point for cardiac index (A), heart rate (B), systolic arterial pressure (C), diastolic arterial pressure (D), mean arterial pressure (E), pulse pressure (F), central venous pressure (G), systemic perfusion pressure (H), systolic pulmonary arterial pressure (I), diastolic pulmonary arterial pressure (J), mean pulmonary arterial pressure (K), and body temperature (L). Patients in the rapid FBT group are represented by a grey line, those in the combined FBT group are represented by a red line.

Supplementary Figure 6. Relative change from baseline of individual haemodynamics and temperature measurements



Data shown is the relative value for each patient, at each time point, for cardiac index (A), heart rate (B), systolic arterial pressure (C), diastolic arterial pressure (D), mean arterial pressure (E), pulse pressure (F), central venous pressure (G), systemic perfusion pressure (H), systolic pulmonary arterial pressure (I), diastolic pulmonary arterial pressure (J), mean pulmonary arterial pressure (K), and body temperature (L). Patients in the rapid FBT group are represented by a grey line, those in the combined FBT group are represented by a red line.

Supplementary Table 1. Characteristics of study patients at the time of fluid bolus administration

	All patients N = 50	Rapid FBT group N = 25	Combined FBT group N = 25	*P*
Mechanical ventilation mode				0.49
SIMV	48 (96%)	23 (92%)	25 (100%)	-
PSV	2 (4%)	2 (8%)	0 (0%)	-
Mechanical ventilation settings				
Tidal volume (mL/kg PBW)§	7.1 [6.5; 8.0]	7.0 [6.3; 8.1]	7.1 [6.5; 7.7]	0.91
PIP (cm H_2O)	18 [16; 20]	18 [16; 21]	18 [16; 19]	0.36
PEEP (cm H_2O)	5 [5; 5]	5 [5; 5]	5 [5; 5]	>0.99
FiO_2	0.3 [0.3; 0.4]	0.3 [0.3; 0.4]	0.3 [0.3; 0.5]	0.18
PaO_2/FiO_2 ratio	337 [255; 399]	343 [255; 390]	332 [261; 402]	0.88
SpO_2 (%)	100 [97; 100]	98 [96; 100]	100 [98; 100]	0.10
$EtCO_2$ (mm Hg)	36 [32; 40]	38 [33; 41]	34 [31; 39]	0.17
Body temperature control				
External body active warming	20 (40%)	11 (44%)	9 (36%)	0.77
Haemodynamic status				
Vasopressor support	8 (16%)	4 (16%)	4 (16%)	>0.99
Milrinone administration	8 (16%)	5 (20%)	3 (12%)	0.70
Heart rhythm				0.57
Paced	26 (52%)	14 (56%)	12 (48%)	-
Sinus rhythm	23 (46%)	10 (40%)	13 (52%)	-
Atrial fibrillation	1 (2%)	1 (4%)	0 (0%)	-
Sedation				
Propofol	47 (94%)	24 (96%)	23 (92%)	>0.99
Propofol dose (mg/h)	120 [100; 150]	100 [100; 150]	150 [100; 150]	0.30
Opioids	4 (8%)	1 (4%)	3 (12%)	0.61
*Biochemistry**				
pH	7.38 [7.34; 7.42]	7.38 [7.34; 7.42]	7.37 [7.33; 7.40]	0.72
PaO_2 (mm Hg)	126 [90; 230]	102 [76; 190]	185 [113; 307]	0.065
$PaCO_2$ (mm Hg)	42 [40; 46]	43 [39; 46]	42 [40; 47]	0.95
Bicarbonate (mmol/L)	25 [23; 26]	24 [23; 26]	25 [24; 26]	0.40
Lactate (mmol/L)	1.1 [0.8; 1.5]	1.2 [0.9; 1.4]	1.1 [0.8; 1.7]	0.79
Creatinine (µmol/L)	71 [61; 78]	71 [60; 76]	71 [61; 81]	0.84
Haemoglobin (g/L)	104 [93; 115]	109 [92; 117]	104 [94; 107]	0.49
Blood sugar level (mmol/L)	6.8 [6.0; 8.0]	6.6 [6.0; 7.8]	7.1 [6.2; 9.1]	0.091

Data is median [interquartile range], or count (percentage).
*: measured on arterial blood gas closest to FBT. §: predicted body weight (ARDS Network formula). P values reflect the between-groups comparison.
$EtCO_2$: end-tidal CO_2 partial pressure; FiO_2: fraction of inspiratory oxygen; $PaCO_2$: arterial partial pressure of CO_2; PaO_2: arterial partial pressure of oxygen; PBW: predicted body weight; PEEP:

positive end-expiratory pressure; PIP: peak inspiratory pressure; PSV: pressure support ventilation; SIMV: synchronized intermittent mandatory ventilation; SpO_2: peripheral capillary oxygen saturation.

Supplementary Table 2. Haemodynamic effect of 4% albumin rapid FBT, and combined FBT (absolute value).

	Time after FB	Rapid FBT group	Combined FBT group
		N = 25	N = 25
Mean arterial pressure (mmHg)	Baseline	73 (13)	71 (13)
	0 min	83 (12)	78 (13)
	15 min	80 (13)	74 (13)
	30 min	81 (13)	75 (13)
Systolic arterial pressure (mmHg)	Baseline	102 (19)	102 (15)
	0 min	116 (18)	112 (15)
	15 min	112 (18)	106 (15)
	30 min	113 (18)	108 (16)
Diastolic arterial pressure (mmHg)	Baseline	59 (12)	56 (11)
	0 min	64 (10)	61 (11)
	15 min	63 (11)	58 (11)
	30 min	64 (12)	58 (11)
Pulse pressure (mmHg)	Baseline	43 (11)	45 (10)
	0 min	51 (11)	51 (12)
	15 min	49 (11)	49 (12)
	30 min	49 (11)	50 (13)
Mean PAP (mmHg)	Baseline	19 (5)	19 (3)
	0 min	23 (4)*	21 (3)*
	15 min	22 (5)	21 (3)
	30 min	22 (4)	22 (3)
Systolic PAP (mmHg)	Baseline	27 (6)	27 (4)
	0 min	32 (6)*	29 (5)*
	15 min	31 (6)	29 (4)
	30 min	30 (5)	30 (4)
Diastolic PAP (mmHg)	Baseline	15 (5)	14 (3)
	0 min	17 (4)	16 (3)
	15 min	17 (5)	15 (3)
	30 min	16 (4)	16 (3)
Central venous pressure (mmHg)	Baseline	9 (5)	8 (4)
	0 min	13 (4)*	10 (3)*
	15 min	11 (5)	10 (3)
	30 min	11 (4)	11 (3)
Heart rate (/min)	Baseline	87 (9)	84 (7)
	0 min	86 (7)	83 (7)
	15 min	87 (9)	84 (6)
	30 min	87 (9)	84 (6)
Cardiac index (L/min/m^2)	Baseline	2.1 (0.4)	2.0 (0.5)
	0 min	2.4 (0.5)	2.1 (0.6)
	15 min	2.5 (0.3)	2.3 (0.6)
	30 min	2.4 (0.4)	2.3 (0.5)
SVRi (dyn.s.cm^{-5}.m^{-2})	Baseline	2528 (696)	2643 (747)
	0 min	2428 (836)	2707 (813)
	15 min	2309 (666)	2392 (760)
	30 min	2436 (719)	2362 (688)

Systemic perfusion pressure (mmHg)	Baseline	64 (12)	63 (12)
	0 min	70 (11)	68 (12)
	15 min	69 (13)	64 (12)
	30 min	70 (13)	64 (11)
Blood temperature (°C)	Baseline	36.3 (0.7)	36.1 (0.8)
	0 min	36.0 (0.7)	35.9 (0.8)
	15 min	36.2 (0.7)	36.0 (0.8)
	30 min	36.4 (0.7)	36.1 (0.8)

Data as mean (standard deviation).
***:** p<0.05: comparison between the bolus group and the slow infusion group at each time point, adjusted for the repetition of measurements in a given patient.
FB: fluid bolus; PAP: pulmonary arterial pressure; SVRi: Systemic vascular resistance index.

Supplementary Table 3. Haemodynamic effect of 4% albumin rapid FBT, and combined (relative change from baseline).

	Time after FB	Rapid FBT group N = 25	Combined FBT group N = 25
Mean arterial pressure (mmHg)			
	0 min	9 (11)	7 (7)
	15 min	7 (12)	3 (8)
	30 min	7 (12)	4 (9)
Systolic arterial pressure (mmHg)			
	0 min	14 (16)	10 (11)
	15 min	10 (17)	5 (12)
	30 min	11 (17)	7 (13)
Diastolic arterial pressure (mmHg)			
	0 min	6 (8)	4 (6)
	15 min	4 (9)	1 (6)
	30 min	5 (9)	2 (6)
Pulse pressure (mmHg)			
	0 min	8 (9)	6 (6)
	15 min	6 (9)	4 (7)
	30 min	6 (9)	5 (7)
Mean PAP (mmHg)			
	0 min	4 (2)*	2 (1)*
	15 min	3 (3)*	2 (2)*
	30 min	2 (2)	2 (2)
Systolic PAP (mmHg)			
	0 min	5 (2)*	2 (2)*
	15 min	4 (4)*	2 (2)*
	30 min	3 (2)	3 (3)
Diastolic PAP (mmHg)			
	0 min	3 (2)*	1 (1)*
	15 min	2 (3)	1 (1)
	30 min	2 (2)	2 (1)
Central venous pressure (mmHg)			
	0 min	4 (3)*	2 (3)*
	15 min	2 (4)	2 (2)
	30 min	2 (3)	2 (2)
Heart rate (/min)			
	0 min	-1 (2)	-1 (2)
	15 min	0 (2)	-1 (2)
	30 min	0 (3)	0 (3)
Cardiac index (L/min/m^2)			
	0 min	0.3 (0.4)	0.1 (0.3)
	15 min	0.3 (0.3)	0.3 (0.3)
	30 min	0.3 (0.3)	0.3 (0.3)
SVRi (dyn.s.cm^{-5}.m^{-2})			
	0 min	-100 (682)	63 (384)
	15 min	-221 (531)	-251 (350)
	30 min	-92 (506)	-281 (294)
Systemic perfusion pressure (mmHg)			
	0 min	6 (12)	5 (8)
	15 min	4 (13)	1 (8)
	30 min	5 (13)	1 (8)

Blood temperature (°C)			
	0 min	-0.3 (0.1)*	-0.2 (0.1)*
	15 min	0.0 (0.2)	-0.1 (0.1)
	30 min	0.1 (0.3)*	-0.1 (0.2)*

Data as mean (standard deviation).
***:** $p<0.05$ between the bolus group and the slow infusion group at each time point, adjusted for the repetition of measurements in a given patient.
FB: fluid bolus; PAP: pulmonary arterial pressure; SVRi: Systemic vascular resistance index.

Supplementary Table 4. Dissipation of the haemodynamic response (MAP and CI)

	Rapid FBT group	Combined FBT group	P
Early MAP responders	N=11	N=11	
Absence of dissipation at 30 minutes*	9 (82%)	9 (82%)	>0.99
Effect dissipated	2 (18%)	2 (18%)	
Time to dissipation (min)	12 [10; 14]	4 [4; 4]	
Early CI responders	N=13	N=5	
Absence of dissipation at 30 minutes§	10 (77%)	4 (80%)	>0.99
Effect dissipated at 15 minutes	1 (8%)	1 (20%)	
Effect dissipated at 30 minutes	2 (15%)	0 (0%)	

Data is median [interquartile range], or count (percentage).
*: defined as a MAP within 3 mm Hg of baseline
§: defined as a CI within 5% of baseline CI
CI: cardiac index; MAP: mean arterial pressure

Chapter 5: Crystalloid vs. 4% albumin vs 20% albumin fluid bolus therapy in cardiac surgery patients

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A comparison of the hemodynamic effects of fluid bolus therapy with crystalloids vs. 4% albumin and vs. 20% albumin in patients after cardiac surgery

Fumitaka Yanase [a, b], Salvatore L Cutuli [a, c, d], Thummaporn Naorungroj [a, e], Laurent Bitker [a, f], Anthony Wilson [a], Glenn M Eastwood [a], Rinaldo Bellomo [a, b, g, *]

[a] Department of Intensive Care, Austin Hospital, Melbourne, VIC, Australia
[b] Australian and New Zealand Intensive Care Research Centre (ANZIC-RC), School of Public Health and Preventive Medicine, Monash University, Australia
[c] Dipartimento di Scienze dell'emergenza, anestesiologiche e della Rianimazione, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy
[d] Università Cattolica del Sacro Cuore, Rome, Italy
[e] Department of Intensive Care, Siriraj Hospital, Mahidol University, Bangkok, Thailand
[f] Service de médecine intensive et réanimation, hôpital de la Croix Rousse, Hospices Civils de Lyon, Lyon, France
[g] Centre for Integrated Critical Care, School of Medicine, University of Melbourne, Parkville, Victoria, Australia

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ABSTRACT

Background: Crystalloids, 4% albumin and 20% albumin are used for fluid bolus therapy (FBT) in patients after cardiac surgery. However, their detailed early (30 min) hemodynamic effects remain unstudied.

Methods: In a comparative prospective observational trial of 120 ventilated, we studied post cardiac surgery patients who received crystalloid 500 ml FBT, 4% albumin 500 ml FBT or 20% albumin 100 ml FBT (40 per group). We recorded second-by-second hemodynamic parameters and 15-minutely cardiac index (CI) data before and for 30 min after FBT. We compared the crystalloid group (reference) vs. the 4% albumin group, and vs. the 20% albumin group.

Results: Immediately after FBT, the mean (standard deviation) CI increase was 0.4 (0.4) L/min/m^2 with crystalloids, 0.4 (0.5) L/min/m^2 with 4% albumin and 0.3 (0.4) L/min/m^2 with 20% albumin, despite the much smaller FBT volume with 20% albumin. Mean arterial pressure (MAP) increase was 11 (10), 12 (9) and 9 (6) mm Hg, respectively. There was no group effect or interaction for changes in CI. However, there were time-group interactions for MAP changes such that crystalloid FBT had faster MAP reduction than 4% ($p < 0.001$) or 20% albumin ($p < 0.001$). Moreover, patients treated with crystalloid FBT showed a faster decline in central venous pressure, perfusion pressure than the two groups. Finally, 20% albumin attenuated the fall in temperature induced by FBT.

Conclusion: In postoperative cardiac surgery patients, after a similar initial CI and MAP response, the MAP effect of crystalloid FBT dissipates faster than that of 4% or 20% albumin FBT. These findings can be used to inform clinical practice.

Introduction

Crystalloid or colloid fluid bolus therapy (FBT) is common after cardiac sugery.[1] Its rationale is based on the notion that FBT increases cardiac preload and, thereby, cardiac index (CI) and mean arterial pressure (MAP).

Several different fluid types can be used for such FBT. They include crystalloids (normal saline or balanced solutions) or colloids (hydroxyethyl starch [HES], gelatin, and human albumin solution). All such fluids have theoretical potential advantages and disadvantages. Physiologically, colloids are thought to increase intravascular volume more than

Abbreviation: AKI, acute kidney injury; CI, cardiac index; CVP, central venous pressure; DAP, diastolic arterial pressure; FBT, fluid bolus therapy; HES, hydroxyethyl starch; HR, heart rate; ICU, intensive care unit; MAP, mean arterial pressure; PA, pulmonary artery; PAP, pulmonary artery pressure; SAP, systolic arterial pressure; SD, standard deviation

* Corresponding author at: Intensive Care Research, Austin Health, Studley Road, Heidelberg 3084, Victoria, Australia.
E-mail address: rinaldo.bellomo@austin.org.au (R. Bellomo).

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0147-9563/

crystalloids because of their oncotic pressure.[2] Among colloids, iso-oncotic (4%) albumin is the solution most frequently used in Australia.[1] This is because HES and gelatin have been found to lead to acute kidney injury (AKI) or coagulopathy, and because albumin is provided free of charge to hospitals by the Australian National Blood Authority.[3–6] More recently, however, hyper-oncotic (20%) albumin has also been used for FBT in cardiac surgery patients and reported to be associated with favorable clinical outcomes.[7] Finally, among crystalloids, balanced solutions such as Hartmann's solution, are preferred because normal saline contains supra-physiologic amounts of chloride, which may also contribute to AKI.[8]

However, despite the above considerations, in patients after cardiac surgery, no head-on comparative studies have assessed the early hemodynamic effects of FBT with these different solutions. Accordingly, we conducted a prospective detailed observational study to compare 500 ml crystalloid FBT vs. 500 ml 4% albumin FBT, and 500 ml crystalloid FBT vs. 100 ml 20% albumin FBT in ventilated patients after cardiac surgery. We hypothesized that the CI and MAP changes induced by FBT and their course over time would differ between the crystalloid group vs. the 4% albumin group, and the crystalloid group vs. the 20% albumin group.

Methods

The study protocol was approved by the Austin Health human research ethics committee in Melbourne, Australia (LNR/16/Austin/358). The need for informed consent was waived because of the observational nature of the study.

Study design

We conducted a single-center, prospective, observational study. This study was a part of broader research program, which aimed to understand the effect of FBT in patients admitted to intensive care unit (ICU) after cardiac surgery and which was conducted from December 2016 to December 2020.[9,10]

We included a convenience sample of adult (aged 18 years or older) patients who were admitted to ICU after on-pump cardiac surgery. At the time of evaluation, all received mechanical ventilation and had a pulmonary artery (PA) catheter in place. They were either prescribed 500 ml crystalloid (Plasmalyte; Plasmalyte 148, Baxter Healthcare, New South Wales, Australia or Hartmann's solution; Compound Sodium Lactate, Baxter Healthcare, New South Wales, Australia), 500 ml 4% albumin (Albumex 4: CSL Behring, Victoria, Australia) or 100 ml 20% albumin (Albumex 20: CSL Behring, Victoria, Australia) for a hemodynamic indication by their treating clinical team.

We excluded patients who were admitted to the ICU after 5 pm during week days or who were admitted to ICU during the weekend. We excluded patients who were admitted to the ICU when the research team were not available to obtain the necessary computerized data set and/or monitor for confounding hemodynamic interventions. We also excluded pregnant patients or those who required mechanical hemodynamic support (intra-aortic balloon counter-pulsation or extracorporeal membrane oxygenation). We excluded patients where the clinicians did not prescribe the above fluids and all patients. We excluded patients from analysis if any intervention with hemodynamic effect was necessary during the 30-minute observation period (Supplementary appendix 1). Finally, once we reached 40 patients in a given group, we excluded any additional patient treated with such fluid and only included patients in the other group until each group had achieved a total number of 40 patients.

Fluid bolus therapy

The intervention was 500 ml Hartmann's solution or 500 ml Plasmalyte FBT in the crystalloid group, 500 ml 4% albumin FBT in the 4% albumin group and 100 ml 20% albumin FBT in the 20% albumin group. FBT was prescribed by the treating clinical team, which operated independently from the research team.

All fluid boluses were infused rapidly (within 15 min) using a hand pump infusion system with a compressible reservoir as is usual care in the study ICU. All fluids were stored at room temperature.

Data collection

We have previously described our detailed data collection methodology.[10] In brief, in all patients, we measured systolic arterial pressure (SAP), diastolic arterial pressure (DAP), MAP, central venous pressure (CVP), systolic pulmonary artery pressure (PAP), diastolic PAP, mean PAP, heart rate (HR), peripheral oxygen saturation and core temperature. We did these measurements on a second-by-second basis using Medicollector logging software (Medicollector LCC, Boston, MA).

We measured CI by continuous or intermittent technique depending on the type of PA catheter. When patients did not have a continuous cardiac output PA catheter, the research team measured CI intermittently at four time points, before FBT, immediately after FBT, 15 min after FBT, and 30 min after FBT.

A research team member observed all study patients for the full period and recorded all interventions. When the patients unexpectedly needed other interventions that met exclusion criteria (Supplementary appendix 1), the patient was removed from analysis. However, when the patient met minor confounders (Supplementary appendix 2), recording continued and the patient was included for analysis.

Power calculation

Based previous studies, we estimated the standard deviation (SD) for CI and MAP at 0.65 and 10, respectively.[10] We thus estimated that this study would have an 80% power (two sided *p*value of 0.05) to detect differences between the two groups equivalent to 63% of the standard deviation (equivalent to a 6.3 mmHg overall MAP difference and a 0.41 L/min/m^2 overall CI difference) if 40 patients were recruited in each group. We reasoned that differences of this magnitude would likely be considered of clinical importance.

Statistical analysis

R software, version 3.5.2 (The R Foundation for Statistical Computing, Vienna, Austria) was used for analysis. We analyzed all data by comparing the crystalloid group vs. the 4% albumin group, and the crystalloid group vs. the 20% albumin group. We reported categorical data as counts (percentages) and analyzed using Fisher's exact test. We reported all baseline hemodynamic data as means (standard deviation) and analyzed them using Student's t test as they were normally distributed. We reported other continuous data as medians [interquartile range] and analyzed using Mann-Whitney U test. Longitudinal hemodynamic variables were analyzed using linear mixed effects models, accounting for within subject repeated measures, and treating time as a continuous variable. We considered a two-sided P value below 0.05 as significant.

Results

Patient characteristics

There were 1600 on-pump cardiac surgeries in the study period. Among them, after applying our exclusion criteria, we were able to en-

roll 179 patients and analyze 120 patients without major confounders (40 patients in each group) (Supplementary figure 1). Main confounders included changes in catecholamine dose or sedative drugs during the observation period.

Patient characteristics are described in Table 1 and Supplementary Table 1. The groups were well balanced and there was no difference in comorbidities, type of surgery or vasopressor use. (Supplementary Table 1). Table 2 shows the hemodynamic characteristics of the three study groups before FBTs and demonstrates no differences in any of the key parameters.

FBT description

Compared to the crystalloid group, the duration of administration of 4% albumin FBT was almost the same, however, for 20% albumin (each bottle contains only 100 ml) it was significantly shorter at 2.6 [2.1; 5.3] min ($p < 0.001$) (Table 3).

CI changes after FBT

There was no group effect or interaction in absolute values or change from baseline for the CI (Figs. 1 and 2). Immediately after FBTs, the CI increased by 0.4 (0.4) L/min/m^2 in the crystalloid group vs. by 0.4 (0.5) L/min/m^2 in the 4% albumin group vs. by 0.3 (0.4) L/min/m^2 in the 20% albumin group (Supplementary Table 2 and Supplementary Table 3). Overall, in the crystalloid group, 11 (28%) patients did not increase their CI immediately after FBT (Table 3). This compared with 11 (28%) in the 4% albumin group ($p > 0.99$) vs. nine (23%) such patients in the 20% albumin group ($p = 0.80$) (Table 3). Thirty minutes after FBTs, the CI increased by 0.2 (0.3) L/min/m^2 in the crystalloid group vs. by 0.2 (0.4) L/min/m^2 in the 4% albumin group vs. by 0.3 (0.4) L/min/m^2 in the 20% albumin group (Supplementary Table 2 and Supplementary Table 3).

During the observation period, in the crystalloid group, 11 (38%) patients who increased CI immediately after FBT returned to their baseline CI at least at one time point compared with four (14%) patients in the 4% albumin group ($p = 0.070$) vs. seven (23%) patients in the 20% albumin group ($p = 0.26$) (Table 3).

MAP changes after FBT

Immediately after FBT, the MAP increased by 11 (10) mmHg in the crystalloid group vs. 12 (9) mmHg in the 4% albumin group vs. 9 (6) mm Hg in the 20% albumin group. (Supplementary Table 2 and Supplementary Table 3). In the crystalloid group two (5.0%) patients who did not increase their MAP immediately after FBT vs. five (13%) in the 4% albumin group ($p = 0.43$) vs. one (2.5%) in the 20% albumin group ($p > 0.99$) (Table 3).

After the initial increase, there was a significant group-time interaction for both absolute values ($p < 0.001$) and relative changes ($p < 0.001$) in MAP, such that the crystalloid group showed a faster decrease in MAP compared with the 4% albumin group (Figs. 1 and 2). There was also a significant group-time interaction for absolute values and relative changes in MAP after FBT such that, after the immediate response, the 20% albumin group showed a slower MAP reduction than the crystalloid group ($p < 0.001$ and $p < 0.001$) (Figs. 1 and 2).

Overall, at 30 min after FBTs, MAP had increased by a mean of 5 (12) mmHg in the crystalloid group vs. 4 (12) mmHg in the 4% albumin group vs. 5 (7) mm Hg in the 20% albumin group (Table 3).

Fifteen (39%) patients who increased MAP immediately after crystalloid FBT returned to the baseline at least one time point in the fol-

Table 1
Baseline characteristics of study patients.

	Crystalloid	**4% Albumin**	*P*	**20% Albumin**	*P*
	N = 40	*N* = 40	Crystalloid vs 4%	*N* = 40	Crystalloid vs 20%
Age (years)	68 [60; 74]	66 [61; 69]	0.40	68 [63; 77]	0.34
Sex (male)	33 (83%)	35 (88%)	0.76	32 (80%)	>0.99
Body mass index (kg/m^2)	27.5 [24.5; 29.5]	28.1 [25.9; 32.3]	0.21	27.7 [25.0; 30.5]	0.52
Comorbidities					
Atrial fibrillation	3 (7.5%)	6 (15%)	0.48	7 (18%)	0.31
COPD	0 (0%)	5 (13%)	0.055	1 (2.5%)	>0.99
Chronic kidney disease	4 (10%)	2 (5.0%)	0.68	3 (7.5%)	>0.99
Diabetes mellitus	15 (38%)	14 (35%)	>0.99	12 (30%)	0.64
Hypertension	28 (70%)	25 (63%)	0.64	25 (63%)	0.64
Ischemic heart disease	32 (80%)	33 (83%)	>0.99	29 (73%)	0.60
EuroSCORE	5 [3, 7]	3 [2, 5]	0.055	5 [4, 7]	0.93
Type of surgery			0.17		0.68
On-pump CABG	24 (60%)	30 (75%)	–	19 (48%)	–
Valve	7 (18%)	7 (18%)	–	11 (28%)	–
CABG + Valve	5 (13%)	3 (7.5%)	–	6 (15%)	–
Other	4 (10%)	0 (0%)	–	4 (10%)	–
CPB duration (min)	114 [87; 143]	110 [97; 136]	0.90	118 [94; 154]	0.50
Aorta clamp duration (min)	85 [66; 114]	89 [71; 106]	0.78	94 [72; 122]	0.40
Post-CPB TEE assessment					
Left ventricular dysfunction	10 (25%)	6 (15%)	0.40	12 (30%)	0.80
Right ventricular dysfunction	4 (10%)	1 (2.5%)	0.36	3 (7.5%)	>0.99

Data is median [interquartile range], or count (percentage). P values reflect the between-groups comparison.
CABG: coronary aortic bypass graft; COPD: chronic obstructive pulmonary disease; CPB: cardio-pulmonary bypass; EuroSCORE: European system for cardiac operative risk evaluation; TEE: trans-esophageal echocardiography.

Table 2
Baseline hemodynamics.

	Crystalloid	**4% Albumin**	*P*	**20% Albumin**	*P*
	N = 40	*N* = 40	Crystalloid vs 4%	*N* = 40	Crystalloid vs 20%
Mean arterial pressure (mmHg)	72 (11)	75 (13)	0.29	72 (13)	0.85
Systolic arterial pressure (mmHg)	101 (16)	105 (17)	0.31	103 (15)	0.67
Diastolic arterial pressure (mmHg)	57 (10)	60 (11)	0.24	57 (11)	0.95
Pulse pressure (mmHg)	44 (12)	45 (11)	0.50	46 (10)	0.40
Mean PAP (mmHg)	20 (5)	20 (4)	0.68	20 (5)	0.97
Systolic PAP (mmHg)	27 (6)	27 (5)	0.81	28 (7)	0.75
Diastolic PAP (mmHg)	15 (5)	15 (4)	0.67	15 (5)	0.82
Central venous pressure (mmHg)	9 (4)	9 (4)	0.73	8 (3)	0.58
Heart rate (beats/min)	85 (11)	87 (7)	0.50	86 (11)	0.90
Cardiac index (L/min/m^2)	2.2 (0.5)	2.2 (0.5)	0.43	2.3 (0.6)	0.67
Stroke volume index (ml/m^2)	26 (6)	25 (5)	0.18	27 (8)	0.70
SVRi (dyn.s/cm^5.m^2)	2383 (740)	2576 (760)	0.26	2439 (916)	0.77
Systemic perfusion pressure (mmHg)	63 (11)	66 (12)	0.32	64 (12)	0.81
Blood temperature (°C)	36.3 (0.8)	36.3 (0.8)	0.86	36.2 (1.0)	0.81

Data is mean (standard deviation). P values reflect the between-groups comparison.
CI: cardiac index; PAP: pulmonary arterial pressure; SVRi: systemic vascular resistance index.

Table 3
Fluid bolus characteristics and hemodynamic response.

	Crystalloid	4% Albumin	P	20% Albumin	P
	N = 40	N = 40	Crystalloid vs 4%	N = 40	Crystalloid vs 20%
Time from ICU admission to FBT (hour)	1.8 [1.0; 2.5]	1.2 [0.8; 2.1]	0.22	1.4 [0.9; 2.5]	0.58
Fluid bolus indication			0.094		0.32
Tachycardia	0 (0%)	0 (0%)	–	1 (2.5%)	
Low cardiac output	13 (33%)	10 (25%)	–	12 (30%)	
Low filling pressures	0 (0%)	5 (13%)	–	2 (5.0%)	
Oliguria	1 (2.5%)	0 (0%)	–	0 (0%)	
Hypotension	26 (65%)	25 (63%)	–	23 (58%)	
Skin mottling	0 (0%)	0 (0%)	–	2 (5.0%)	
Duration of fluid bolus infusion (min)	4.2 [3.5; 6.6]	4.4 [3.5; 7.1]	0.49	2.6 [2.1; 5.3]	<0.001
Did not increase MAP immediately after FBT	2 (5.0%)	5 (13%)	0.43	1 (2.5%)	>0.99
MAP returned to the baseline after FBT*	15 (39%)	11 (31%)	0.63	11 (28%)	0.34
Did not increase CI immediately after FBT	11(28%)	11(28%)	>0.99	9 (23%)	0.80
CI returned to the baseline after FBT§	11 (38%)	4 (14%)	0.070	7 (23%)	0.26
Occurrence of a confounding event					
Minor event†	7 (18%)	5 (13%)	0.76	2 (5.0%)	0.15

Data is median [interquartile range], or count (percentage). *p* values reflect the between-groups comparison.
CI: cardiac index; CVP: central venous pressure; FBT: fluid bolus therapy; ICU: intensive care unit; MAP: mean arterial pressure.
* Percentage of people whose MAP returned to baseline among those whose MAP increased from baseline immediately after FBT.
§ Percentage of people whose CI returned to baseline among those whose CI increased from baseline immediately after FBT.
† Refer to Supplementary Appendix 2 for definitions.

lowing 30 min vs. 11 (28%) patients in the 4% albumin group ($p = 0.63$) vs. 11 (28%) patients in the 20% albumin group ($p = 0.34$)

Other hemodynamic changes and temperature changes

There were significant group-time interactions for CVP and perfusion pressure ($p = 0.025$ in both absolute value and relative change in CVP, and $p = 0.001$ in absolute value and $p = 0.002$ in relative change in perfusion pressure). Thus, after the initial response, the crystalloid group showed a faster decline in CVP and perfusion pressure than the 4% albumin group (Figs. 1 and 2).

There were also significant group-time interactions in CVP and perfusion pressure ($p<0.001$ in both absolute value and relative change in CVP, and $P = 0.025$ in absolute value and $p = 0.035$ in relative change in perfusion pressure). Thus, after the initial response, crystalloid FBT showed a faster reduction in CVP and perfusion pressure than 20% albumin FBT.

For blood temperature, there were significant group-time interactions for absolute values and relative changes (group effect; $p<0.001$ in relative change, and interaction; $p<0.001$ in both absolute value and relative change) such that 20% albumin FBT showed the least effect on lowering body temperature (Fig. 2).

Discussion

Key findings

We conducted a prospective observational trial to compare the hemodynamic effects of 500 ml crystalloid FBT with 500 ml 4% albumin FBT, and with 100 ml 20% albumin FBT. We found that the initial response was similar despite the fact that 20% albumin delivered only 20% of the volume delivered with crystalloid or 5% FBT. However, after the initial response, patients treated with crystalloid FBT also showed a faster MAP, CVP and perfusion pressure decline compared with both the 4% albumin and the 20% albumin group. Also, 20% albumin attenuated the rise in CVP seen with FBT and fall in temperature typically induced by crystalloid FBT.

Relationship to previous studies

No controlled study has compared FBT with crystalloid vs. albumin solutions (both 4% and 20%) and described their hemodynamic response over 30 min in patients after cardiac surgery despite the apparently widespread use of these fluids. Even in sepsis where FBT is ubiquitous, essentially all studies of FBT do not provide minutely data and stop their analysis at the end of the bolus or at 15 min thereafter, making our study unique.[11] A single center, before-and-after study compared crystalloid therapy with 20% albumin FBT in patients after cardiac surgery.[7] It found that 20% albumin therapy was associated with a less positive fluid balance, a smaller dose of norepinephrine, and a shorter duration of norepinephrine treatment than the crystalloid group. However, the investigators did not report on the direct hemodynamic effects of these solutions.[7]

Use of 4% albumin in patients after cardiac surgery appears relatively common;[1] however, its benefits or hemodynamic effects in comparison with crystalloid FBT are unclear. One propensity-matched cohort study concluded that 5% albumin use was associated with a reduction of in-hospital mortality.[12] On the other hand, another retrospective study concluded that use of 4% albumin was associated with more complications.[13] Regrettably, these are retrospective studies that did not describe any actual hemodynamic effect of FBT with 4% albumin.

Study implications

Our findings imply that, in post-cardiac surgery patients, compared with crystalloid FBT, FBT with 4% albumin and 20% albumin achieves more sustained effects on MAP and perfusion pressure. In addition, 20% albumin achieved these hemodynamic effects with one-fifth (100 ml) of infused volume and with less FBT induced hypothermia.

Strengths and limitations

Our study has several strengths. Our data are robust to other interventions because at least one researcher observed all patients and excluded data when hemodynamic confounders occurred. Moreover, our study reflected clinical practice in many centers where FBT is given rapidly with the choice of fluid left to clinical preference. Finally, our data are based on detailed second-by-second collection of data (with the exception of the cardiac index) which provides a high level of granularity and dynamic assessment of changes in key circulatory parameters.

We acknowledge several limitations. Our study was not a randomized controlled trial However, baseline characteristics were well-balanced between the groups. Our study was a single center study and included a limited number of patients. In particular, other clinicians in different institutions may tolerate greater levels of hypotension or be more liberal in the use of vasopressor drugs. However, this is a physiological study over 30 min with detailed data collection, which makes it

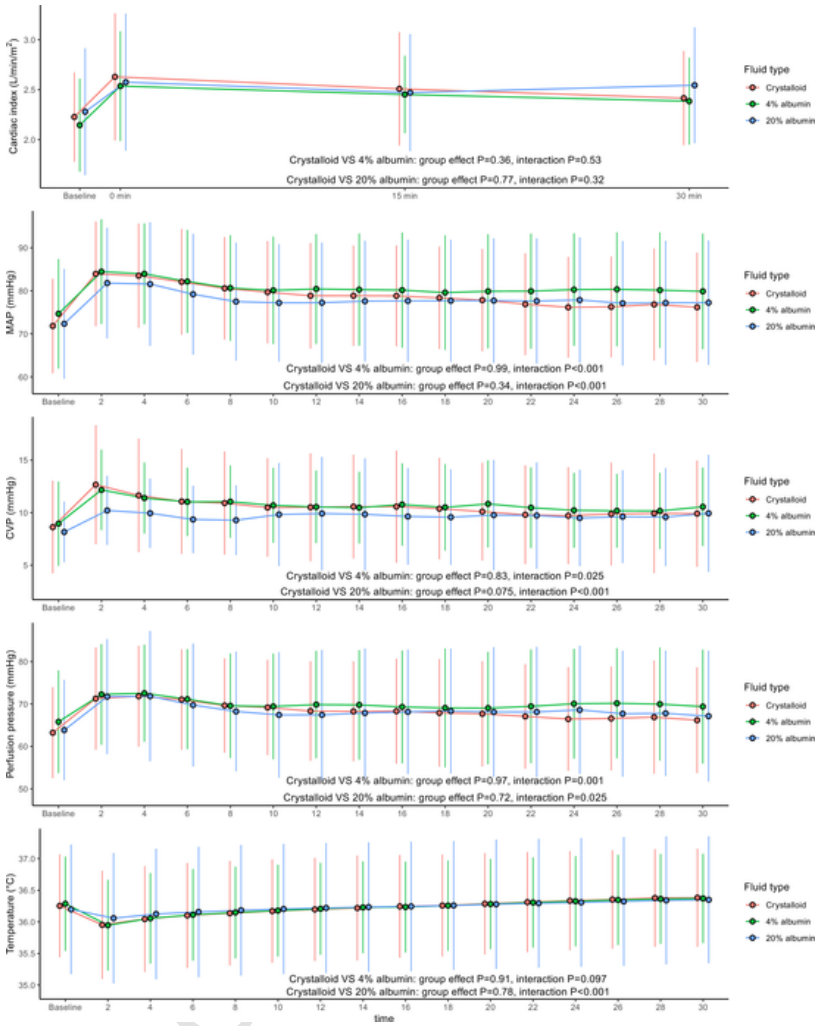

Fig. 1. Comparison of the hemodynamic response (absolute value) to a 500 ml crystalloid fluid bolus (red), a 500 ml 4% albumin fluid bolus (green) and to a 100 ml 20% albumin fluid bolus (blue). Data are shown as means and standard deviations. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

unlikely that particular practice patterns would influence our findings. In such detailed data collection is labor-intensive making it difficult to do a large-scale study of hundreds of patients. Moreover, plausible and physiologically logical significant differences were observed. We did not study patients after the first 30 min. However, we found that after 30 min, multiple confounders rapidly accrued (changes in sedation, positioning, ventilation and vasopressor therapy and make assessment the FBT effect unreliable) making assessment of the fluid effect per se highly flawed. Finally, this study did not report clinical outcomes and the clinical meaning of the physiological changes seen with these three types of fluids remains unclear. However, an ongoing randomized controlled trial comparing crystalloid FBT vs. 20% albumin FBT for patients after cardiac surgery in five different hospitals in Australia is currently under way to address this question in the near future.[14]

Conclusion

In conclusion, after an initially similar effect, crystalloid FBT leads to significantly greater dissipation of the MAP and perfusion pressure effect than 4% or 20% albumin FBT. Moreover, 20% albumin achieves the same effect as crystalloid therapy without the same loss of effect on MAP and is equivalent to 4% albumin in all aspects but at only one fifth of the volume and without the infusion associated rapid fall in body temperature. These findings inform clinicians' expectations of FBT ef-

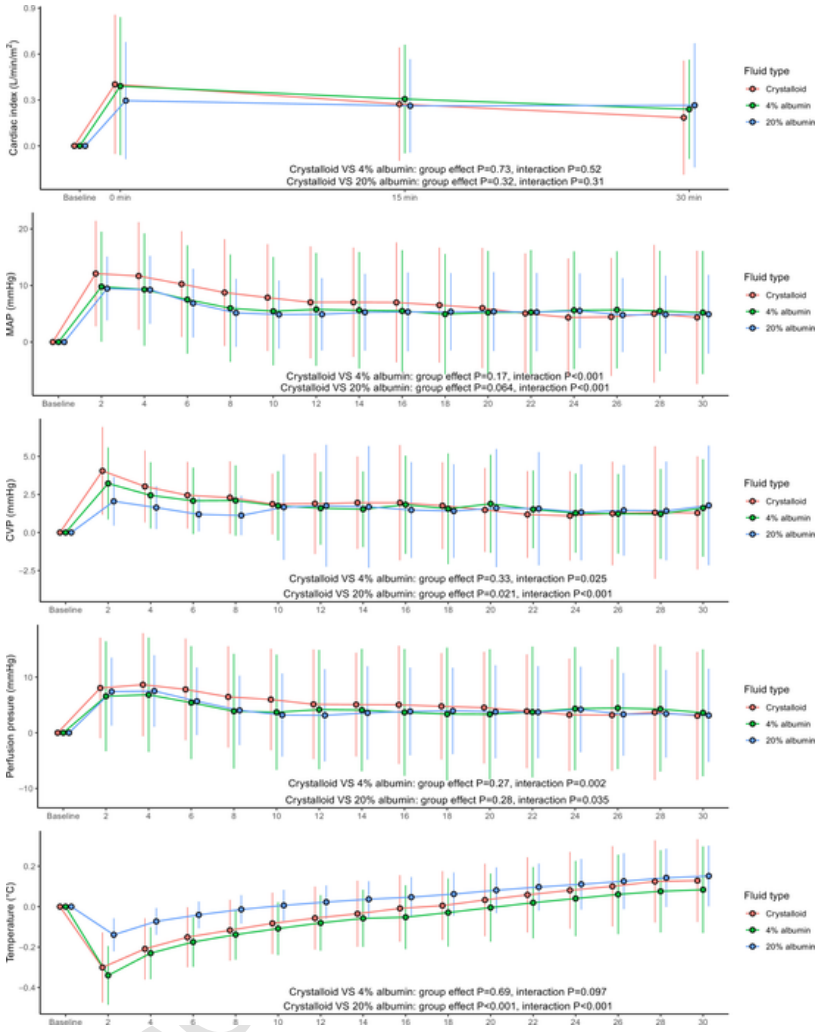

Fig. 2. Comparison of the hemodynamic response (as absolute change from baseline) to a 500 ml crystalloid fluid bolus (red), a 500 ml 4% albumin fluid bolus (green) and to a 100 ml 20% albumin fluid bolus (blue). Data are shown as means and standard deviations. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

fects and can be used guide choice of fluid type for FBT in cardiac surgery patients.

Declaration of Competing Interest

None.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.hrtlng.2021.07.014.

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Supplementary Appendix Item 1. Detailed exclusion criteria.

- Additional bolus: Additional fluid bolus was prescribed during the observation period.
- Catecholamine: Any change (bolus or continuous infusion) of catecholamine.
- Sedative drug: Any change (bolus or continuous infusion) of sedative drugs.
- Muscle relaxant: Any dose of muscle relaxant infusion.
- Pacing: Any change of pacing setting.
- Ventilation setting: Any change of ventilator parameters or ventilation setting except for FiO_2 setting.
- Other: Care givers' intervention that may affect patients' hemodynamic parameters, for example, tracheal suctioning, patients' movement or movement of transducers.

Supplementary Appendix Item 2. Minor confounders in the study patients.

- Patients awoke during the study periods, but kept stable without any change of sedative drug use.
- When patients awoke, bedside nurse talked to patients without touching.

Supplementary Table 1. Characteristics of study patients at the time of fluid bolus administration

	Crystalloid N = 40	**4% Albumin** N = 40	P Crystalloid vs 4%	**20% Albumin** N = 40	P Crystalloid vs 20%
Mechanical ventilation mode			>0.99		>0.99
SIMV	38 (95%)	38 (95%)	-	38 (95%)	-
PSV	2 (5.0%)	2 (5.0%)	-	2 (5.0%)	-
Mechanical ventilation settings					
Tidal volume (mL/kg PBW)§	7.3 [6.5; 8.2]	6.8 [6.3; 8.1]	0.38	7.2 [6.4; 8.1]	0.90
PIP (cm H_2O)	17 [16; 19]	18 [16; 21]	0.16	19 [16; 21]	0.22
PEEP (cm H_2O)	5 [5; 5]	5 [5; 5]	0.36	5 [5; 5]	0.40
FiO_2	0.32 [0.30; 0.50]	0.30 [0.21; 0.40]	0.32	0.40 [0.30; 0.50]	0.34
SpO_2 (%)	99 [96; 100]	99 [97; 100]	0.82	99 [97; 100]	0.73
$EtCO_2$ (mm Hg)	34 [31; 38]	37 [34; 40]	0.054	34 [31; 38]	0.97
Body temperature control					
External body active warming	11 (28%)	14 (35%)	0.63	17 (43%)	0.24
Hemodynamic status					
Vasopressor support	11 (28%)	8 (20%)	0.60	11 (28%)	>0.99
Milrinone administration	8 (20%)	7 (18%)	>0.99	8 (20%)	>0.99
Heart rhythm			0.37		0.66
Paced	18 (45%)	22 (55%)	-	20 (50%)	-
Sinus rhythm	22 (55%)	17 (43%)	-	19 (48%)	-
Atrial fibrillation	0 (0%)	1 (2.5%)	-	1 (2.5%)	-
Sedation					
Propofol	35 (88%)	37 (93%)	0.71	39 (98%)	0.20
Propofol dose (mg/h)	100 [58; 150]	100 [100; 200]	0.087	110 [80; 150]	0.19
Opioids	1 (2.5%)	2 (5.0%)	>0.99	3 (7.5%)	0.62
*Biochemistry**					
pH	7.40 [7.37; 7.43]	7.36 [7.34; 7.42]	0.015	7.41 [7.35; 7.43]	0.69
PaO_2 (mm Hg)	127 [87; 174]	102 [78; 177]	0.24	125 [83; 204]	0.95
$PaCO_2$ (mm Hg)	41 [37; 42]	43 [40; 46]	0.003	43 [41; 45]	0.014
Bicarbonate (mmol/L)	24 [22; 26]	24 [23; 26]	0.82	26 [24; 27]	0.10
Lactate (mmol/L)	1.2 [1.0; 1.7]	1.2 [0.9; 1.4]	0.26	1.1 [0.9; 1.6]	0.45
Creatinine (µmol/L)	70 [64; 86]	72 [62; 78]	0.80	77 [67; 105]	0.23
Hemoglobin (g/L)	106 [94; 112]	106 [100; 115]	0.57	98 [89; 106]	0.13
Blood sugar level (mmol/L)	7.5 [6.4; 8.7]	6.8 [6.0; 7.8]	0.14	7.0 [6.2; 8.2]	0.45

Data is median [interquartile range], or count (percentage).
*: measured on arterial blood gas closest to FBT. §: predicted body weight (ARDS Network formula).
P values reflect the between-groups comparison.
$EtCO_2$: end-tidal CO_2 partial pressure; FiO_2: fraction of inspiratory oxygen; $PaCO_2$: arterial partial pressure of CO_2; PaO_2: arterial partial pressure of oxygen; PBW: predicted body weight; PEEP: positive end-expiratory pressure; PIP: peak inspiratory pressure; PSV: pressure support ventilation; SIMV: synchronized intermittent mandatory ventilation; SpO_2: peripheral capillary oxygen saturation.

Supplementary Table 2. Hemodynamic effect of crystalloid FBT, 4% albumin FBT, and 20% albumin FBT (absolute value).

	Time after FB	Crystalloid	4% Albumin	20% Albumin
		N = 40	N = 40	N = 40
Mean arterial pressure (mmHg)	Baseline	72 (11)	75 (13)	72 (13)
	0 min	84 (12)	84 (12)	82 (13)
	15 min	79 (12)	80 (13)	78 (14)
	30 min	76 (13)	80 (13)	77 (15)
Systolic arterial pressure (mmHg)	Baseline	101 (16)	105 (17)	103 (15)
	0 min	119 (18)	119 (17)	115 (16)
	15 min	111 (18)	113 (19)	110 (18)
	30 min	108 (19)	112 (19)	109 (18)
Diastolic arterial pressure (mmHg)	Baseline	57 (10)	60 (11)	57 (11)
	0 min	64 (11)	65 (10)	63 (11)
	15 min	61 (10)	62 (11)	60 (12)
	30 min	59 (11)	63 (11)	60 (12)
Pulse pressure (mmHg)	Baseline	43 (12)	45 (11)	46 (10)
	0 min	55 (16)	54 (12)	52 (10)
	15 min	50 (15)	50 (13)	50 (11)
	30 min	48 (15)	49 (14)	50 (11)
Mean PAP (mmHg)	Baseline	20 (5)	20 (4)	20 (5)
	0 min	24 (7)	24 (4)	23 (6)
	15 min	22 (5)	22 (4)	22 (6)
	30 min	21 (5)	21 (4)	21 (6)
Systolic PAP (mmHg)	Baseline	27 (6)	27 (5)	28 (7)
	0 min	33 (8)	33 (6)	32 (8)
	15 min	30 (7)	31 (6)	30 (8)
	30 min	29 (6)	30 (5)	30 (8)
Diastolic PAP (mmHg)	Baseline	15 (5)	15 (4)	15 (5)
	0 min	18 (6)	18 (4)	17 (5)
	15 min	16 (5)	16 (4)	16 (6)
	30 min	16 (5)	16 (4)	16 (5)
Central venous pressure (mmHg)	Baseline	9 (4)	9 (4)	8 (3)
	0 min	13 (6)	12 (4)	10 (3)
	15 min	10 (5)	11 (4)	9 (4)
	30 min	10 (5)	11 (4)	10 (6)
Heart rate (/min)	Baseline	85 (11)	87 (7)	86 (11)
	0 min	85 (12)	86 (7)	86 (11)
	15 min	85 (11)	86 (7)	86 (10)
	30 min	85 (12)	87 (8)	86 (10)
Cardiac index (L/min/m^2)	Baseline	2.2 (0.4)	2.1 (0.5)	2.3 (0.6)
	0 min	2.6 (0.6)	2.5 (0.5)	2.6 (0.7)
	15 min	2.5 (0.6)	2.5 (0.4)	2.5 (0.6)
	30 min	2.4 (0.5)	2.4 (0.4)	2.5 (0.6)
SVRi (dyn.s/cm^5.m^2)	Baseline	2383 (740)	2576 (760)	2439 (916)
	0 min	2320 (774)	2424 (845)	2401 (870)
	15 min	2238 (764)	2319 (654)	2430 (927)
	30 min	2276 (690)	2394 (663)	2290 (907)

Systemic perfusion pressure (mmHg)	Baseline	63 (11)	66 (12)	64 (12)
	0 min	71 (12)	72 (12)	72 (14)
	15 min	68 (13)	69 (14)	68 (15)
	30 min	66 (12)	69 (13)	67 (16)
Blood temperature (°C)	Baseline	36.3 (0.8)	36.3 (0.7)	36.2 (1.0)
	0 min	36.0 (0.9)	35.9 (0.7)	36.1 (1.0)
	15 min	36.3 (0.8)	36.2 (0.7)	36.3 (1.0)
	30 min	36.4 (0.8)	36.4 (0.7)	36.3 (1.0)

Data as mean (standard deviation).
FBT: fluid bolus therapy; PAP: pulmonary arterial pressure; SVRi: Systemic vascular resistance index.

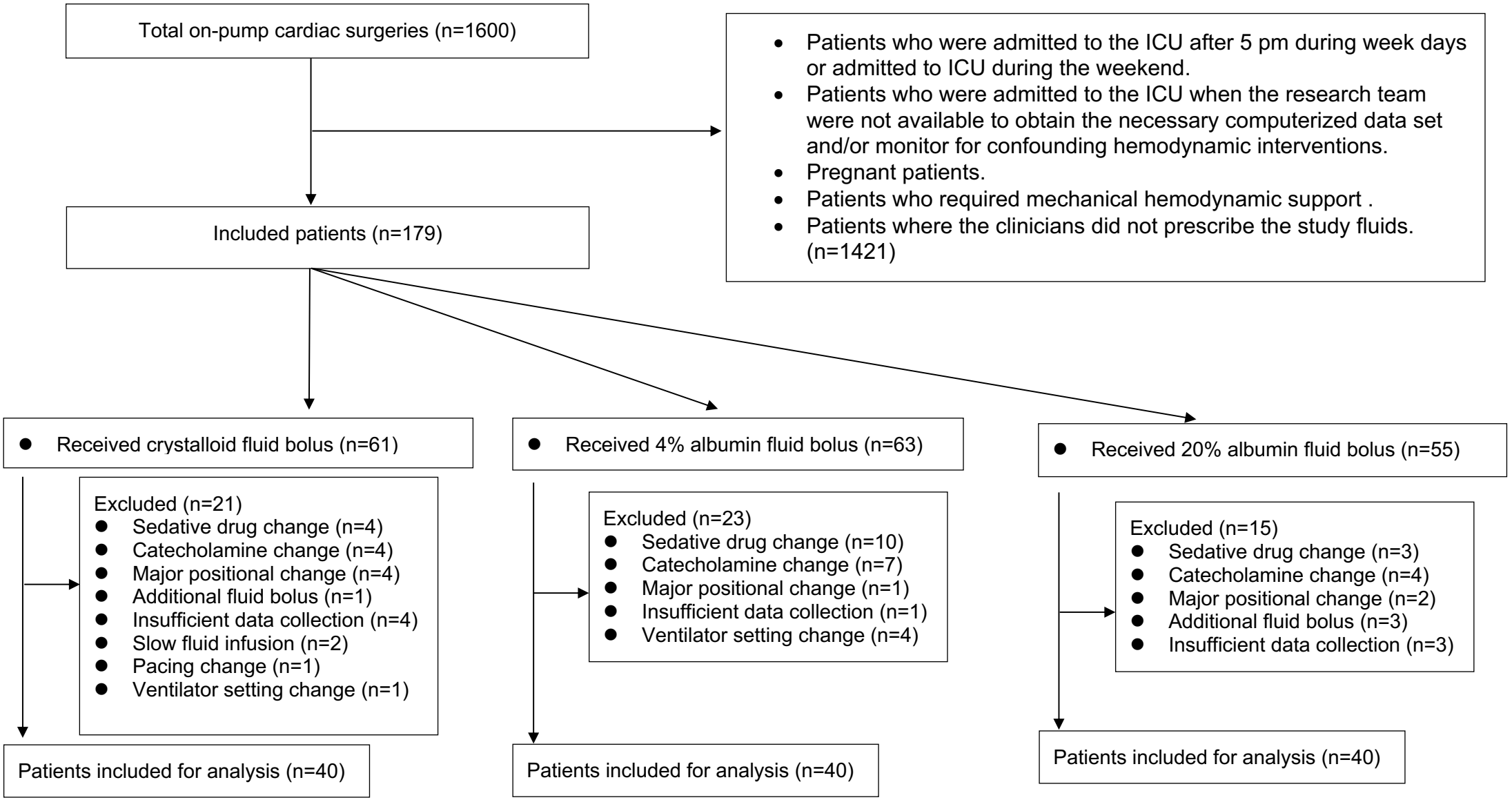
Supplementary Table 3. Haemodynamic effect of crystalloid FBT, 4% albumin FBT, and 20% albumin FBT (relative change from baseline).

	Time after FB	Crystalloid	4% Albumin	20% Albumin
		N = 40	N = 40	N = 40
Mean arterial pressure (mmHg)				
	0 min	11 (10)	12 (9)	9 (6)
	15 min	6 (10)	7 (10)	5 (7)
	30 min	5 (12)	4 (12)	5 (7)
Systolic arterial pressure (mmHg)				
	0 min	16 (14)	18 (13)	13 (8)
	15 min	9 (15)	10 (14)	7 (11)
	30 min	7 (17)	7 (16)	7 (11)
Diastolic arterial pressure (mmHg)				
	0 min	7 (7)	8 (7)	6 (4)
	15 min	4 (8)	4 (8)	3 (5)
	30 min	3 (8)	3 (9)	3 (5)
Pulse pressure (mmHg)				
	0 min	9 (8)	10 (8)	6 (5)
	15 min	5 (8)	5 (6)	4 (8)
	30 min	4 (9)	4 (8)	4 (7)
Mean PAP (mmHg)				
	0 min	4 (2)	4 (3)	3 (1)
	15 min	2 (3)	2 (2)	2 (2)
	30 min	1 (2)	1 (2)	1 (3)
Systolic PAP (mmHg)				
	0 min	5 (3)	5 (4)	4 (2)
	15 min	3 (3)	2 (3)	2 (2)
	30 min	2 (3)	2 (3)	2 (4)
Diastolic PAP (mmHg)				
	0 min	3 (2)	3 (2)	2 (1)
	15 min	1 (3)	1 (3)	1 (2)
	30 min	1 (3)	0 (3)	1 (2)
Central venous pressure (mmHg)				
	0 min	4 (3)	4 (3)	2 (2)
	15 min	2 (3)	2 (3)	1 (3)
	30 min	2 (3)	1 (3)	2 (4)
Heart rate (/min)				
	0 min	-1 (3)	0 (3)	0 (2)
	15 min	0 (2)	0 (2)	0 (1)
	30 min	0 (3)	0 (2)	0 (2)
Cardiac index (L/min/m^2)				
	0 min	0.4 (0.4)	0.4 (0.5)	0.3 (0.4)
	15 min	0.3 (0.4)	0.3 (0.4)	0.3 (0.3)
	30 min	0.2 (0.3)	0.2 (0.4)	0.3 (0.4)
SVRi (dyn.s/cm^5.m^2)				
	0 min	-110 (548)	-68 (407)	-32 (457)
	15 min	-193 (425)	-103 (320)	-89 (408)
	30 min	-135 (406)	-85 (300)	-195 (393)
Systemic perfusion pressure (mmHg)				
	0 min	7 (10)	8 (9)	7 (6)
	15 min	4 (11)	5 (10)	4 (8)
	30 min	3 (11)	3 (11)	3 (9)

Blood temperature (°C)				
	0 min	-0.3 (0.2)	-0.3 (0.2)	-0.1 (0.1)
	15 min	0.0 (0.2)	0.0 (0.2)	0.1 (0.1)
	30 min	0.1 (0.2)	0.1 (0.2)	0.2 (0.2)

Data as mean (standard deviation).
FB: fluid bolus; PAP: pulmonary arterial pressure; SVRi: Systemic vascular resistance index.

Supplementary Figure 1. Flow chart of study inclusion process



Chapter 6: High-dose intravenous vitamin C in cardiac surgery

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Original Article

A Pilot, Double-Blind, Randomized, Controlled Trial of High-Dose Intravenous Vitamin C for Vasoplegia After Cardiac Surgery

Fumitaka Yanase, MD[*,†], Laurent Bitker, MD[*,‡], Lara Hessels, MD[*,§], Eduardo Osawa, MD[*], Thummaporn Naorungroj, MD[*,‖], Salvatore L. Cutuli, MD[*], Paul J. Young, MD, PhD[¶], Jay Ritzema, MD[¶], Georgia Hill, MClinlm[¶], Charlotte Latimer-Bell, MClinlm[¶], Anna Hunt, RN, BN, PGDip, HSM[¶], Glenn M. Eastwood, PhD[*], Andrew Hilton, MD[*], Rinaldo Bellomo, MD, PhD[*,†,#,1]

[*]*Department of Intensive Care, Austin Hospital, Melbourne, Australia*
[†]*Australian and New Zealand Intensive Care Research Centre, Monash University School of Public Health and Preventive Medicine, Melbourne, Australia*
[‡]*Service de médecine intensive et réanimation, Hôpital de la Croix Rousse, Hospices Civils de Lyon, Lyon, France*
[§]*Department of Critical Care, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands*
[‖]*Department of Intensive Care, Siriraj Hospital, Mahidol University, Bangkok, Thailand*
[¶]*Department of Intensive Care, Wellington Hospital, Wellington, New Zealand*
[#]*Centre for Integrated Critical Care, Department of Medicine & Radiology, University of Melbourne, Melbourne, Australia*

Objective: To conduct a pilot feasibility and physiologic efficacy study of high-dose vitamin C in patients with vasoplegia after cardiac surgery.
Design: Prospective, double-blind, randomized, controlled trial.
Setting: Two tertiary intensive care units (ICUs).
Participants: Post-cardiac surgery patients with vasoplegia.
Interventions: The authors randomly assigned the patients to receive either high-dose intravenous vitamin C (1,500 mg every 6 hours) or placebo. The primary outcome was time from randomization to resolution of vasoplegia. Secondary outcomes included total norepinephrine equivalent dose in the first 2 days, ICU length of stay, ICU mortality, and in-hospital mortality.
Measurements and Main Results: The authors studied 50 patients (25 patients in each arms). The mean (standard deviation) time to resolution of vasoplegia was 27.0 (16.5) hours in the vitamin C group versus 34.7 (41.1) hours in the placebo group (mean decrease with vitamin C of 7.7 hours, 95% confidence interval −10.5 to 25.9, p = 0.40). The median (interquartile range) norepinephrine equivalent dose in the first 2 days was 64.9 (23.5-236.5) μg/kg versus 47.4 (21.4-265.9) μg/kg in the vitamin C and placebo group (p = 0.75). The median duration of ICU admission was similar (1.4 [0.5-2.5] days and 1.5 [0.5-3.3] days in the vitamin C and placebo group; p = 0.36). Only 1 patient, in the vitamin C arm, died.

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[1]*Address reprint requests to* Rinaldo Bellomo, MD, PhD, Director, Intensive Care Research, Austin Health, 145 Studley Road, Heidelberg, Melbourne, Victoria, Australia, 3084.
E-mail address: rinaldo.bellomo@austin.org.au (R. Bellomo).

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Conclusion: In patients with post-cardiac surgery vasoplegia, high-dose vitamin C infusion was feasible, appeared safe, and, within the limitations of a pilot study, did not achieve statistically faster resolution of vasoplegia.

Key Words: cardiopulmonary bypass; hypotension; post-cardiac surgery; postoperative care; vasoplegia; vitamin C

Vasoplegia is a recognized complication of cardiac surgery, and its incidence varies from 5% to 25%.[1] It is characterized by a preserved or increased cardiac index, vasodilatation, and hypotension sufficient to warrant vasopressor therapy.[2] Possible triggers for such a vasoplegic state include an inflammatory reaction to the bypass circuit, ischemia-reperfusion injury, and operative tissue trauma.[3] These processes appear to induce the release of cytokines and other inflammatory mediators that lead to the activation of vasodilatory pathways, diminished response to the effect of vasopressor hormones and drugs, and the consumption of endogenous antioxidants in response to increased reactive oxygen species generation.[1]

Vitamin C has potent immunomodulatory and antioxidant properties, acting as scavenger for both reactive oxygen and nitrogen species. High oxidative stress is implicated in the pathogenesis of vasoplegia after cardiac surgery and in sepsis-induced hypotension.[4] Thus, vitamin C is a potential treatment for patients with vasoplegia after cardiac surgery.[3,5] In this regard, a previous study of high-dose intravenous vitamin C (6,000 mg per day) combined with hydrocortisone and high-dose thiamine showed decreased vasopressor requirement and diminished mortality in septic patients with sepsis-associated severe vasoplegia.[6] In cardiac surgery patients, however, corticosteroids and thiamine already have been examined and found to have no effect on vasoplegia.[7,8] In contrast, to date, the hemodynamic effects of high-dose intravenous vitamin C in patients with post-cardiac surgery vasoplegia have not been studied.

Accordingly, the authors conducted a pilot feasibility, safety, and physiologic efficacy trial to investigate the effect of high-dose vitamin C in patients with vasoplegia after cardiac surgery, with the aim of establishing whether larger trials could be conducted successfully and justified by promising preliminary findings. The authors hypothesized that in patients admitted to intensive care unit (ICU) after cardiac surgery, high-dose vitamin C therapy would be associated with earlier resolution of postoperative vasoplegia.

Methods

Trial Design and Ethical Oversight

This study was a randomized, 2-center, double-blind feasibility, safety, and physiological efficacy pilot trial comparing high-dose vitamin C to placebo in the treatment of vasoplegia in patients admitted to the ICU after cardiac surgery.

The study was conducted in 2 tertiary ICUs, 1 in Australia and 1 in New Zealand. The study was approved by the Austin Hospital Human Ethics Committee in Australia (reference number DT 17/162) and Health and Disability Ethics Committees in New Zealand (reference number 17/NTA/212), and written informed consent was obtained from all participants participating in the trial. The trial was registered with the Australian New Zealand Clinical Trial Registry prior to patient enrollment (ACTRN12617000793314). The report of the present study adheres the Consolidated Standards of Reporting Trials (CONSORT) 2010 statement.[9]

Informed Consent

Research staff in New Zealand obtained consent before surgery and, if the patient met inclusion criteria in the ICU, they started the intervention. Therefore, when patients met exclusion criteria before surgery, for example by receiving steroid therapy before surgery, they were not enrolled. The research team in Australia enrolled patients after surgery. They obtained written consent after ICU admission when the patient's legally responsible decision maker was in the ICU or the patient was extubated and able to provide such consent. If the patient was intubated and their legally responsible person was not in the hospital, the patient was enrolled in the trial. Delayed written consent to use the data and to continue treatment was obtained later when the patient was extubated and able to provide such consent or when the legally responsible person was available (such approach to consent is permitted for time-sensitive intervention in the state of Victoria, Australia, for Ethics Committee-approved studies)

Patients

The authors included adult patients (≥18 years of age) who underwent on-pump cardiac surgery, were admitted to the ICU, and met the study enrollment criteria for postoperative vasoplegia. The authors included patients only when they met inclusion criteria and could be randomized within 6 hours after ICU admission. As no consensus definition exists, for the purpose of this study and in keeping with key characteristics used to describe this condition in previous literature, the authors defined post-cardiac surgery vasoplegia as hypotension with normal or increased cardiac index and a low systemic vascular resistance.[1] Specifically, for the purpose of inclusion in this trial, the authors operatively defined vasoplegia by the above criteria and the need for any dose of continuous vasopressor (norepinephrine, vasopressin, dopamine, phenylephrine, epinephrine, or metaraminol) infusion to a maintain mean arterial pressure (MAP) >65 mmHg, in the setting of a cardiac index ≥2.2 L min/m and/or of a central venous oxygen saturation >60%.

Exclusion criteria were pregnancy, the use of vasopressor or inotropic drugs in the preoperative period, off-pump cardiac surgery, corticosteroid use prior to or after surgery, a history of oxalate nephropathy, hemochromatosis, and glucose 6 phosphate dehydrogenase deficiency. The authors also excluded patients if the treating clinician believed there was an additional cause for

hypotension other than vasoplegia (bleeding, fluid requirement, pneumothorax, pacemaker issues, heart failure, or infection).

Randomization and Protocol

The authors randomly assigned eligible patients to receive either vitamin C or placebo, with a 1:1 ratio. Randomization was performed by means random number sequences using permuted blocks of variable size, and allocation was concealed using sequentially numbered sealed envelopes. Each envelope contained the patient's study number and the study arm allocation.

Patients received either 1,500 mg of vitamin C (vitamin C, Rotexmedica GmbH Arzneimittelwerk, Trittau, Germany in Australia or Ascor L 500, McGuff Pharmaceuticals, Inc, California, in New Zealand) in normal saline (100-mL bag) administered 6 hourly in the vitamin C group or normal saline (100-mL bag) administered 6 hourly in the placebo group. The study drug was administered over 1 hour.

The ICU research staff members, who were not involved in direct patient care, prepared all study drugs. All 100-mL study solutions were labeled as "vitamin C 1,500 mg or placebo." Thus, patients and clinical staff were kept blinded to treatment allocation. Infusion of the study drug was commenced rapidly after randomization. The study drug was given until the resolution of the vasoplegic state. This was defined as no vasopressor being administered to maintain a MAP >65 mmHg for a consecutive period of 4 hours, or if 96 hours had passed since randomization.

All other perioperative patient treatment, including hemodynamic management and sedation, was based on usual care and ICU protocols and dictated by the attending clinicians, who were blinded to treatment allocation. In the 2 study centers, anesthesia and perfusion techniques were similar (Supplementary Material S1). The surgery plan was dependent on the consultant surgeon.

Data Collection

All patient information was recorded in the electronic charting systems. Vasopressor dose was recorded as hourly dose in the ICU charting system. Vasopressin, dopamine, and epinephrine dose were converted to equivalent dose of norepinephrine to derive a cumulative dose of vasopressors, as previously described (Supplementary Table S3).[10]

Outcomes

The primary physiological efficacy outcome was time to resolution of postoperative vasoplegia. Secondary efficacy outcomes included total norepinephrine equivalent dose given in the first 2 days after randomization, ICU length of stay, ICU mortality, and hospital mortality. Feasibility outcomes included recruitment rate, eligibility rate, randomization to eligibility rate, protocol compliance, follow-up rate, and adverse event rate.

Sample Size

For this pilot study, which was focused on preliminary evidence of physiological effect, feasibility, safety, recruitment, and compliance, the authors aimed to enroll 50 patients over 2 sites. Such a study would provide a greater than 78% power to detect a difference in the duration of vasopressor therapy equal to that reported in a recent study of septic vasopelgia.[6] The authors considered that such sample size also would provide sufficient information to assess feasibility, a point estimate of a possible physiological effect, and sufficient pilot data to estimate an appropriate sample size for such studies.

Statistical Analysis

Analysis was performed on an intention-to-treat basis. A 2-sided p value less than 0.05 was considered statistically significant. Normally distributed continuous variables were compared using Student's *t* test or analysis of variance, and skewed variables were compared with either a Mann-Whitney U test or Kruskall-Wallis test, or log-transformed and compared by parametric tests. Categorical data were compared by Fisher's exact test. Time to shock resolution was compared using the log-rank test. Cox proportional hazard analysis then was performed, to adjust for possible confounders. Baseline variables with a univariate $p < 0.15$ on univariable analysis and the European System for Cardiac Operative Risk Evaluation (EuroSCORE) were included in the Cox regression analysis. All analyses were performed using *R* version 3.4.2 (R Foundation for Statistical Computing, Vienna, Austria).

(https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?id=373018&isReview=true

Results

Patients

The authors screened 456 patients from November 8, 2017 to October 29, 2018, and enrolled 50 patients (25 patients in each group) in the study (Fig 1).

Study patient characteristics are presented in Table 1. The median age was 67.0 years in the vitamin C group and 64.0 years in the placebo group. The median EuroSCORE was 5.0 in both groups, and 96.0% of the patients received norepinephrine (Supplementary Table S1) as the primary vasopressor drug. The median baseline norepinephrine equivalent dose was the same in both groups at 0.06 μg/kg/min of norepinephrine, equivalent to approximately 5 μg/min in an 80-kg patient.

Feasibility Outcomes and Protocol Compliance

Of 456 patients, 52 (11.4%) were eligible and 50 (11.0%) were included. Overall, 23 (46.0%) patients were included in Australia and 27 (54.0%) in New Zealand. All included patients completed the study protocol, and no patient was lost to follow-up or discontinued study treatment. Of the 50 patients, 48 (96.0%) patients stopped the study drug after postoperative vasoplegia resolution. One patient in the vitamin C group stopped the study drug because of death, and 1 patient in the placebo group stopped the study drug after 96 hours from randomization.

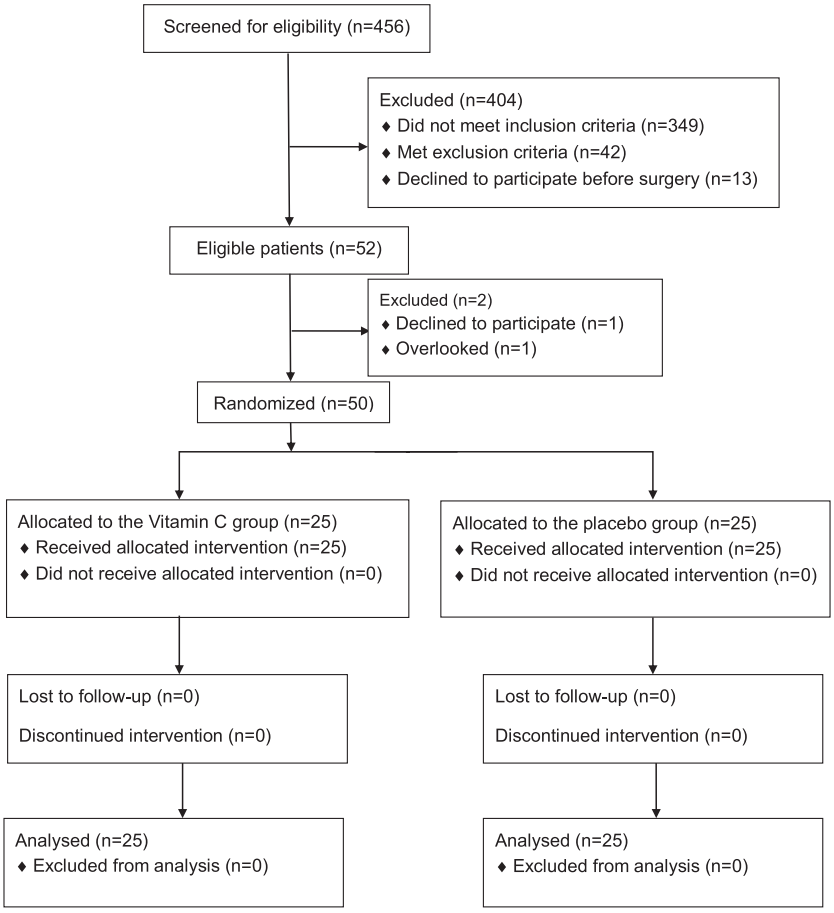

Fig 1. Trial screening and enrollment flowchart.

There were 2 cases (4.0%) of protocol deviation where extra doses of the study drug were given incorrectly after vasoplegia resolution (1 patient in each group).

There was no adverse event related to blood glucose measurements or other possible vitamin C-related events (hemolysis, kidney stones). One patient (4.0%) died of cardiogenic shock in the vitamin C group.

Primary Outcome

The mean (standard deviation) to vasoplegic shock resolution was 27.0 (16.5) hours in the vitamin C group and was 34.7 (41.1) hours in the placebo group (mean difference: 7.7 hours, 95% confidence interval [CI], −10.5 to 25.9 hours) (Table 2). There was no difference between the 2 groups for time to postoperative vasoplegia resolution ($p = 0.51$) (Fig 2). On Cox proportional hazard regression analysis, adjusted for age, sex, left ventricular ejection fraction, New York Heart Association class, and EuroSCORE (Table 3), high-dose vitamin C was not associated with earlier shock resolution (hazard ratio = 1.19; 95% CI, 0.55-2.55; $p = 0.66$).

Secondary Outcomes

There was no statistical difference in the median (interquartile range [IQR]) total norepinephrine equivalent dose administered on the first 2 calendar days after randomization (64.9 [IQR, 23.5-236.5] μg/kg for vitamin C and 47.4 [IQR, 21.4-265.9] μg/kg for placebo, respectively; $p = 0.75$). The median ICU length of stay was similar in the 2 groups (1.4 [IQR, 0.45-2.5] days in the vitamin C group and 1.5 [IQR, 0.5-3.3] days in the placebo group; $p = 0.36$). Other clinical outcomes were also similar in the 2 groups (Table 2). Moreover, MAP, heart rate, cumulative norepinephrine equivalent dose, and fluid administration in the first 3 days showed no difference between the 2 groups (Supplementary Fig S1).

Table 1
Baseline Characteristics of Study Patients

	Vitamin C	Placebo	p Value
Age, y	67.0 (82.0-74.0)	64.0 (59.0-69.0)	0.11
Sex, male	15/25 (60.0%)	23/25 (92.0%)	0.02
BMI,* kg/m^2	29.8 (26.8-32.9)	27.2 (24.4-30.7)	0.38
Admission category, urgent	7/25 (28.0%)	10/25 (40.0%)	0.55
EuroSCORE	5.0 (4.0-8.0)	5.0 (3.0-7.0)	0.51
LVEF†			0.12
<30%	1/22 (4.5%)	1/23 (4.3%)	
30%-49%	5/22 (22.7%)	12/23 (52.2%)	
≥50%	16/22 (72.7%)	10/23 (43.5%)	
NYHA class (%)			0.04
Class I	10/25 (40.0)	13/25 (52.0)	
Class II	6/25 (24.0)	11/25 (44.0)	
Class III	8/25 (32.0)	1/25 (4.0)	
Class IV	1/25 (4.0)	0/25 (0.0)	
Preoperative comorbidities (%)			
Hypertension	15/25 (60.0)	16/25 (64.0)	>0.99
Diabetes	8/25 (32.0)	12/25 (48.0)	0.39
Hyperlipidemia	8/25 (32.0)	9/25 (36.0)	>0.99
Previous myocardial infarction	6/25 (24.0)	9/25 (36.0)	0.54
Cerebrovascular disease	1/25 (4.0)	2/25 (8.0)	>0.99
Peripheral vascular disease	1/25 (4.0)	1/25 (4.0)	>0.99
Previous cardiac surgery	3/25 (12.0)	1/25 (4.0)	0.60
Chronic lung disease	3/25 (12.0)	3/25 (12.0)	>0.99
Atrial fibrillation	6/25 (24.0)	3/25 (12.0)	0.46
Ischemic heart disease	8/25 (32.0)	10/25 (40.0)	0.77
Severe pulmonary hypertension	3/25 (12.0)	1/25 (4.0)	0.60
Preoperative medication (%)			
ACEi/ARB	13/25 (52.0)	17/25 (68.0)	0.39
Beta-blocker	12/25 (48.0)	16/25 (64.0)	0.39
Calcium-channel blocker	5/25 (20.0)	7/25 (28.0)	0.74
Nitrate	4/25 (16.0)	3/25 (12.0)	>0.99
Statin	17/25 (68.0)	14/25 (56.0)	0.56
Serum creatinine, μmol/L	85.0 (75.0-98.0)	92.0 (80.0-100.0)	0.35
Type of surgery (%)			0.62
CABG	13/25 (52.0)	15/25 (60.0)	
Valvular surgery or valve and CABG	11/25 (44.0)	8/25 (32.0)	
Other	1/25 (4.0)	2/25 (8.0)	
Cross-clamp time, min	76.0 (69.0-107.0)	94.0 (78.3-109.3)	0.31
Cardiopulmonary bypass time, min	119.0 (97.0-172.0)	119.5 (104.8-159.3)	0.84
Intraoperative RBC transfusion	3/25 (12.0%)	1/25 (4.0%)	0.61
Baseline vasopressor			
Norepinephrine (μg/kg/min)	0.06 (0.05-0.11)	0.06 (0.04-0.14)	0.87
Norepinephrine equivalent dose‡ (μg/kg/min)	0.06 (0.05-0.11)	0.06 (0.04-0.16)	0.81

NOTE. Categorical data are presented as number/total number (%); continuous data are presented as median (25th-75th percentiles). Age, sex, NYHA, and LVEF had a $p < 0.15$ for the difference between the 2 study groups, and hence were added to the multivariate Cox regression model of time to shock resolution, along with study group allocation.
ACEi/ARB, angiotensin converting enzyme inhibitor/angiotensin receptor blocker; BMI, body mass index; CABG, coronary artery bypass graft; EuroSCORE II, European System for Cardiac Operative Risk Evaluation; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association; RBC, red blood cell.
* BMI is the weight in kilograms divided by the square of the height in meters.
† LVEF value before surgery.
‡ Vasopressin, dopamine, and epinephrine were converted to equivalent dose of norepinephrine.[10]

Table 2
Primary Secondary and Other Outcomes

	Vitamin C	Placebo	p
Primary outcome			
Time to vasoplegia resolution,* h	27.0 (16.5)	34.7 (41.1)	0.40
Secondary outcomes			
Cumulative norepinephrine equivalent dose in the first 2 d,* μg/kg	64.9 (23.5-236.5)	47.4 (21.4-265.9)	0.75
ICU length of stay, d	1.4 (0.5-2.5)	1.5 (0.5-3.3)	0.36
Hospital length of stay, d	13.2 (7.9-20.2)	12.5 (8.1-16.7)	0.92
ICU mortality	1/25 (4.0%)	0/25 (0.0%)	>0.99
Hospital mortality	1/25 (4.0%)	0/25 (0.0%)	>0.99
Other outcomes			
Hospital length of stay, d	13.1 (7.9-20.2)	12.5 (8.1-16.7)	0.91
AKI			0.76
No AKI†	11/25 (44.0%)	13/25 (52.0%)	
Stage 1	3/25 (12.0%)	1/25 (4.0%)	
Stage 2	10/25 (40.0%)	9/25 (36.0%)	
Stage 3	1/25 (4.0%)	2/25 (8.0%)	
Atrial fibrillation‡	11/25 (44.0%)	8/25 (32.0%)	0.56
Time to extubation,§ h	16.1 (7.2-20.0)	10.1 (5.4-18.9)	0.48

NOTE. Categorical data are presented as number/total number (%); continuous data are presented as median (25th-75th percentiles) except for time to shock resolution (mean [standard deviation]), because it is used for sample size calculation.
AKI, acute kidney injury.
* Vasopressin dose was converted to equivalent dose of norepinephrine.[7]
† Acute kidney injury defined as modified Kidney Disease Improving Global Outcomes guideline.
‡ Incidence of newly diagnosed atrial fibrillation in intensive care unit.
§ Time from intensive care unit admission to extubation.

Discussion

Key Findings

In this double-blind, randomized, placebo-controlled pilot feasibility and physiologic efficacy study of high-dose vitamin C for patients with vasoplegia after cardiac surgery, the authors found that resolution of the vasoplegic state occurred relatively rapidly with standard care. In this setting, the administration of high-dose vitamin C did not lead to a faster resolution of postoperative vasoplegia or a decrease in the total norepinephrine equivalent dose administered in the first 2 calendar days after randomization. Finally, the observed 95% CIs were consistent with vitamin C therapy having an effect on duration of shock that ranged from a 25.9-hour reduction to a 10.5-hour increase, an effect size that the authors submit is unlikely to be clinically important in this population.

Relationship to Previous Studies

High-dose vitamin C has a strong biological rationale in post-cardiac surgery vasoplegia. Vitamin C scavenges reactive oxygen species, which likely contribute to vasoplegia after cardiac surgery; stimulates norepinephrine synthesis; and decreases the incidence of atrial fibrillation, cardiac enzyme release, and the level of markers of oxidative stress.[11,12] In addition, cardiac surgery patients have decreased vitamin C levels compared to preoperative values, suggesting increased consumption and/or dilution.[13]

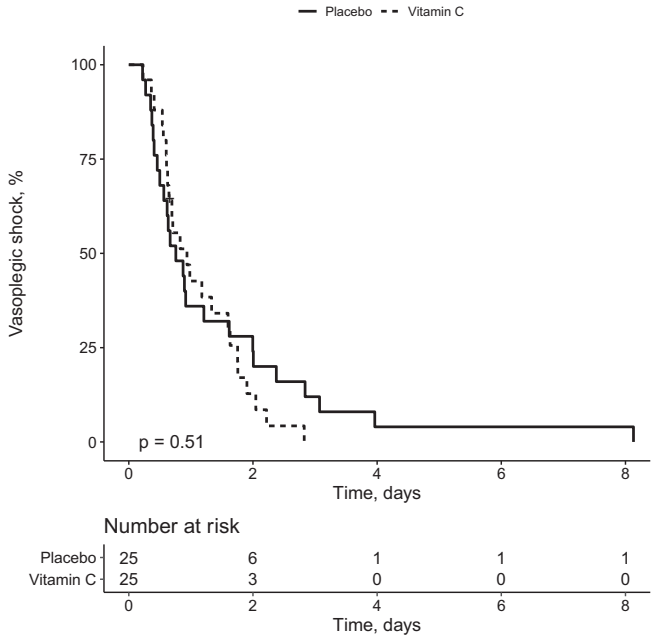

Fig 2. Time to vasoplegia resolution according to treatment.

Previous studies of vitamin C in cardiac surgery patients have not focused on postoperative vasoplegia and on the duration of vasopressor therapy and have not administered high-dose vitamin C.[14-19] Instead, they investigated the use of vitamin C in unselected patients undergoing cardiopulmonary bypass and focused on creatinine kinase and the development of atrial fibrillation. In addition, vitamin C was given enterally in 3 of the 6 studies reported so far.[14-19] This is problematic, because vitamin C absorption after enteral high-dose administration is limited by intestinal transporter (sodium-vitamin C transporter-1) function, and peak plasma vitamin C levels after enteral intake in healthy volunteers are one-sixth of those measured after the same dose given by intravenous infusion.[20] Thus, enteral therapy is unlikely to deliver sufficient amounts of vitamin C to these patients.[6,21,22]

Table 3
Cox Proportional Hazard Regression Analysis for Time to Resolution of Post-Cardiac Surgery Vasodilatory Shock With 95% CI

Variables	Hazard Ratio (95% CI)	p
Study group		0.66
Placebo	1	
Vitamin C	1.19 (0.55-2.55)	
NYHA		0.97
Class I or II	1	
Class III or IV	0.98 (0.32-2.97)	
Age, 1 y	1.02 (0.97-1.06)	0.41
Sex		0.62
Female	1	
Male	1.24 (0.53-2.90)	
EuroSCORE	0.95 (0.79-1.14)	0.58
LVEF		0.74
≥50%	1	
<50%	0.88 (0.42-1.86)	

NOTE. LVEF was missing for 5 patients (10.0%).
EuroSCORE, European System for Cardiac Operative Risk Evaluation; LVEF, left ventricular ejection fraction value before surgery; NYHA, New York Heart Association.

In addition, with the exception of 1 study, vitamin C has been given at a much lower dose, and, in the only high-dose study prior to the authors' investigation, it was given as a single dose prior to cardiopulmonary bypass.[14-19]

Of relevance to this study, high-dose intravenous vitamin C has recently been used to treat vasodilatory septic shock, another type of vasoplegic state.[6,22,23] Such studies used high-dose (6000 mg per day in 4 divided doses) vitamin C in association with thiamine and hydrocortisone and found a decrease in vasopressor therapy duration. However, because corticosteroid or thiamine therapy for cardiac surgery patients has been shown to have no effect on hemodynamic or clinical outcomes, the authors decided to use high-dose intravenous vitamin C monotherapy in this study.[7,8]

Implications of Study Findings

This study implies that postoperative vasoplegia, as defined in the study, resolves within 1 to 2 days with usual care. It also implies that, in such unselected patients, high-dose intravenous

vitamin C is unlikely to have a clinically important effect on the duration of the vasoplegic state. Moreover, given the point estimate of its effect, hundreds of similar patients would be needed to provide evidence of statistical significance.

Strengths and Limitations

To the authors' knowledge, this is the first double-blind, randomized, controlled study of high-dose intravenous vitamin C study for post-cardiac surgery vasoplegia. The authors enrolled essentially all eligible patients, and the study protocol was delivered successfully in essentially all patients, with complete follow-up and no adverse events related to the study drug. It provided information on the duration of postoperative vasoplegia in an unselected population and evidence that, in such patients, high-dose vitamin C is unlikely to have a clinically important effect. Moreover, these results provide clear evidence of feasibility and an estimate of sample size for a future trial. As such, they suggest that hundreds of patients would be needed to demonstrate an effect of vitamin C equal to the point estimate found in the authors' pilot study on the duration of vasopressor therapy in this setting. The authors acknowledge several limitations. One-third of study patients received milrinone as an inotropic drug, and such treatment may have caused vasodilation and contributed to hypotension. However, the proportion of patients with milrinone was the same in both groups. The authors did not use the cardiac index to defined the resolution of vasoplegia. Instead, the authors chose a practical approach to vasoplegia resolution, because the authors expected that several patients would have their pulmonary artery catheter removed while on low-dose vasopressor therapy. In addition, when measured, the systemic vascular resistance index was the same in the 2 groups. One patient treated with vitamin C died from cardiogenic shock, and this was not considered a drug-related side effect by the treating clinical team. The average EuroSCORE in the study patients was 5. Thus, the expected mortality rate was 5%. Finally, the authors did not investigate very high doses (≥1 g/kg per day) of vitamin C given intravenously as has been done in other conditions. Whether such very high doses may prove more useful remains untested.[24,25]

Conclusion

In conclusion, in a pilot double-blind randomized trial in cardiac surgery patients with postoperative vasoplegia, the authors found that resolution of the vasoplegic state occurred on average within 1.5 days. The authors also found that high-dose intravenous vitamin C did not decrease the duration of vasopressor therapy significantly and appeared unlikely to have a clinically important impact on this outcome. Investigation of patients with much a higher intensity of postoperative vasoplegia may be warranted.

Declaration of Competing Interest

The authors declare no conflicts of interest.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1053/j.jvca.2019.08.034.

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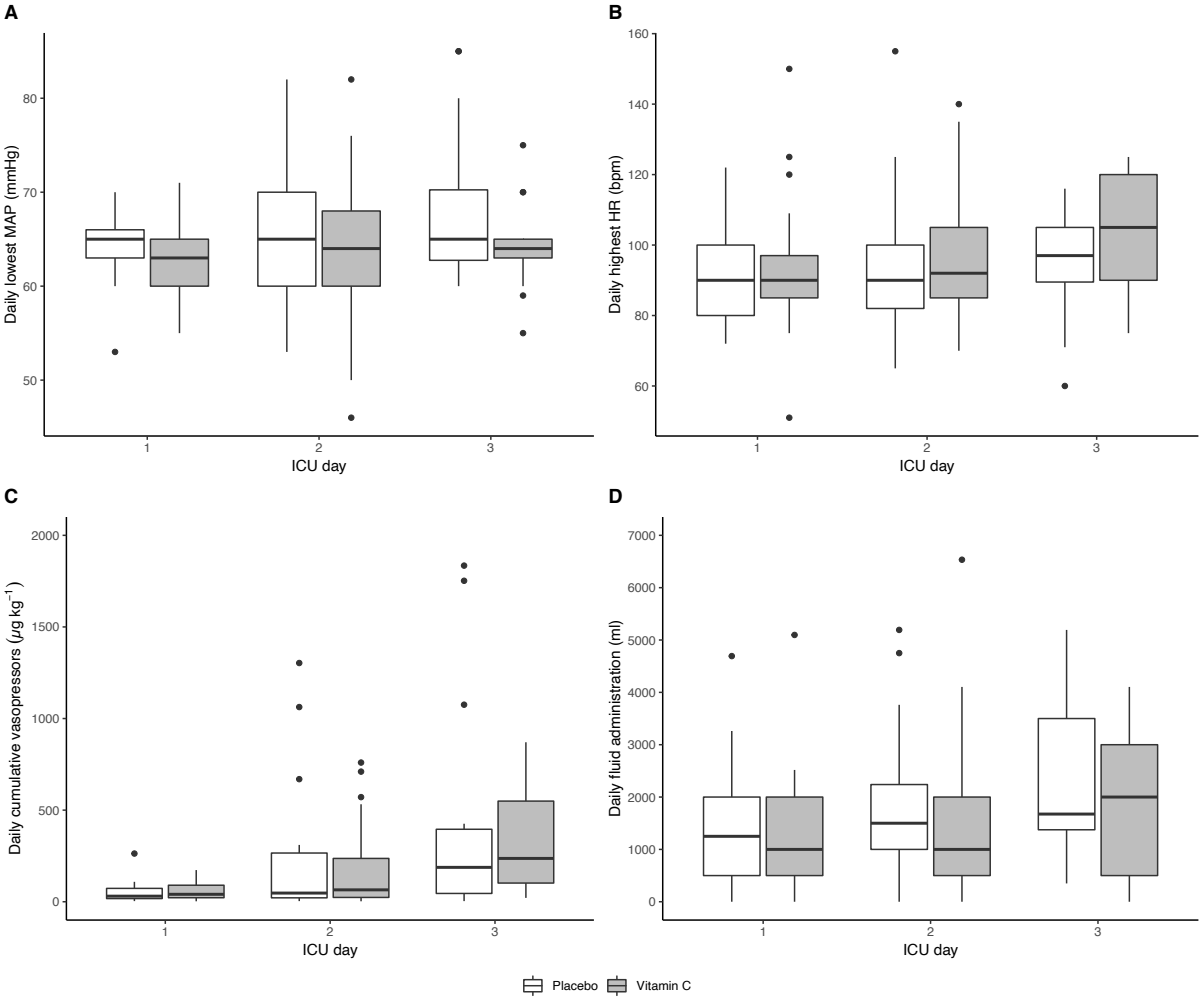
SUPPLEMENTARY APPENDIX

Supplementary Figure 1. Box plot of daily lowest mean arterial pressure (MAP), highest heart rate (HR), cumulative norepinephrine equivalent dose and cumulative fluid administration for the first three days of ICU stay.



Supplementary Table1. Baseline use of vasopressors and inotropes at the time of randomization.

	Vitamin C	Placebo
Measure	25	25
Norepinephrine	24/25 (96.0%)	24/25 (96.0%)
Vasopressin	1/25 (4.0%)	2/25 (8.0%)
Dopamine	0/25 (0.0%)	2/25 (8.0%)
Epinephrine	4/25 (16.0%)	2/25 (8.0%)
Milrinone	8/25 (32.0%)	6/25 (24.0%)
Phenylephrine	0/25 (0.0%)	0/25 (0.0%)
Metaraminol	0/25 (0.0%)	0/25 (0.0%)
Dobutamine	0/25 (0.0%)	0/25 (0.0%)
Levosimendan	0/25 (0.0%)	0/25 (0.0%)

Supplementary Table 2. Baseline characteristics of the baseline high cumulative vasopressor group (over median dose).

	Vitamin C	Placebo
Age, years	70.5 (61.5, 77.0)	65.0 (57.0, 67.0)
Sex, male	7/12 (58.3%)	10/12 (83.3%)
BMI*, kg m^{-2}	29.9 (28.8, 32.8)	24.9 (23.8, 27.3)
Admission category, urgent	3/12 (25.0%)	7/12 (58.3%)
EuroSCORE†	5.5 (4.0, 8.0)	5.0 (3.0, 7.0)
LVEF‡		
<30%	0/12 (0.0%)	1/11 (9.1%)
30-49%	2/12 (18.2%)	4/11 (36.4%)
≥50%	9/12 (81.8%)	6/11(54.5%)
NYHA class§		
Class I	5/12 (41.7%)	6/12 (50.0%)
Class II	3/12 (25.0%)	5/12 (41.7%)
Class III	4/12 (33.3%)	1/12 (8.3%)
Class IV	0/12 (0.0%)	0/12 (0.0%)
Preoperative comorbidities		
Hypertension	7/12 (58.3%)	6/12 (50.0%)
Diabetes	4/12 (33.3%)	3/12 (25.0%)
Hyperlipidemia	3/12 (25.0%)	5/12 (41.7%)
Previous myocardial infarction	4/12 (33.3%)	5/12 (41.7%)
Cerebrovascular disease	1/12 (8.3%)	1/12 (8.3%)
Peripheral vascular disease	0/12 (0.0%)	1/12 (8.3%)
Previous cardiac surgery	2/12 (16.7%)	0/12 (0.0%)
Chronic lung disease	2/12 (16.7%)	2/12 (16.7%)
Atrial fibrillation	4/12 (33.3%)	2/12 (16.7%)
Ischemic heart disease	4/12 (33.3%)	4/12 (33.3%)
Severe pulmonary hypertension	2/12 (16.7%)	0/12 (0.0%)
Preoperative medication		
ACEi/ARB¶	6/12 (50.0%)	7/12 (58.3%)
Beta blocker	4/12 (33.3%)	5/12 (41.7%)
Calcium-channel blocker	3/12 (25.0%)	2/12 (16.7%)
Nitrate	3/12 (25.0%)	0/12 (0.0%)
Statin	9/12 (75.0%)	7/12 (58.3%)
Serum creatinine, μmol L^{-1}	79.0 (72.5, 86.8)	84.5 (79.8, 108.0)
Type of surgery		
Coronary artery bypass graft (CABG)	7/12 (58.3%)	8/12 (66.7%)

	Vitamin C	Placebo
Valvular surgery or valve and CABG	5/12 (41.7%)	3/12 (25.0%)
Other	0/12 (0.0%)	1/12 (8.3%)
Number of distal grafts	3.0 (3.0, 3.0)	3.0 (3.0, 3.0)
Use of internal thoracic-artery grafts	0.0 (0.0, 0.5)	0.0 (0.0, 0.3)
Cross-clamp time, minutes	88.5 (66.8, 124.0)	89.0 (79.0, 95.8)
Cardiopulmonary bypass time, minutes	148.5 (128.0, 174.5)	116.5 (104.8, 133.0)
Baseline drug infusion as categorical data		
Norepinephrine	12/12 (100.0%)	12/12 (100.0%)
Vasopressin	1/12 (8.3%)	2/12 (16.7%)
Dopamine	0/12 (0.0%)	2/12 (16.7%)
Epinephrine	2/12 (16.7%)	1/12 (8.3%)
Milrinone	4/12 (33.3%)	4/12 (33.3%)
Baseline vasopressor		
Norepinephrine (μg kg^{-1} min^{-1})	0.16 (0.09, 0.23)	0.15 (0.09, 0.21)
Norepinephrine equivalent dose** (μg kg^{-1} min^{-1})	0.16 (0.09, 0.24)	0.16 (0.09, 0.24)

Categorical data are presented as number/total number (%); continuous data are presented as median (25th, 75th percentiles).
* Body-mass index is the weight in kilograms divided by the square of the height in meters. † European System for Cardiac Operative Risk Evaluation (EuroSCORE). ‡ Left ventricular ejection fraction (LVEF) value before surgery. One patient in placebo group had a missing value. § New York Heart Association class. ¶ Angiotensin converting enzyme inhibitor / Angiotensin receptor blocker. ** Vasopressin, dopamine and epinephrine were converted to equivalent dose of norepinephrine.[10]

Supplementary Table 3. Conversion to norepinephrine equivalent dose.

Drug	Dose	Norepinephrine equivalent dose
Epinephrine	0.1 μg/kg/min	0.1 μg/kg/min
Norepinephrine	0.1 μg/kg/min	0.1 μg/kg/min
Dopamine	15 μg/kg/min	0.1 μg/kg/min
Phenylephrine	1.0 μg/kg/min	0.1 μg/kg/min
Vasopressin	0.04 U/min	0.1 μg/kg/min

Supplementary Table 4. Comparison of baseline characteristics between the two hospitals.

	Austin (n=23)	Wellington (n=27)	P value
Age, years	66.0 [60.0, 71.5]	66.0 [59.0, 71.5]	0.87
Sex, male	18 (78.3)	20 (74.1)	>0.99
BMI*, kg m^{-2}	29.8 [23.4, 35.0]	28.4 [25.5, 31.6]	0.85
Admission category, urgent	5 (21.7)	12 (44.4)	0.14
EuroSCORE†	6.0 [4.0, 7.0]	5.0 [3.0, 7.0]	0.25
LVEF‡			0.59
<30%	0 (0.0)	2 (7.4)	
30-49%	8 (44.4)	9 (33.3)	
≥50%	10 (55.6)	16 (59.3)	
NYHA class§			0.008
Class I	5 (21.7)	18 (66.7)	
Class II	11 (47.8)	6 (22.2)	
Class III	6 (26.1)	3 (11.1)	
Class IV	1 (4.3)	0 (0.0)	
Preoperative comorbidities			
Hypertension	13 (56.5)	18 (66.7)	0.56
Diabetes	11 (47.8)	9 (33.3)	0.39
Hyperlipidemia	11 (47.8)	6 (22.2)	0.08
Previous myocardial infarction	2 (8.7)	13 (48.1)	0.004
Cerebrovascular disease	1 (4.3)	2 (7.4)	>0.99
Peripheral vascular disease	1 (4.3)	1 (3.7)	>0.99
Previous cardiac surgery	2 (8.7)	2 (7.4)	>0.99
Chronic lung disease	3 (13.0)	3 (11.1)	>0.99
Atrial fibrillation	6 (26.1)	3 (11.1)	0.27
Ischemic heart disease	10 (43.5)	8 (29.6)	0.38
Severe pulmonary hypertension	1 (4.3)	3 (11.1)	0.61
Preoperative medication			
ACEi/ARB¶	12 (52.2)	18 (66.7)	0.39
Beta blocker	12 (52.2)	16 (59.3)	0.78
Calcium-channel blocker	6 (26.1)	6 (22.2)	>0.99
Nitrate	4 (17.4)	3 (11.1)	0.69
Statin	14 (60.9)	17 (63.0)	>0.99
Serum creatinine, μmol L^{-1}	93.0 [78.0, 103.0]	86.0 [79.0, 97.5]	0.54
Type of surgery			0.42

	Austin (n=23)	Wellington (n=27)	P value
Coronary artery bypass graft (CABG)	11 (47.8)	17 (63.0)	
Valvular surgery or valve and CABG	11 (47.8)	8 (29.6)	
Other	1 (4.3)	2 (7.4)	

Categorical data are presented as number (%); continuous data are presented as median (25th, 75th percentiles).
* Body-mass index is the weight in kilograms divided by the square of the height in meters. † European System for Cardiac Operative Risk Evaluation (EuroSCORE). ‡ Left ventricular ejection fraction (LVEF) value before surgery. One patient in placebo group had a missing value. § New York Heart Association class. ¶ Angiotensin converting enzyme inhibitor / Angiotensin receptor blocker.

Supplementary Material 1. Detailed anesthesia and perfusion technique.

General anesthesia was managed by a group of cardiac anesthesiologists using a protocol designed to standardize care. All participants were fasted for 6-hours for solids and 2-hours for clear fluids. Prior to anesthesia, participants did not receive any intravenous fluid loading. Induction of anesthesia consisted of a standard technique using propofol (0.5-1 mg kg^{-1}), fentanyl (5-10 ug kg^{-1}) and a non-depolarizing neuromuscular blocker. Maintenance of anesthesia was achieved using sevoflurane or propofol in 50% oxygen: 50% air ratio titrated to a patient state index (PSI) of 25 to 50 or bispectral Index (BIS) of 40 to 60. Prior to initiation of CPB fluid intervention was generally restricted but where clinically necessary, crystalloid solutions were permitted. There was no use of pre-bypass colloid solutions.
CPB was performed using a membrane oxygenator and the pump was primed with 1,500 to 2,000 ml balanced crystalloid. The pump rate was set at 2.4 l/m^2/min and body temperature was kept around 34°C. If volume supplementation was required during CPB, fluid intervention was completely at the discretion of the attending clinical perfusionist and anesthesiologist. Post CPB use of fluids and vasoactive medications was at the discretion of the attending anesthesiologist. After heparin reversal with a standardised dose of protamine, and correction of activated clotting (ACT) time to below 150 seconds, maintenance of anesthesia was also achieved using sevoflurane or propofol in 50% oxygen: 50% air ratio.

Chapter 7: The safety of high-dose vitamin C

Harm of IV High-Dose Vitamin C Therapy in Adult Patients: A Scoping Review

Fumitaka Yanase, MD[1,2]; Tomoko Fujii, MD, PhD[2,3]; Thummaporn Naorungroj, MD[1,4]; Alessandro Belletti, MD[1,5]; Nora Luethi, MD[2,6]; Anitra C. Carr, BSc (Hons), PhD[7]; Paul J. Young, MB, ChB, PhD[8,9]; Rinaldo Bellomo, MD, PhD[1,2,10]

Objectives: The potential harm associated with the use of IV vitamin C has not been systematically assessed. We aimed to review the available evidence on harm related to such treatment.

Data Sources: We searched MEDLINE, EMBASE, Cochrane Library, National Institute of Health Clinical Trials Register, and World Health Organization International Clinical Trials Registry Platform.

Study Selection: We included studies in adult population that reported harm related to IV high-dose vitamin C which we defined as greater than or equal to 6 g/d, greater than or equal to 75 mg/kg/d, or greater than or equal to 3 g/m^2/d.

Data Extraction: Two independent investigators screened records and extracted data.

Data Synthesis: We identified 8,149 reports, of which 650 full text were assessed for eligibility, leaving 74 eligible studies. In these studies, 2,801 participants received high-dose vitamin C at a median (interquartile range) dose of 22.5 g/d (8.25–63.75 g/d), 455 mg/kg/d (260–925 mg/kg/d), or 70 g/m^2/d (50–90 g/m^2/d); and 932 or more adverse events were reported. Among nine double-blind randomized controlled trials (2,310 patients), adverse events were reported in three studies with an event rate per patient for high-dose vitamin C identical to placebo group in one study (0.1 [1/10] vs 0.1 [1/10]), numerically lower in one study (0.80 [672/839] vs 0.82 [709/869]), and numerically higher in one study (0.33 [24/73] vs 0.23 [17/74]). Six double-blind randomized controlled trials reported no adverse event in either group. Five cases of oxalate nephropathy, five cases of hypernatremia, three cases of hemolysis in glucose-6-phosphate dehydrogenase deficiency patients, two cases of glucometer error, and one case of kidney stones were also reported overall.

Conclusions: There is no consistent evidence that IV high-dose vitamin C therapy is more harmful than placebo in double-blind randomized controlled trials. However, reports of oxalate nephropathy, hypernatremia, glucometer error, and hemolysis in glucose-6-phosphate dehydrogenase deficiency patients warrant specific monitoring. (*Crit Care Med* 2020; 48:e620–e628)

Key Words: adverse event; ascorbic acid; glucose-6-phosphate dehydrogenase deficiency; harm; oxaluria; vitamin C

Vitamin C is an essential water-soluble vitamin responsible for vital biochemical functions in the human body. As humans cannot synthesize vitamin C, exogenous supplementation is required to prevent vitamin C deficiency.

The biological properties of vitamin C have been considered promising as an adjunct or individual therapy for several syndromes including sepsis, to the common cold, and cancer (1–3). Recently, high-dose IV vitamin C (≥ 50 mg/kg/d) has been investigated as a part of "metabolic resuscitation" for sepsis with the expectation that its anti-inflammatory and antioxidant properties and its role as co-factor in several biological reactions might improve hemodynamic stability and reduce mortality (1, 4–6). Furthermore, in vitro experiments using cancer cell lines and animal experiments suggested that vitamin C works pro-oxidatively and is cytotoxic to cancer cells at high doses, although this has not yet been demonstrated in humans (7–9).

Several systematic reviews have focused on the possible efficacy of vitamin C for sepsis or cancer (10–12). However, there has been no comprehensive review of the possible harm

[1]Department of Intensive Care, Austin Hospital, Heidelberg, VIC, Australia.
[2]Australian and New Zealand Intensive Care Research Centre, Monash University, Melbourne, VIC, Australia.
[3]Department of Health Promotion and Human Behavior, Kyoto University Graduate School of Medicine, Kyoto, Japan.
[4]Department of Intensive Care, Siriraj Hospital, Mahidol University, Bangkok, Thailand.
[5]Department of Anesthesia and Intensive Care, IRCCS San Raffaele Scientific Institute, Milan, Italy.
[6]Institute of Social and Preventive Medicine, University of Bern, Bern, Switzerland.
[7]Nutrition in Medicine Research Group, Department of Pathology and Biomedical Science, University of Otago, Christchurch, New Zealand.
[8]Intensive Care Unit, Wellington Hospital, Wellington, New Zealand.
[9]Medical Research Institute of New Zealand, Wellington, New Zealand.
[10]Centre for Integrated Critical Care, Department of Medicine & Radiology, University of Melbourne, Melbourne, VIC, Australia.

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or adverse effects of high-dose IV vitamin C therapy, despite occasional reports of adverse events. Oxalate nephropathy and hemolytic events among glucose-6-phosphate dehydrogenase (G6PD) deficiency patients are well known adverse events (13–15). Also, vitamin C is known to affect point of care glucose monitoring (16). Such knowledge is increasingly important as critical care researchers explore its potential beneficial effects in critically ill patients.

Accordingly, we aimed to systematically review all currently available reports of IV high-dose vitamin C therapy to inform clinicians and researchers on the possible harm associated with such therapy.

MATERIALS AND METHODS

Study Design

We conducted a systematically structured scoping review (SR), using the guidelines from the Cochrane Collaboration and Centre for Reviews and Dissemination, and reported the results according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline and its extension for SRs (17, 18).

Search Strategy

The search strategy was developed in consultation with a research librarian at the Alfred Hospital, a tertiary hospital affiliated to Monash University, Melbourne, Australia. We searched all relevant studies from the following databases in June 2019: MEDLINE (Ovid), EMBASE (Ovid), and Cochrane Library using a list of keywords, medical subject heading terms, truncations, and Boolean operators. For ongoing trials, we searched National Institute of Health Clinical Trials Register (https://clinicaltrials.gov/) and World Health Organization International Clinical Trials Registry Platform (http://www.who.int/ictrp/en/). The detailed search syntax is shown in **Supplementary material 1** (Supplemental Digital Content 1, http://links.lww.com/CCM/F510). Publication status of the trials retrieved from the clinical trial registries was followed-up on February 11, 2020.

Definition of Harms in This SR

Harm in this SR was defined as any observation that was adjudicated and reported to be unfavorable by the original authors with keywords related to harm, that is, adverse events, side effects, complications, safety, or tolerability. If reported adverse events were graded by any version of the Common Terminology Criteria for Adverse Events, we extracted grade 3 or higher severe adverse events (19–22). This threshold has been used in previous clinical research to define clinically important adverse events (23). When adverse events were graded, but the grading method was not clearly defined or referenced, we extracted grade 3 or more severe adverse events. When not graded, we extracted all adverse events as reported.

Inclusion and Exclusion Criteria

We included all randomized controlled trials (RCTs), non-RCTs, observational studies, case series, or case reports that mentioned any harm related to the administration of IV high-dose vitamin C in adult patients.

We included studies reported as full text, those published as abstract only, and unpublished data. We included studies for adult patients who were 18 years old or older. Studies that included a minority of patients (< 10%) under 18, or studies with median or mean of age of patients over 20 were also included. As there is no consensus on the definition of high-dose vitamin C, in this SR, we operationally defined the high dose as 6 g/d or more on the first day of treatment regardless of the frequency or duration. The cutoff dose was chosen with a view to inform a number of clinical trials investigating effects of vitamin C with the dose in thousands of patients with sepsis of possible safety concerns (1, 24–26). Where doses were defined in other units, we used 75 mg/kg/d or 3 g/m^2/d as thresholds for high dose. Mega-dose IV vitamin C was arbitrary defined using thresholds of greater than or equal to 20 g/d, 250 mg/kg/d, or 10 g/m^2/d.

Studies were excluded when the dose or the route was not reported, or the original authors did not report whether harm occurred or not.

Data Collection

Screening titles and abstracts of all studies retrieved from the database search was conducted independently and in duplicate by the review authors (F.Y., T.F., T.N., A.B.). We classified them as "retrieve" (eligible or potentially eligible/unclear) or "do not retrieve." We retrieved full texts, determined study eligibility, and recorded reasons for exclusion of the ineligible studies. We resolved any disagreement through discussion, or, if required, we consulted a fifth reviewer (R.B.).

We extracted the name of the author, year of publication, study design, characteristics of the patient population, total number of patients included in the study, number of patients in each treatment group, total dose of vitamin C on the first day of the treatment, rate of vitamin C infusion, other interventions in each group, diagnosis of harm, the number of each harmful event, and funding source of the study. When a series of patients who received various doses of vitamin C were reported in a study, we classified them into five groups; placebo group, no intervention group (did not receive either vitamin C or placebo), low-dose group (< 6 g/d), high-dose group (≥ 6, < 20 g/d), and mega-dose group (≥ 20 g/d). For adverse event, we extracted adverse events only when the original authors adjudicated that the events were related to vitamin C. We did not extract events which they concluded were not related to vitamin C. When the authors did not make any judgment, we extracted all reported events in the study.

RESULTS

The database search retrieved 8,149 citations, and 650 full-text articles were assessed for eligibility. Among these, 576 articles were excluded for a variety of reasons and 74 studies (4,678 patients) met our eligibility criteria (**Fig. 1**). The characteristics of the included studies are summarized in **Table 1** and **Supplementary Table 1** (Supplemental Digital Content 1,

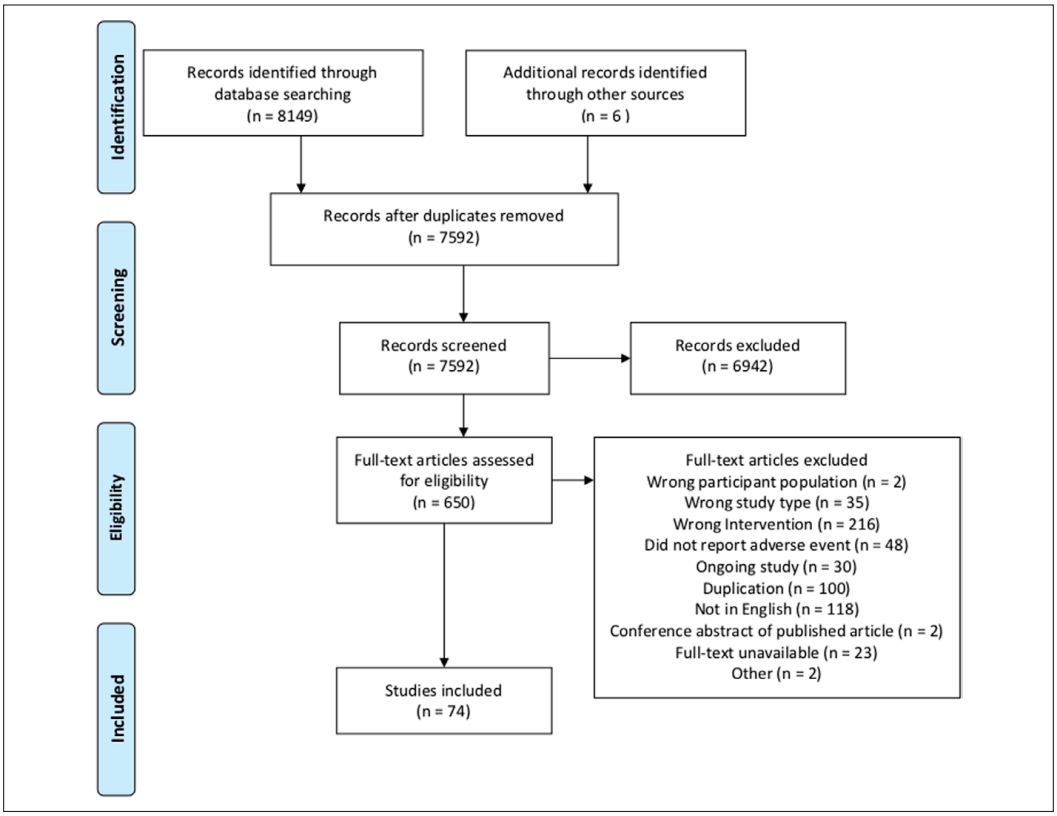

Figure 1. Flow chart showing the selection process.

http://links.lww.com/CCM/F510) according to target condition (infectious disease, malignancy, and other diseases) (6, 16, 27–97).

When given at a fixed dose, the median (interquartile range) dose of high-dose vitamin C was 22.5 g/d (8.25–63.75 g/d) (16, 25, 27–30, 32, 33, 36, 38–44, 46, 47, 49–56, 58, 68–78, 81–84, 87–92, 97), or 455 mg/kg/d (260–925 mg/kg/d) (6, 31, 34, 37, 48, 59, 62, 63, 75, 79, 80, 85, 86, 93, 96) or 70 g/m^2/d (50–90 g/m^2/d) (67) and the infusion speed ranged from IV bolus to continuous infusion for 24 hours (70, 96). In total, 932 adverse events and some cases of fluid retention, edema, thirst, or polyuria (numbers not reported) were identified in 2,801 patients who received high-dose vitamin C (**Table 2**; and **Supplementary Table 2**, Supplemental Digital Content 1, http://links.lww.com/CCM/F510). Among the 722 patients who received mega-dose vitamin C (20 g/d or more, 250 mg/d or more, or 10 g/m^2/d or more), 101 adverse events and some cases of fluid retention, edema, thirst, or polyuria were reported (35, 36, 41–67, 81–97).

In 12 studies conducted in patients with infectious disease, 492 patients received high-dose IV vitamin C, with only eight adverse events reported (Table 2). Three double-blind RCTs (DB-RCTs) compared high-dose vitamin C (100 mg/kg/d in one study [31], or 200 mg/kg/d in two studies [6, 34]) with placebo. Vitamin C (200 mg/kg) was infused over 30 minutes, every 6 hours in the two studies (6, 34). All the three papers reported no harm in either group (6, 31, 34).

In 31 studies with various study design conducted in patients with malignancies, 544 patients received high-dose vitamin C, and most patients also received chemotherapy (Supplementary Tables 1 and 2, Supplemental Digital Content 1, http://links.lww.com/CCM/F510). The median dose for high-dose vitamin C was 50 g/d (22.5–75 g/d) (38–44, 46, 47, 49–56, 58), 710 mg/kg/d (430–1,200 mg/kg/d) (48, 59, 62, 63), or 70 g/m^2/d (50–90 g/m^2/d) (65) and the infusion speed was mainly 0.5–1 g/min (49–52, 54, 55, 57–63, 66, 67). There was no DB-RCT but one open-label RCT (OL-RCT). In the OL-RCT that compared a high-dose vitamin C group (50–80 mg/kg/d and chemotherapy) and a nonvitamin C group (chemotherapy), the adverse event rate per patient in the vitamin C group was numerically slightly higher than that in nonvitamin C group (2.5 vs 2.4 adverse events per patient) (37). When all the malignancy treatment studies are considered, there were four cases of hypernatremia (67), two cases of allergic reaction

TABLE 1. Characteristics of Eligible Studies in Infectious Diseases

References	Design	Target Condition	Total No.	Vitamin C Dose	Other Intervention	Industry Funding
Fujii et al (25)	OL-RCT	Septic shock	211	Nil[a]; 6 g/d	Hydrocortisone; hydrocortisone, thiamine	No
Kim et al (27)	Before-after cohort study	Nonsepsis infection	99	Nil[a]; 6 g/d	Usual pneumonia treatment; thiamine, hydrocortisone, usual pneumonia treatment	No
Schencking and Kraft (28)	Case series	Nonsepsis infection	1	7.5 g/d	Cantharidin patch	NR
Schencking et al (29)	Prospective observational	Nonsepsis infection	67	7.5 g/d	Standard anti-herpetic treatment	Yes
Hagel et al (30)	Prospective observational	Nonsepsis infection Allergic disease	89	7.5 g/d; 7.5 g/d	NR; NR	NR
Zabet et al (31)	DB-RCT	Septic shock	28	Placebo; 100 mg/kg/d	Standard sepsis treatment	None
de Grooth et al (32)	OL-RCT	Sepsis, major surgery, trauma	20	2 g/d; 2 g/d; 10 g/d; 10 g/d	Standard concomitant treatment	No
Schencking et al (33)	Case series	Nonsepsis infection	2	15 g/d	Brivudine, macrogol aryl ether, zinc oxide, metamizole, gabapentin	No
Fowler et al (34)	DB-RCT	Severe sepsis	24	Placebo; 50 mg/kg/d; 200 mg/kg/d	Usual sepsis treatment	No
Fowler et al (6)	DB-RCT	Sepsis with ARDS	167	Placebo; 200 mg/kg/d	Standard sepsis and ARDS treatment	Yes
Marcial-Vega et al (35)	Case series	Nonsepsis infection	56	20–50 g/d	Magnesium chloride, vitamin B	NR
Gonzalez et al (36)	Case series	Nonsepsis infection	1	100 g/d	None	NR

ARDS = acute respiratory distress syndrome, DB-RCT = double-blind randomized controlled trial, NR = not reported, OL-RCT = open-label randomized controlled trial.

[a]Did not receive any dose of vitamin C or placebo.

When patients were allocated to two or more different doses of vitamin C group, the doses are reported in ascending order and other interventions are reported, respectively, separated by a semicolon.

(66), two cases of acute oxalate nephropathy (44, 51), one case of kidney stone (48), one case of renal failure (66), and one case of glucometer error (43).

In 31 studies conducted in patients with other diseases, 1,765 patients received high-dose vitamin C, and at least 745 adverse events were reported (Supplementary Tables 1 and 2, Supplemental Digital Content 1, http://links.lww.com/CCM/F510). The median dose for high-dose vitamin C was 10 g/d (7.5–45 g/d) (16, 68–78, 81–84, 87–92, 97) or 320 mg/kg/d (260–480 mg/kg/d) (79, 80, 85, 86, 93, 96), and the infusion speed varied from IV bolus of 7 g/d to continuous 24-hour infusion of 1,584 mg/kg/d (70, 96). Six DB-RCTs were included. Of these, one study in previous myocardial infarction patients reported 672 adverse events in the high-dose vitamin C group and 709 adverse events in the placebo group (71). The adverse event rates in the high-dose vitamin C group (7 g/d, infusion speed not reported) was numerically lower than in the placebo group (0.80 vs 0.82 adverse events per patient) (71). One study in healthy volunteers reported a numerically higher adverse event rate in the high-dose vitamin C group (10 g/d, infusion speed not reported) than the placebo group (0.33 vs 0.23 adverse events per patient) (78), and one study in atrial fibrillation patients (vitamin C 50 mg/kg over 30 min, 6 hr) reported identical adverse event rates (0.10 vs 0.10 adverse events per patient) (80). Three DB-RCTs (one in patients after cardiac surgery, one in patients with traumatic brain injury, and one in patients who underwent coronary angiography) reported no harm in either group (69, 77, 89). In the case series, four patients with G6PD deficiency received high-dose vitamin C and three developed hemolysis, and one had disseminated intravascular coagulation and renal failure (82, 90–92). Three cases of oxalate nephropathy, one case of hypernatremia, and one case of falsely elevated glucometer measurement were also reported (16, 83, 88, 97).

There was only one DB-RCT of mega-dose vitamin C versus placebo in acute myocardial infarction patients undergoing percutaneous coronary angioplasty (89). Mega-dose vitamin C (53 g/d) was given over 3 hours, and there was no harm reported in either group.

TABLE 2. Reported Harm and the Number of Events in Patients With Infectious Diseases

References	No. in Group	Vitamin C Dose	Infusion Time	Reported Harm (No. of Events)
Fujii et al (25)	104	Nil[a]	–	Gastrointestinal bleeding (1)
	107	6 g/d (H)	1.5 g every 6 hr, infusion over 1 hr	Fluid overload (1), hyperglycemia (1)
Kim et al (27)	46	Nil[a]	–	None
	53	6 g/d (H)	NR	None
Schencking and Kraft (28)	1	7.5 g/d (H)	NR	None
Schencking et al (29)	67	7.5 g/d (H)	NR	Itching/burning sensation on injection site (1), paresthesia (1), drug-induced urticaria (1)
Hagel et al (30)	70	7.5 g/d (H)	60 min	Slight abdominal pain, pruritus, and moderate dyspnea (1)
	19	7.5 g/d (H)	60 min	
Zabet et al (31)	14	Placebo	Every 6 hr, infusion over 30 min	None
	14	100 mg/kg/d (H)	4 equal doses every 6 hr, infusion over 30 min	None
de Grooth et al (32)	5	2 g/d (L)	1 g every 12 hr, infusion over 15 min	None
	5	2 g/d (L)	24 hr	None
	5	10 g/d (H)	5 g every 12 hr, infusion over 15 min	None
	5	10 g/d (H)	24 hr	None
Schencking et al (33)	2	15 g/d (H)	NR	None
Fowler et al (34)	8	Placebo	4 equal doses every 6 hr, infusion over 30 min	None
	8	50 mg/kg/d (L)	4 equal doses every 6 hr, infusion over 30 min	None
	8	200 mg/kg/d (H)	4 equal doses every 6 hr, infusion over 30 min	None
Fowler et al (6)	83	Placebo	Every 6 hr, infusion over 30 min	None
	84	200 mg/kg/d (H)	4 equal doses every 6 hr, infusion over 30 min	None
Marcial-Vega et al (35)	56	20–50 g/d (M)	2–4 hr	None
Gonzalez et al (36)	1	100 g/d (M)	NR	None

(H) = high-dose vitamin C group, (L) = low-dose vitamin C group, (M) = mega-dose vitamin C group, NR = not reported.
[a]Did not receive any dose of vitamin C or placebo.
Each intervention group corresponds to that in Table 1.

DISCUSSION

Key Findings

In this systematically structured SR, we studied the frequency and nature of harm associated with high-dose IV vitamin C therapy. We identified 74 relevant articles reporting adverse events, but most studies did not have control or placebo group. In the nine DB-RCTs that compared a high-dose IV vitamin C group to placebo group, the adverse event rate was essentially the same. Furthermore, six DB-RCTs, including one mega-dose vitamin C study, reported no harm in either group. However, specific adverse events attributed to high-dose IV vitamin C therapy, included five cases of oxalate nephropathy, five cases of hypernatremia, three cases of hemolysis in patients with

G6PD deficiency, two cases of glucometer error, and one case of kidney stones.

Relationship to Previous Studies

This is the first study to systematically review whether high-dose IV vitamin C therapy is associated with harm. We identified a number of reports on oxalate nephropathy, hypernatremia, kidney stones, glucometer errors, and hemolysis in patients with G6PD deficiency as rare adverse events of high-dose vitamin C. These specific adverse events appeared attributable to vitamin C therapy and were reported only in patients who received high-dose vitamin C.

As oxalate is a metabolite produced in the vitamin C oxidation pathway and is excreted in urine, oxalate nephropathy, or kidney stones were considered as specific adverse events of high-dose IV vitamin C therapy (98). Supplemental thiamine may mitigate further oxalate production, however, its clinical effect is unknown (99). The sodium level might also be expected to increase when vitamin C was infused in high doses as commercially available products of vitamin C are marketed as sodium ascorbate. Finally, high-dose vitamin C, which has a property of an electron donor, could result in falsely elevated point of care glucometer, as electrical currents in glucometers are interfered with by vitamin C derived electrons (100). In our results, there was no report of falsely elevated glucose levels measured by a central laboratory.

Some oxidant foods (fava beans, red wine, tonic water, and menthol) and medications (methylene blue, dapsone, nitrofurantoin, primaquine, rasburicase, and nonsteroidal anti-inflammatory drugs) are known to cause hemolysis in patients with G6PD deficiency (90). The mechanism of such hemolysis is not fully understood. As ascorbic acid is a single electron donor and can reduce oxygen radicals, it could be theoretically protective for G6PD deficiency at a supraphysiological concentration (101). In fact, IV vitamin C (4 g/d and 20 g/d) has been used to treat drug-induced methemoglobinemia and hemolysis in patients with G6PD deficiency without any harm in two case reports (82, 102). However, in a study with rats, high-dose vitamin C decreased RBC life span in G6PD deficiency (103). Our findings imply that some patients with G6PD deficiency who receive 75–80 g/d of IV vitamin C might develop hemolysis (90–92). In addition, two children with G6PD deficiency in India developed hemolysis after taking 4–6 g/d of oral vitamin C, which would have achieved lower blood vitamin C levels compared with IV administration (104, 105). These reports suggest that high-dose vitamin C should be avoided in patients with G6PD deficiency.

Implications of Study Findings

Glucometer error reporting pseudo-hyperglycemia may lead to iatrogenic hypoglycemia through inappropriate administration of insulin (16). Our findings imply that glucose levels in patients on high-dose IV vitamin C therapy should be monitored with other devices such as the central laboratory using the hexokinase spectrophotometric method for safety (100). This technique is reportedly not affected by high-dose vitamin C infusion (100). Finally, despite overall safety, specific side effects warrant vigilance during such therapy.

Strength and Limitations

High-dose vitamin C has been investigated as a promising therapy in various fields and, more recently as an adjunctive treatment for septic shock. Recent trials reported inconsistent findings on the effect on mortality, and this uncertainty warrants further studies both on the benefit and harm of vitamin C (6, 25). And there is a lack of evidence regarding its safety. Thus, our study provides the most comprehensive assessment of safety so far. Furthermore, it should facilitate communication with potential research subjects, and prevent its use in patients with G6PD deficiency.

Our study has some limitations. First, we conducted a SR without formal meta-analysis. This was because we expected considerable heterogeneity in the way adverse events would be reported, a concern confirmed by our review. Furthermore, it was not feasible to calculate pooled estimates of mortality, as death due to the target condition frequently occurred in most patient populations receiving high-dose IV vitamin C therapy, acting as a competing risk for adverse events. Considering the focus of the current study on the issue of safety, we included case reports and case series in the review. However, this design enabled us to report rare harmful events that might occur in patients with rare comorbidities (e.g., G6PD deficiency). Second, we collected data on events that were adjudicated and reported as harms related to vitamin C therapy by the original authors. The causality of high-dose IV vitamin C could not be confirmed and the reported numbers might be over or underestimated. Finally, we only included studies in the English language and excluded 118 papers written in other languages. However, there is no evidence of a systematic bias when non-English papers are excluded (106).

CONCLUSIONS

In 74 eligible studies, at least 932 adverse events were reported in 2,801 patients who received high-dose IV vitamin C. However, in the nine DB-RCTs available, the adverse event rates in the high-dose vitamin C groups were equivalent to those in the control group. Oxalate nephropathy, hypernatremia, and glucometer error should be monitored when patients receive high-dose IV vitamin C and such therapy should be avoided in patients with known or suspected G6PD deficiency. Considering the limited data in the critically ill patients, the lack of prevalent adverse events does not endorse the use of vitamin C in critically ill patients.

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For information regarding this article, E-mail: Rinaldo.BELLOMO@austin.org.au

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Supplementary Table 1. Characteristics of eligible studies in patients with other target conditions than infectious diseases.

Author and year	Design	Target condition	Total No.	Vitamin C dose	Other intervention	Industry funding
Zhao(37) 2018	OL-RCT	Malignancy	73	Nil[a]; 50-80 mg/kg/day	Chemotherapy	No
Rodríguez(38) 2017	Prospective observational	Malignancy	48	7.5 g/day	Conventional oncological therapy	Yes
Vollbracht(39) 2011	Retrospective observational	Malignancy	125	Nil[a]; 7.5 g/day	Chemotherapy, radiotherapy	N/R
Yeom(40) 2007	Prospective observational	Malignancy	39	10 g/day	N/R	Yes
Campbell(41) 1979	Case series	Malignancy	3	10 g/day; 20 g/day	Supportive care; chemotherapy	No
Seo(42) 2015	Case series	Malignancy	1	20 g/day	Magnesium sulphate	N/R
Bahr(43) 2015	Case series	Malignancy	1	25 g/day	N/R.	N/R
Nangia(44) 2011	Case series	Malignancy	1	30 g/day	Not reported	N/R
Cameron(45) 1974	Case series	Malignancy	50	0-6 g/day; 6-20 g/day; 20-45 g/day	Oral ascorbic mixture, sodium bicarbonate	N/R
Carr(46) 2014A	Case series	Malignancy	1	30 g/day	N/R	N/R
Carr(47) 2014B	Case series	Malignancy	1	50 g/day	Chemotherapy	N/R
Riordan(48) 2005	Case series	Malignancy	24	150 mg/kg/day; 290 mg/kg/day; 430 mg/kg/day; 570 mg/kg/day; 710 mg/kg/day	Standard cancer treatment	No
Nielsen(49) 2015	Case series	Malignancy	10	60 g/day	Standard care	No
Nielsen(50) 2017	Case series	Malignancy	23	60 g/day	Standard care	No
Wong(51) 1994	Case series	Malignancy	1	60 g/day	Diclofenac	N/R
Kawada(52) 2014	Single arm trial	Malignancy	3	75 g/day	Chemotherapy	N/R
Baillie(53) 2018	Case series	Malignancy	1	85 g/day	None	Yes
Mitchell(54) 2010	Prospective observational	Malignancy	5	100 g/day	Chemotherapy	N/R
Monti(55) 2012	Prospective observational	Malignancy	14	50 g/day; 75 g/day; 100 g/day	Chemotherapy	No
Polireddy(56) 2017	Prospective observational	Malignancy	14	100 g/day	Chemotherapy	No

Raymond(57) 2016	Case series	Malignancy	9	25-100 g/day	None; none; chemotherapy; supplemental therapy; chemotherapy; other therapy; N/R; N/R; N/R	None
Riordan(58) 2004	Case series	Malignancy	7	15 g/day; 30 g/day; 50 g/day; 65 g/day; 75 g/day; 100 g/day; 100 g/day	Radiotherapy; not reported; none; not reported; chemotherapy; none; chemotherapy and oral vitamin/nutrients	N/R
Hoffer(59) 2008	Controlled trial	Malignancy	24	400 mg/kg/day, 600 mg/kg/day, 900 mg/kg/day, 1500 mg/kg/day	Multivitamin tablet and d-α-tocopherol and oral ascorbic acid	No
Hoffer(60) 2015	Controlled trial	Malignancy	14	1.5 g/kg/day when BMI ≤ 30 kg/m^2, BMI 24 kg/m^2 when BMI > 30	Chemotherapy	Yes
Robitaille(61) 2009	Case series	Malignancy	16	100-1500 mg/kg/day	N/R	No
Ou(62) 2017	RCT	Malignancy	15	1000 mg/kg/day; 1200 mg/kg/day; 1500 mg/kg/day	Modulated electrohyperthermia	No
Wang(63) 2019	Prospective observational	Malignancy	36	200-1500 mg/kg/day; 1500 mg/kg/day	Chemotherapy	No
Drisko(64) 2018	Case series	Malignancy	1	75-125 g/day	None	N/R
Welsh(65) 2013	Prospective observational	Malignancy	9	15-125 g/day	Chemotherapy	No
Bazzan(66) 2018	Retrospective observational	Malignancy	86	50-150 g/day	Magnesium chloride and calcium gluconate; magnesium chloride, calcium gluconate and chemotherapy	No
Stephenson(67) 2013	Prospective observational	Malignancy	17	30 g/m^2/day; 50 g/m^2/day; 90 g/m^2/day; 70 g/m^2/day; 110 g/m^2/day	Supportive medications, nutritional interventions	No
Talbot(68) 2014	Crossover RCT	Healthy volunteer	7	Placebo; 6 g/day	Induction of hypoxia	No
Yanase(69) 2019	DB-RCT	Cardiac surgery	50	Placebo; 6 g/day	Standard perioperative care	No
Komiyama(70) 2017	OL-RCT	CAG and CKD	429	Nil[a]; 7 g/day	Hydration	None
Lamas[71] 2013	DB-RCT	Previous MI	1708	Placebo; 7 g/day	None; EDTA, magnesium chloride, procaine hydrochloride, heparin, potassium chloride, sodium bicarbonate, pantothenic acid, thiamine, pyridoxine	No
Mühlhöfer (72) 2004	Crossover RCT	Healthy volunteer	6	0.75 g/day; 7.5 g/day	None	Yes

Vollbracht(73) 2018	Prospective observational	Allergy	71	7.5 g/day	Antiallergic drug, antibiotics	Yes
Kodama(74) 2006	Case series	Interstitial pneumonia	1	8 g/day	Antibiotics	N/R
Hong(75) 2002	Prospective observational	Healthy volunteers; paraquat poisoning	17	50 mg/kg/day; 9 g/day	None; N/R	N/R
Laskowski(76) 1995	OL-RCT	AMI	84	Nil[a]; 10 g/day	Thrombolytic therapy for AMI	N/R
Razmkon(77) 2010	DB-RCT	Traumatic brain injury	100	Placebo; 0.5 g/day; 10 g/day	ICP-targeted strategy, vitamin E	N/R
Sharma(16) 2018	Case series	Cardiac surgery	1	10 g/day	N/R	N/R
Suh(78) 2012	DB-RCT	Healthy volunteers	147	Placebo; 10 g/day	None	N/R
Kang(79) 2013	OL-RCT	Sudden sensorineural hearing loss	72	Nil[a]; 200 mg/kg/day	Steroid 1mg/kg	N/R
NCT03148236(80) 2017	DB-RCT	AF ablation	20	Placebo; 200 mg/kg/day	N/R	N/R
Lonsdale(81) 1999	OL-RCT	Various acute/chronic conditions requiring nutritional therapy	64	6.66 g/day; 20 g/day	Magnesium chloride, calcium gluconate, pyridoxine hydrochloride, dexpanthenol, hydroxocobalamin, thiamine, zinc, copper, manganese, chromium, selenium; Magnesium chloride, potassium chloride, dexpanthenol, folic acid, manganese chloride, zinc chloride, selenium, chromium, adenosine 5' monophosphate, procaine, pyridoxine hydrochloride, hydroxocobalamin, vitamin B, thiamine, riboflavin 5' phosphate sodium, pyridoxine, dexpanthenol, niacinamide	No
Reeves(82) 2016	Case series	G6PD deficiency	1	20 g/day	Hemodialysis	N/R
Badrick(83) 1992	Case series	N/R	1	30 g/day	N/R	N/R
Engelhart(84) 2003	Case series	Healthy volunteers	1	30 g/day	None	N/R
Aschauer(85) 2014	Crossover	Healthy volunteers and LPS infusion	36	Placebo; 320 mg/kg/day; 480 mg/kg/day	None	Yes
Weisshaar(86) 2013	Crossover RCT	Healthy volunteer and LPS infusion	14	320 mg/kg/day; 480 mg/kg/day	None	N/R
Park(87) 2014	Case series	Dapsone-induced methemoglobinemia	1	40 g/day	Standard care	No

Lawton(88) 1985	Case series	Primary amyloidosis	1	45 g/day	chemotherapy	N/R
Ramos(89) 2017	DB-RCT	CAG	99	Placebo; 53 g/day	PCI and STEMI standard treatment	No
Quinn(90) 2017	Case series	G6PD deficiency, RA, CLL/SLL, HTN	1	75 g/day	N/R	N/R
Rees(91) 1993	Case series	G6PD deficiency, HIV	1	80 g/day	N/R	N/R
Campbell(92) 1975	Case series	G6PD deficiency	1	80 g/day	N/R	N/R
Virno(93) 1966	Case series	Glaucoma	21	280 mg/kg/day; 400-1000 mg/kg/day	Glycerol; None	N/R
Prier(94) 2018	Prospective observational	Glaucoma	157	15-100 g/day	N/R	N/R
Ray(95) 1977	Prospective observational	Glaucoma	5	1000-1500 mg/kg/day	Glycerol	N/R
Tanaka(96) 2000	OL-RCT	Burn	37	Nil[a]; 1584 mg/kg/day	Usual burn treatment	N/R
Buehner(97) 2016	Case series	Burn	2	101 g/day; 224 g/day	Usual burn treatment	N/R

AF denotes atrial fibrillation; AMI, acute myocardial infarction; CAG, coronary angiogram; CKD, chronic kidney disease; CLL, chronic lymphocytic leukemia; DB-RCT, double-blind randomized controlled trial; EDTA, ethylenediaminetetraacetic acid; G6PD, glucose-6-phosphate dehydrogenase; HIV, human immunodeficiency virus; HTN, hypertension; ICP, intra cranial pressure; LPS, Lipopolysaccharide; MI, myocardial infarction; N/R; not reported; OL-RCT, open-label randomized controlled trial; PCI, percutaneous coronary intervention; RA, rheumatoid arthritis; SLL, small lymphocytic lymphoma; STEMI, ST-elevation myocardial infarction.
When patients were allocated to two or more different doses of vitamin C group, the doses are reported in ascending order and other interventions are reported respectively, separated by semicolon.
[a]Did not receive any dose of vitamin C or placebo.

Supplementary Table 2. Reported harm and the number of events in patients with other target conditions than infectious diseases. Each intervention group corresponds to that in Supplementary Table 1.

Author and year	Number in group	Vitamin C dose	Infusion time	Reported harm (No. of events) Grade ≥ 3 or all events of harm when not graded.
Zhao(37) 2018	34	Nil[a]	—	Neutropenia (20), thrombocytopenia (29), infection (31)[b]
	39	50-80 mg/kg/day (H)	N/R	Neutropenia (29), thrombocytopenia (36), infection (34)[b]
Rodríguez(38) 2017	48	7.5 g/day (H)	N/R	None
Vollbracht(39) 2011	72	Nil[a]	—	None
	53	7.5 g/day (H)	N/R	None
Yeom(40) 2007	39	10 g/day (H)	N/R	None
Campbell(41) 1979	1	10 g/day (H)	N/R	Fever with pain in the mass of Hodgkin's tissue (1)
	2	20 g/day (M)	N/R	Exacerbation of the malignant tumors (2)
Seo(42) 2015	1	20 g/day (M)	N/R	Thirst (1)
Bahr(43) 2015	1	25 g/day (M)	N/R	No reaction with glucose strip test (1)
Nangia(44) 2011	1	30 g/day (M)	N/R	Acute oxalate nephropathy requiring RRT (1)
Cameron(45) 1974	22	0-6 g/day (L)	N/R	Fluid retention (number not reported), dependent edema (number not reported)[c]
	24	6-20 g/day (H)	N/R	
	4	20-45 g/day (M)	N/R	
Carr(46) 2014A	1	30 g/day (M)	N/R	None
Carr(47) 2014B	1	50 g/day (M)	N/R	None
Riordan(48) 2005	3	150 mg/kg/day (H)	N/R	None[b]
	7	290 mg/kg/day (M)	N/R	Kidney stone (1) (possibly related)[b]
	6	430 mg/kg/day (M)	N/R	None[b]
	3	570 mg/kg/day (M)	N/R	Hypokalemia (1) (possibly related)[b]
	5	710 mg/kg/day (M)	N/R	None[b]
Nielsen(49) 2015	10	60 g/day (M)	1 hour	None[b]
Nielsen(50) 2017	23	60 g/day (M)	1 hour	Hypertension (1), urinary tract infection (1), transurethral prostate resection (3), metastatic affection of medulla spinalis (3), pulmonary embolism (2), pneumonia (1), anemia (1)[b]
Wong(51) 1994	1	60 g/day (M)	2 hours	Acute oxalate nephropathy (1)
Kawada(52) 2014	3	75 g/day (M)	75 min	None
Baillie(53) 2018	1	85 g/day (M)	N/R	None

Mitchell(54) 2010	5	100 g/day (M)	90 min	Diarrhea (1), jaundice (1)[b]
Monti(55) 2012	4	50 g/day (M)	90 min	Low hemoglobin (2), low absolute neutrophil count (1), ileus (1), urinary tract infection (1), pulmonary emboli (2), death (3)[b,c]
	5	75 g/day (M)	90 min	
	5	100 g/day (M)	90 min	
Polireddy(56) 2017	14	100 g/day (M)	N/R	None related to vitamin C[b]
Raymond(57) 2016	1	25-100 g/day (M)	0.5 g/min	Jarisch-Herxheimer reaction (1)
	1	25-100 g/day (M)	0.5 g/min	None
	1	25-100 g/day (M)	0.5 g/min	None
	1	25-100 g/day (M)	0.5 g/min	None
	1	25-100 g/day (M)	0.5 g/min	None
	1	25-100 g/day (M)	0.5 g/min	None
	1	25-100 g/day (M)	0.5 g/min	None
	1	25-100 g/day (M)	0.5 g/min	None
	1	25-100 g/day (M)	0.5 g/min	None
Riordan(58) 2004	1	30 g/day (M)	N/R	None
	1	65 g/day (M)	N/R	None
	1	100 g/day (M)	N/R	None
	1	75 g/day (M)	N/R	None
	1	15 g/day (H)	N/R	None
	1	50 g/day (M)	N/R	None
	1	100 g/day (M)	2 hours	None
Hoffer(59) 2008	5	400 mg/kg/day (M)	Doses up to 90 g were infused at >90 min; doses >90 g were infused over 120 min.	None[b]
	6	600 mg/kg/day (M)	Doses up to 90 g were infused at >90 min; doses >90 g were infused over 120 min.	None[b]
	7	900 mg/kg/day (M)	Doses up to 90 g were infused at >90 min; doses >90 g were infused over 120 min.	None[b]

	6	1500 mg/kg/day (M)	Doses up to 90 g were infused at >90 min; doses >90 g were infused over 120 min.	None[b]
Hoffer(60) 2015	14	1.5 g/kg/day when BMI ≤ 30 kg/m^2, BMI 24 kg/m^2 when BMI > 30 (M)	90 minutes for doses up to 90 g, and over a period 120 minutes for doses > 90 g	Nausea and occasional vomiting (1), thirst, unpleasant fluttering sensation in the upper abdomen and mentally heavy feeling (1), chills, thirst headache and a rumbling feeling and increased leg edema (1)
Robitaille(61) 2009	16	100-1500 mg/kg/day (M)	90-120 min	Thirst and polyuria (number not reported)
Ou(62) 2017	5	1000 mg/kg/day (M)	2 hours	None[b]
	5	1200 mg/kg/day (M)	2 hours	None[b]
	5	1500 mg/kg/day (M)	2 hours	Diarrhea (3)[b]
Wang(63) 2019	21	200-1500 mg/kg/day (M)	3 hours	Peripheral sensory neuropathy (1), vomiting (1), neutropenia (6), leukopenia (1), diarrhea (1)[b]
	15	1500 mg/kg/day (M)	0.6-1 g/min	
Drisko(64) 2018	1	75-125 g/day (M)	N/R	None
Welsh(65) 2013	9	15-125 g/day (M)	N/R	None related to vitamin C[b]
Bazzan(66) 2018	32	50-150 g/day (M)	Over 2 to 3 hours. Adjusted to tolerance by the patient.	Allergic reaction (2), anemia (1), hyponatremia (1), hypotension (1), renal failure (1) [b]
	54	50-150 g/day (M)	Over 2 to 3 hours. Adjusted to tolerance by the patient.	Platelet count decreased (2), anemia (3), neutrophil count decreased (5), hypotension (2), nausea/vomiting (2), biliary obstruction (3), ileus (1), colonic hemorrhage (1)[b]
Stephenson(67) 2013	3	30 g/m^2/day (M)	1 g/min	None[b]
	5	50 g/m^2/day (M)	1 g/min	None[b]
	3	90 g/m^2/day (M)	1 g/min	None[b]
	3	70 g/m^2/day (M)	1 g/min	Hypokalemia (2), hypernatremia (2), headache (1)[b]
	3	110 g/m^2/day (M)	1 g/min	Hypernatremia (2)[b]
Talbot(68) 2014	7	Placebo	—	None
	7	6 g/day (H)	10 min	None
Yanase(69) 2019	25	Placebo	—	None
	25	6 g/day (H)	60 min	None
Komiyama(70) 2017	218	Nil[a]	—	None
	211	7 g/day (H)	IV bolus	None

Lamas(71) 2013	869	Placebo	—	Serious adverse event (127), non-serious adverse event (582)[d]
	839	7 g/day (H)	N/R	Serious adverse event (100), non-serious adverse event (572)[d]
Mühlhöfer(72) 2004	6	0.75 g/day (L)	10 min	Increased thirst (number not specified)
	6	7.5 g/day (H)	30 min	Thrombophlebitis (1), increased thirst (number not reported)
Vollbracht(73) 2018	71	7.5 g/day (H)	N/R	Sensation of cold after infusion (1), tiredness on next morning (1)
Kodama(74) 2006	1	8 g/day (H)	N/R	None
Hong(75) 2002	7	50 mg/kg/day (L)	N/R	None
	10	9 g/day (H)	N/R	None
Laskowski(76) 1995	42	Nil[a]	—	Left ventricular insufficiency (5), ventricular ectopic beats (IVa Lown class or higher) (7), supraventricular tachyarrhythmias (1), angina (21), reocclusion (4)
	42	10 g/day (H)	4 hours	Left ventricular insufficiency (2), ventricular ectopic beats (IVa Lown class or higher) (1), supraventricular tachyarrhythmias (1), atrioventricular block (second and third degree) (1), angina (16), reocclusion (2)
Razmkon(77) 2010	25	Placebo	—	None
	25	Placebo	—	None
	25	0.5 g/day (L)	N/R	None
	25	10 g/day (H)	N/R	None
Sharma(16) 2018	1	10 g/day (H)	N/R	Falsely elevated glucose levels on finger stick resulting in inappropriate insulin administration (1)
Suh(78) 2012	74	Placebo	—	Itching sense/pain at injection site (11), dry mouth (2), others (3), common cold symptoms (1)
	73	10 g/day (H)	N/R	Itching sense/pain at injection site (10), dry mouth (8), others (4), diarrhea (1), common cold symptoms (1)
Kang(79) 2013	36	Nil[a]	—	None
	36	200 mg/kg/day (H)	N/R	None
NCT03148236(80)	10	Placebo	—	Pericarditis (1)
	10	200 mg/kg/day (H)	50 mg/kg for 30 min 6 hourly	Pericarditis (1)
Lonsdale(81) 1999	30	6.66 g/day (H)	30 min	Vomiting (1)
	34	20 g/day (M)	3 hours	Vomiting (1)
Reeves(82) 2016	1	20 g/day (M)	N/R	None
Badrick(83) 1992	1	30 g/day (M)	N/R	Abnormal laboratory values (Low urate, cholesterol and triglyceride. Hypernatremia and increased anion gap. High total iron binding capacity) (1)

Engelhart(84) 2003	1	30 g/day (M)	N/R	None
Aschauer(85) 2014	36	Placebo	—	None
	36	320 mg/kg/day (M)	160 mg/kg for 30 min, 160 mg/kg for 90 min	None
	36	480 mg/kg/day (M)	240 mg/kg for 30 min, 240 mg/kg for 90 min	None
Weisshaar(86) 2013	7	320 mg/kg/day (M)	2 hours	None
	7	480 mg/kg/day (M)	2 hours	None
Park(87) 2014	1	40 g/day (M)	10 g for 10 min 6 hourly	Epigastric discomfort (1), nausea (1)
Lawton(88) 1985	1	45 g/day (M)	N/R	Acute oliguric renal failure with tubular obstruction by calcium oxalate (1)
Ramos(89) 2017	53	Placebo	—	None
	46	53 g/day (M)	3 hours	None
Quinn(90) 2017	1	75 g/day (M)	N/R	Intravascular hemolysis (1)
Rees(91) 1993	1	80 g/day (M)	N/R	Hemolysis (1)
Campbell(92) 1975	1	80 g/day (M)	N/R	Intravascular hemolysis, disseminated intravascular coagulation and renal failure (1)
Virno(93) 1966	16	280 mg/kg/day (M)	N/R	None
	5	400-1000 mg/kg/day (M)	N/R	None
Prier(94) 2018	157	15-100 g/day (M)	N/R	None
Ray(95) 1977	5	1000-1500 mg/kg/day (M)	N/R	Chills (3), hematuria (1), nausea/vomiting (2), headache (1), confusion (1), tremor (1)
Tanaka(96) 2000	18	Nil[a]	—	None
	19	1584 mg/kg/day (M)	24 hours	None
Buehner(97) 2016	1	101 g/day (M)	18 hours	Birefringent calcium oxalate crystals in kidneys (1)
	1	224 g/day (M)	20 hours	AKI with calcium oxalate crystals in kidneys (1)

AKI denotes acute kidney injury, (H); high dose vitamin C group, IV; intra venous, (L); low dose vitamin C group, (M); mega dose vitamin C group, N/R; not reported.

When adverse events were reported with grading system, we reported adverse events graded 3 or more and their reported numbers. When not graded, we reported all adverse events and their reported numbers. When authors concluded reported all adverse events were not related to vitamin C treatment, we reported them as “none related to vitamin C”.

[a]Did not receive any dose of vitamin C or placebo.

[b]Only Grade ≥ 3 adverse events were reported.

[c]Adverse events were reported as a whole study group.

[d]Detailed serious and non-serious adverse events were provided in the original report.

Supplementary material 1. Detailed search strategies.

Database: Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Daily and Versions(R) <1946 to June 14, 2019>

Search Strategy:
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1 Ascorbic Acid/ (41096)
2 (ascor* or l-ascor* or acidylina* or adenex* or afj c or agrumina* or alle?corb* or antiscorb* or arcavit* c or arkovital* c or ascelat* or ascofar* or ascomed* or asconvita* or askorbin* or austrovit* c or bentavit* c or c crivit* or c ine or c level or c lisa or c long or c monovit* or c prana or c rivitin* or c sol or c tamin* or c tonic or c tron or c vescent or c vicotrat* or c?vimin* or c vit* or c-will or cantan or cantaxin or catavin c or ce arom or ce major or ce quin* or ce?vi?sol or ce vit* or cebetate or cebicure or cebion* or cecap or cecon* or cecorb* or cecrisina or cedon* or cedoxon* or cee-500 or ceevifil or cegiolan or celaskon* or celin* or cenetone or cenol* or cequinyl or cereon or cergona or cescorbat or cetami* or cetebe or ceterapion* or cetrinets or ceva?in* or cevex or cevibram or cevig* or cevilat or cevimin* or cevisol or cevit* or cewin or chewcee or chivibit c or ci drol or ciamin* or ciergin or cifilina or cipca or cisir or cital or citamino or cith or citoascorbina or citoxyl or citran or citravite or citritabs or citrovitamina or civigor or civitin* or co biagini or concemin or cortalex).mp. (68553)
3 (xylo?ascorb* or dagrascorb* or dagravit c or dancimin c or davitamon c or dayvital or delo c or difvitamin c or dumovit* c or erftamin* c or esuron or esurvit* or flavettes or godabion c or gregovite c or hicee or hybrin* or ido c or ikacee or inovitan c or irocevit or irocevite or jarexin* or keto hexuronic acid lactone or lacivit* or laroscorbine or leder?c or lemascorb or limcee or limo ce or liqui cee or magnorbin* or mega-c?a or myascorbin or natrascorb or nybadol or paa 500 or parkovit c or pharmascorb* or pharmatovit* c or planavit* c or plivit* c or pro-c or proscorbin* or redoxon or ribena or scorbacid* or scorbettes or scorbex or scorbin c or scorbitol or scorbumine or scottavit* c or secorbate or sevalin or sigmavit* c or sodascorb* or sweetcee or synum c or take-c or tanvimil-c or testascorb* or ucemine c or upsa?c or vi?ci sin or vi dom c or vi-c 500 or vicef or vicelat or vicetrin or vici monico or viciman or vicin or vicitina or vicon or viforcit* or viscorin* or vita-cedol orange or vitac or vitace or vitacee or vitaci* or vitamin* C or vitaplex c or vitapric or vitapur c or vitasan c or vitascorb* or vitelix c or vitocee or vorange or wandervit* c or witamina c or xitix or xon-ce).mp. (24927)
4 or/1-3 (77268)
5 Administration, Intravenous/ (7644)
6 infusions, parenteral/ or infusions, intravenous/ (79210)
7 (intravenous* or i?v? or infus* or inject* or intraport* or intravascul* or parenteral* or vein or venous*).mp. (2074773)
8 or/5-7 (2074773)
9 4 and 8 (6591)
10 exp animals/ not humans.sh. (4589476)
11 9 not 10 (3895)

Database: Embase Classic+Embase <1947 to 2019 June 18>
Search Strategy:
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1 Ascorbic Acid/ (98411)
2 (ascor* or l-ascor* or acidylina* or adenex* or afj c or agrumina* or alle?corb* or antiscorb* or arcavit* c or arkovital* c or ascelat* or ascofar* or ascomed* or asconvita* or askorbin* or austrovit* c or bentavit* c or c crivit* or c ine or c level or c lisa or c long or c monovit* or c prana or c rivitin* or c sol or c tamin* or c tonic or c tron or c vescent or c vicotrat* or c?vimin* or c vit* or c-will or cantan or cantaxin or catavin c or ce arom or ce major or ce quin* or ce?vi?sol or ce vit* or cebetate or cebicure or cebion* or cecap or cecon* or cecorb* or cecrisina or cedon* or cedoxon* or cee-500 or ceevifil or cegiolan or celaskon* or celin* or cenetone or cenol* or cequinyl or cereon or cergona or cescorbat or cetami* or cetebe or ceterapion* or cetrinets or ceva?in* or cevex or cevibram or cevig* or cevilat or cevimin* or cevisol or cevit* or cewin or chewcee or chivibit c or ci drol or ciamin* or ciergin or cifilina or cipca or cisir or cital or citamino or cith or citoascorbina or citoxyl or citran or citravite or citritabs or citrovitamina or civigor or civitin* or co biagini or concemin or cortalex).mp. (122362)

3 (xylo?ascorb* or dagrascorb* or dagravit c or dancimin c or davitamon c or dayvital or delo c or difvitamin c or dumovit* c or erftamin* c or esuron or esurvit* or flavettes or godabion c or gregovite c or hicee or hybrin* or ido c or ikacee or inovitan c or irocevit or irocevite or jarexin* or keto hexuronic acid lactone or lacivit* or laroscorbine or leder?c or lemascorb or limcee or limo ce or liqui cee or magnorbin* or mega-c?a or myascorbin or natrascorb or nybadol or paa 500 or parkovit c or pharmascorb* or pharmatovit* c or planavit* c or plivit* c or pro-c or proscorbin* or redoxon or ribena or scorbacid* or scorbettes or scorbex or scorbin c or scorbitol or scorbumine or scottavit* c or secorbate or sevalin or sigmavit* c or sodascorb* or sweetcee or synum c or take-c or tanvimil-c or testascorb* or ucemine c or upsa?c or vi?ci sin or vi dom c or vi-c 500 or vicef or vicelat or vicetrin or vici monico or viciman or vicin or vicitina or vicon or viforcit* or viscorin* or vita-cedol orange or vitac or vitace or vitacee or vitaci* or vitamin* C or vitaplex c or vitapric or vitapur c or vitasan c or vitascorb* or vitelix c or vitocee or vorange or wandervit* c or witamina c or xitix or xon-ce).mp. (32822)
4 or/1-3 (127933)
5 Administration, Intravenous/ (382730)
6 infusions, parenteral/ or infusions, intravenous/ (382325)
7 (intravenous* or i?v? or infus* or inject* or intraport* or intravascul* or parenteral* or vein or venous*).mp. (3527984)
8 or/5-7 (3527984)
9 exp animals/ not humans.sh. (26353065)
10 4 and 8 (14630)
11 10 not 9 (2842)

Database: EBM Reviews - Cochrane Database of Systematic Reviews <2005 to June 14, 2019>, EBM Reviews - ACP Journal Club <1991 to May 2019>, EBM Reviews - Database of Abstracts of Reviews of Effects <1st Quarter 2016>, EBM Reviews - Cochrane Clinical Answers <May 2019>, EBM Reviews - Cochrane Central Register of Controlled Trials <May 2019>, EBM Reviews - Cochrane Methodology Register <3rd Quarter 2012>, EBM Reviews - Health Technology Assessment <4th Quarter 2016>, EBM Reviews - NHS Economic Evaluation Database <1st Quarter 2016>
Search Strategy:
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1 Ascorbic Acid/ (2020)
2 (ascor* or l-ascor* or acidylina* or adenex* or afj c or agrumina* or alle?corb* or antiscorb* or arcavit* c or arkovital* c or ascelat* or ascofar* or ascomed* or asconvita* or askorbin* or austrovit* c or bentavit* c or c crivit* or c ine or c level or c lisa or c long or c monovit* or c prana or c rivitin* or c sol or c tamin* or c tonic or c tron or c vescent or c vicotrat* or c?vimin* or c vit* or c-will or cantan or cantaxin or catavin c or ce arom or ce major or ce quin* or ce?vi?sol or ce vit* or cebetate or cebicure or cebion* or cecap or cecon* or cecorb* or cecrisina or cedon* or cedoxon* or cee-500 or ceevifil or cegiolan or celaskon* or celin* or cenetone or cenol* or cequinyl or cereon or cergona or cescorbat or cetami* or cetebe or ceterapion* or cetrinets or ceva?in* or cevex or cevibram or cevig* or cevilat or cevimin* or cevisol or cevit* or cewin or chewcee or chivibit c or ci drol or ciamin* or ciergin or cifilina or cipca or cisir or cital or citamino or cith or citoascorbina or citoxyl or citran or citravite or citritabs or citrovitamina or civigor or civitin* or co biagini or concemin or cortalex).mp. (5438)
3 (xylo?ascorb* or dagrascorb* or dagravit c or dancimin c or davitamon c or dayvital or delo c or difvitamin c or dumovit* c or erftamin* c or esuron or esurvit* or flavettes or godabion c or gregovite c or hicee or hybrin* or ido c or ikacee or inovitan c or irocevit or irocevite or jarexin* or keto hexuronic acid lactone or lacivit* or laroscorbine or leder?c or lemascorb or limcee or limo ce or liqui cee or magnorbin* or mega-c?a or myascorbin or natrascorb or nybadol or paa 500 or parkovit c or pharmascorb* or pharmatovit* c or planavit* c or plivit* c or pro-c or proscorbin* or redoxon or ribena or scorbacid* or scorbettes or scorbex or scorbin c or scorbitol or scorbumine or scottavit* c or secorbate or sevalin or sigmavit* c or sodascorb* or sweetcee or synum c or take-c or tanvimil-c or testascorb* or ucemine c or upsa?c or vi?ci sin or vi dom c or vi-c 500 or vicef or vicelat or vicetrin or vici monico or viciman or vicin or vicitina or vicon or viforcit* or viscorin* or vita-cedol orange or vitac or vitace or vitacee or vitaci* or vitamin* C or vitaplex c or vitapric or vitapur c or vitasan c or vitascorb* or vitelix c or vitocee or vorange or wandervit* c or witamina c or xitix or xon-ce).mp. (3773)
4 or/1-3 (7089)
5 Administration, Intravenous/ (937)

6 infusions, parenteral/ or infusions, intravenous/ (11538)
7 (intravenous* or i?v? or infus* or inject* or intraport* or intravascul* or parenteral* or vein or venous*).mp. (280728)
8 or/5-7 (280728)
9 4 and 8 (1340)
10 exp animals/ not humans.sh. (44)
11 9 not 10 (1340)

Trial Registry: ClinicalTrials.gov

https://clinicaltrials.gov/
Date searched: 19 June 19
Advanced Search >
Study Type: All Studies
Study Results: All Studies
Interventions: Vitamin C OR ascorbic acid OR ascorbate

Trial Registry: World Health Organization, International Clinical Trials Registry Platform

http://apps.who.int/trialsearch/AdvSearch.aspx
Date searched: 19 June 2019
Advanced Search >
Intervention: Vitamin C OR ascorbic acid OR
Recruitment status: ALL
Phases: All

FEATURE REVIEW ARTICLE

Efficacy and Safety of Parenteral High-Dose Vitamin C Therapy in Pediatric Patients: A Scoping Review

Fumitaka Yanase, MD[1,2]

Sainath Raman, MRCPCH, PhD, FCICM[3,4]

Thummaporn Naorungroj, MD[1,5]

Avril McCarthy, MB, BCh, BAO[6]

Michele Cree, BSci, BPharm, GradDipClinPharm, AdvPracPharm[3,4,7]

Luregn J. Schlapbach, MD, PhD, FCICM[3,4,8]

Rinaldo Bellomo, MD, PhD[1,2,9]

OBJECTIVES: Recently, several adult trials have investigated the potential benefit of high-dose vitamin C therapy in critically ill patients. In pediatric patients, little is known on the efficacy, safety, and risk of high-dose vitamin C therapy. We aimed to review the efficacy and potential harm associated with high-dose vitamin C treatment.

DATA SOURCES: We searched MEDLINE, EMBASE, Cochrane Library, and National Institute of Health Clinical Trials Register.

STUDY SELECTION: We included studies in neonatal and pediatric patients who received IV or intra-arterial high-dose vitamin C (ascorbic acid) defined as greater than or equal to 75 mg/kg/d.

DATA EXTRACTION: Two independent investigators screened articles and extracted data.

DATA SYNTHESIS: We found 1,364 articles, assessed 193 full texts for eligibility, and identified 12 eligible studies. These studies included 855 patients, with 194 receiving high-dose vitamin C. The age of patients who received high-dose vitamin C ranged from 2 hours after delivery to 8.4 years (median 2.4 yr), and the vitamin C dose ranged from 100 to 1,500 mg/kg/d (median 260.5 mg/kg/d). Four studies were double-blind randomized controlled trials, and no clinical efficacy outcome was reported in favor of or against vitamin C. Furthermore, no adverse event or signal of harm was reported with high-dose vitamin C.

CONCLUSIONS: In 12 studies with 194 children treated with parenteral high-dose vitamin C, there was no evidence of clinical efficacy or inferior clinical outcomes in double-blind randomized controlled trials, and no reported harmful effects. These findings justify further investigations of this treatment in children.

KEY WORDS: ascorbic acid; child; efficacy; harm; newborn; vitamin C

Vitamin C (ascorbic acid) is an essential water soluble vitamin contained in certain types of food, which cannot be synthesized by humans. It has multiple anti-inflammatory and antioxidant properties and is a cofactor in the synthesis of vasopressin and catecholamines (1, 2). Although the effect of severe vitamin C deficiency on health has been well described for centuries, more recently, interest in high-dose vitamin C as adjunctive treatment for a number of severe diseases has emerged (3). The rationale to investigate the potential benefit of high-dose vitamin C in critical illness stems from in vitro studies demonstrating an important role of this antioxidant in

DOI: 10.1097/PCC.0000000000002686

steroid and catecholamine pathways, endothelial regulation, immunomodulation, and nonspecific effects in reducing oxidative stress (4), all common features of many critical illness states. In addition, in many types of critical illness, serum vitamin C levels are low, and IV vitamin C supplementation is required to achieve their correct such hypovitaminosis (5–12). Accordingly, these preclinical studies have led to trials in sepsis including coronavirus disease 2019 (6, 13), cardiac surgery (14), and trauma/burns (15).

High-dose IV vitamin C has been recently studied in septic adults and children as "metabolic resuscitation" in the form of a combination therapy of hydrocortisone, ascorbic acid, and thiamine (HAT) for septic shock (5, 12, 16, 17). Furthermore, meta-analyses have indicated a potential effect on shorter duration of organ dysfunction (18). In addition, previous trials have been performed with high-dose vitamin C in patients with other diseases. Vitamin C is known to be cytotoxic to cancer cells in in vitro and animal studies. These observations have prompted testing of high-dose vitamin C therapy as part of anticancer regimens (19–21). In addition, vitamin C has been used as a scavenger of free radicals in the setting of methemoglobinemia in a manner similar to methylene blue (22, 23). This is because vitamin C restores ferrous (Fe^{2+}) iron levels to normal, thus preventing further hypoxia (24, 25).

Although the benefit of vitamin C is subject to ongoing controversy, a systematic review of 74 studies in critically ill adult patients reported that IV high-dose vitamin C therapy has an adverse event profile similar to placebo (26). However, it also suggested caution in patients at risk of oxalate nephropathy, in those with hypernatremia and in those with glucose-6-phosphate dehydrogenase deficiency. As trials are now being designed to investigate high-dose vitamin C in critically ill children, better data on the efficacy, safety profile, and potential for harm of high-dose vitamin C in children are required (27). Accordingly, we aimed to systematically review all published literature providing information on the efficacy and harm related to high-dose vitamin C therapy in neonatal and pediatric patients.

METHOD

Study Design

We conducted a scoping review (SR), according to the guidelines from the Cochrane Collaboration and Centre for Reviews and Dissemination, and reported the results following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines and their extension for SRs (28, 29).

Search Strategy

The search strategy was developed with a librarian at the Austin hospital, Melbourne, VIC, Australia. We searched all relevant studies available from MEDLINE (Ovid), EMBASE (Ovid), and Cochrane Library published by June 2020. For ongoing trials, we searched National Institute of Health Clinical Trials Register (https://clinicaltrials.gov/). Detailed search syntax used in this study is shown in **Supplementary material 1** (Supplemental Digital Content 1, http://links.lww.com/PCC/B699).

Definition of Efficacy

In this SR, we reported the primary outcome of each studies as main outcome. If the original authors did not mention a primary outcome, reviewers summarized the results.

Definition of Harm

In this SR, harm was defined as any unfavorable outcome judged by the original authors with key words related to harm, such as adverse events, side effects, complications, safety, or tolerability.

Inclusion and Exclusion Criteria

We included all relevant randomized controlled trials (RCTs), non-RCTs, observational studies, case series, or case reports of treatment with IV or intra-arterial high-dose vitamin C in neonates and/or pediatric patients.

We included published full text articles, conference abstracts, and unpublished data in clinical trial registries. We included studies for both neonates and pediatric patients (newborn to < 18 yr old).

Studies that included a minority (< 10%) of patients 18 years old or more, or studies with median or mean of age of patients less than 18 were also included. There is no agreed definition of high-dose vitamin C. For the purpose of this study, we therefore defined high dose as 75 mg/kg/d parenteral vitamin C by extrapolating such value from the 6 g/d regimen used in adult sepsis patients, which equals to 75 mg/kg/d in a 80-kg patient (5–10, 26).

When vitamin C was given as fixed dose, such as 2 g/d, the dose was converted to mg/kg/d using the patient's body weight where feasible or mean or median body weight for other studies where individual data were not available. When actual body weight was not reported, we used the 50th percentile male body weight for the patient's age or the patients' mean or median ages, respectively (30).

We excluded reports of oral or enteral vitamin C was or reports where dose or route were not reported. We also excluded articles on neonates where vitamin C had been administered to pregnant mothers prior to delivery.

Data Collection

A title and abstract screening process was conducted independently and in duplicate by the review authors (F.Y., S.R., T.N., A.M., M.C., L.J.S.). We classified all of extracted data as "eligible for full text screening" (unclear, potentially eligible or eligible) or "not eligible for full text screening" (definitely ineligible). We downloaded full texts of "eligible for full text screening" and assessed the eligibility of the studies. We also recorded reasons for exclusion of the excluded studies. Any disagreement was solved by discussion, or, if required, we consulted a third person (R.B.).

We extracted the name of the author, publication year, study design, funding source of the study, characteristics of the included patient, total number of patients included in the study, number of patients in each group, age of patients, body weight of patients, total dose of vitamin C administered, vitamin C treatment protocol, other interventions in addition to IV or intra-arterial vitamin C in each group, main clinical outcomes in each group, reported harm, and the number of each harmful event. For adverse events, we only reported vitamin C–related adverse events.

RESULTS

From the database search, we retrieved 1,360 titles leading to the selection of 193 articles, which were included in the full text screening process (**Fig. 1**). Twelve original studies met the inclusion criteria (16, 24, 31–40) (**Table 1**). Of these, six studies reported both efficacy and harm related to high-dose vitamin C (24, 31–35), and the other six original investigations reported treatment with high-dose IV vitamin C but did not comment on harm (16, 36–40) (**Table 2**).

The 12 studies included 855 patients. Of these, 194 received high-dose vitamin C, and 661 patients received placebo or usual care (Table 1). The main conditions investigated were hypoxic ischemic encephalopathy in neonates, preterm birth, and viral encephalitis or Kawasaki disease (31–33, 37, 38). There were also three studies that reported on high-dose vitamin C for the treatment of methemoglobinemia (24, 35, 36).

The age of patients who received high-dose vitamin C ranged from within 2 hours after delivery to 8.4 years old (median 2.4 yr) (Table 2). Four articles were double-blind RCTs, and one was an open-label RCT (31–33, 37, 38). The four double-blind RCTs included a total of 189 patients, of whom 93 were treated with high-dose vitamin C.

The vitamin C doses administered in these studies ranged from 100 to 1,500 mg/kg/d (median 260.5 mg/kg/d) (Table 2). There was no report of harm related to IV or intra-arterial high-dose vitamin C (Table 2). In comparison, one open-label RCT paper reported nine cases of transient flushing in the control group (33).

The efficacy outcomes are shown in Table 2. The four double-blind RCTs showed no clinical outcome differences in favor or against high-dose vitamin C group. In term infants with hypoxic ischemic encephalopathy, the neurologic outcome and a mortality at discharge were the same between the placebo and the high-dose vitamin C group (100 mg/kg/d) (31). In premature infants, intra-arterial vitamin C (100 mg/kg/d) delivered the same creatinine value between the two groups (32). Furthermore, two studies in patients with Kawasaki disease reported a significant increase of the percentage change in diameter of the brachial artery induced by reactive hyperemia in the high-dose vitamin C group (37, 38). In addition, three studies in patients with methemoglobinemia reported successful recoveries in all patients (24, 35, 36). In one study, methemoglobin level decreased from 33.4% to 3.5% after 24 hours of high-dose vitamin C therapy (24). Finally, a propensity-matched retrospective study reported a 90-day mortality and identified a decreased 90-day mortality among pediatric patients with septic shock in the high-dose vitamin group (15/43 [35%] in the control group, 16/43 [37%] in the hydrocortisone only group, and 6/43 [14%] in the HAT group after propensity matching) (16).

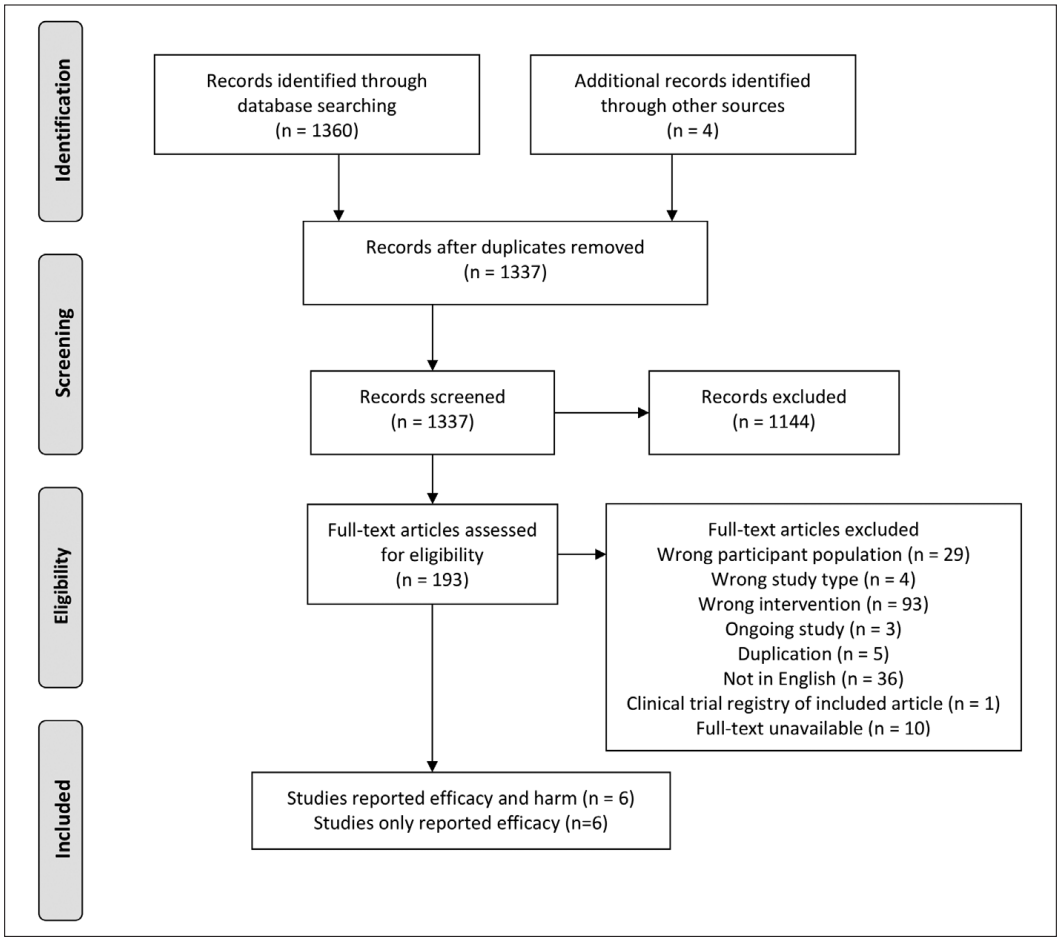

Figure 1. Flow chart of the study screening.

DISCUSSION

Key Findings

In this SR, we studied the efficacy and safety of IV or intra-arterial high-dose vitamin C in neonatal and pediatric patients. We identified 12 studies and a total of 194 patients treated with high-dose vitamin C. The studies assessed a broad range of patients, diseases, study designs, and outcomes. With regard to harm, there was no report of adverse events or complications or side effects related to high-dose vitamin C. With regard to benefit, the studies did not consistently assess efficacy and used different outcomes such as neurologic improvement, changes in serum creatinine, resolution of methemoglobinemia, changes in vascular responsiveness, and mortality. Unfortunately, such heterogeneity precluded any meta-analysis or conclusions about efficacy.

Relationship to Previous Studies

In the past few years, a rapidly increasing number of studies, including high-quality RCTs, have been published in critically ill adult patients investigating the potential benefit of high-dose IV vitamin C (6). The issue of whether high-dose vitamin C has efficacy in the variety of conditions treated with it in adult patients

TABLE 1.
Characteristics of Included Studies

References	Design	Target Condition	Total Number	Vitamin C (mg/kg/d)	Other Intervention	Industry Funding
Aly et al (31)	DB-RCT	Term infants with hypoxic ischemic encephalopathy	60	Placebo	Placebo	N/R
				100	Oral ibuprofen	
Bass et al (32)	DB-RCT	Premature infants	51	Placebo	Standard care	N/R
				100	Standard care	
Boran et al (36)	Case report	Methemoglobinemia	1	300	Standard care	N/R
Deng et al (37)	DB-RCT	Kawasaki disease 1 yr previously	39	Placebo	None	Nonindustrial funding
				130[a]	None	
Deng et al (38)	DB-RCT	Kawasaki disease 1 yr previously	39	Placebo	None	Nonindustrial funding
				130[a]	None	
Jiao et al (33)	Open-label randomized controlled trial	Children with viral encephalitis	99	Nil[b]	Ligustrazini hydrochlorioi (Chinese herb), standard encephalitis treatment	None [c]
				105–158[a]	Coenzyme A, adenosine triphosphate, standard encephalitis treatment	
Mikirova et al (34)	Case report	Neurofibromatosis type 1 and optic pathway glioma	1	385–824[b]	Chemotherapy	N/R
Ried and Fakler (39)	Case report	Juvenile arthritis	1	750–1500[a]	vitamin D, B-vitamins, zinc, magnesium, glutathione	None [c]
Rino et al (24)	Case series	Methemoglobinemia	5	129–606[a]	Oral vitamin C 300–600 mg/d	N/R
Solis-Nolasco et al (40)	Case report	Glioma	1	933[a]	IV chemotherapy and endolaser therapy	None [c]
Uslu and Comert (35)	Case report	Methemoglobinemia	1	300	Standard care	N/R
Wald et al (16)	Retrospective observational	Septic shock	557	Nil[b]	Standard sepsis care	N/R
				Nil[b]	Hydrocortisone, standard sepsis care	
				120	Hydrocortisone, thiamine, standard sepsis care	

DB-RCT = double-blind randomized controlled trial, N/R = not reported.
[a]When vitamin C was given as g/d, dose was divided by reported body weight or predicted body weight.
[b]Did not receive any dose of vitamin C or placebo.
[c]Did not receive any funding.

TABLE 2.
Reported Clinical Outcomes and Harms in Included Studies

References	No. in Group	Age	Body Weight	Vitamin C (mg/kg/d)	Infusion Time	Main Outcomes	Reported Harm (No. of Events)
Aly et al (31)	30	Gestational age 38 ± 1.1 wk, within 2 hr after delivery	2.9 ± 0.4 kg	Placebo	Every 24 hr	DDST II at 6 mo normal 40%, mortality at discharge 33%	None
	30	Gestational age 38.3 ± 1.2 wk, within 2 hr after delivery	2.8 ± 0.3 kg	100	100 mg/kg every 24 hr for 3 d	DDST II at 6 mo normal 47%, mortality at discharge 37%	None
Bass et al (32)	26	Gestational age 27.0 ± 1.9 wk	946 ± 261 g	Placebo	Every 24 hr, given intra-arterial for 7 d	Maximum creatinine in the first month 1.2 ± 0.28	None
	25	Gestational age 27.1 ± 2.0 wk	951 ± 260 g	100	100 mg/kg/d every 24 hourly, given intra-arterial for 7 d	Maximum creatinine in the first month 1.1 ± 0.33	None
Boran et al (36)	1	42 d	4.8[c] kg	300	Single dose	Resolution of methemoglobinemia	N/R
Deng et al (37)	20	7.1 ± 2.7[a] yr	23.0[c] kg	Placebo	Single dose over 10 min	No significant increase of the percent change in diameter of brachial artery induced by reactive hyperemia	N/R
	19			130 [d]	Single dose over 10 min	Significant increase of the percent change in diameter of brachial artery induced by reactive hyperemia	N/R
Deng et al (38)	20	7.1 ± 2.7[a] yr	23.0[c] kg	Placebo	Single dose over 10 min	No significant increase of the percent change in diameter of brachial artery induced by reactive hyperemia	N/R
	19			130[d]	Single dose over 10 min	Significant increase of the percent change in diameter of brachial artery induced by reactive hyperemia	N/R

(*Continued*)

TABLE 2. (*Continued*).
Reported Clinical Outcomes and Harms in Included Studies

References	No. in Group	Age	Body Weight	Vitamin C (mg/kg/d)	Infusion Time	Main Outcomes	Reported Harm (No. of Events)
Jiao et al (33)	51	5.98 ± 8.21 yr	20.5[c] kg	Nil[e]	–	Very effective response 39 (76.47%)[f]	Transient flushing (9)
	48	5.38 ± 7.33 yr	19.0[c] kg	105–158[d]	2–3 g every 24 hr for 7 d	Very effective response 21 (43.75%)[f]	None
Mikirova et al (34)	1	5 yr	18.2[c] kg	385–824[d]	7–15 g/d wk for 100 times	Optic glioma shrunk	None
Ried and Fakler (39)	1	9 yr	20 kg	750–1,500[d]	15–30 g/d mo for 1 yr	Improved activity of daily living	N/R
Rino et al (24)	1	46 mo	15.52 kg	129[d]	0.5 g every 6 hr for 4 d	Recovery from methemoglobinemia	None
	1	2 mo	4.4 kg	341[d]	0.5 g every 8 hr for 4 d	Recovery from methemoglobinemia	None
	1	12 mo	9.05 kg	221[d]	0.5 g every 6 hr for 4 d	Recovery from methemoglobinemia	None
	1	1 mo	3.3 kg	606[d]	0.5 g every 6 hr for 4 d	Recovery from methemoglobinemia	None
	1	1 mo	3.25 kg	308[d]	0.33 g every 8 hr for 4 d	Recovery from methemoglobinemia	None
Solis-Nolasco et al (40)	1	5 yr	26.8 kg (59 pounds)	933[d]	25 g/d twice a week for 18 treatments	Reduction in tumor size	N/R
Uslu and Comert (35)	1	Full term, 6 hr after delivery	3.3 kg	300	300 mg/kg/d single dose	Recovery from methemoglobinemia	None
Wald et al (16)	333	10.1 (3–15)[b] yr	32.0[c] kg	Nil[e]	–	90 d mortality, 30/333 (9%), 15/43 (35%)[g]	N/R
	181	10.5 (4–15)[b] yr	32.2[c] kg	Nil[e]	–	90 d mortality, 39/188 (22%), 16/43 (37%)[g]	N/R
	43	8.4 (4–14)[b] yr	27.0[c] kg	120	30 mg/kg every 6 hr for 4 d	90 d mortality, 6/43 (14%), 6/43 (14%)[g]	N/R

DDST II = Denver developmental screening test II, N/R = not reported.
[a]Mean ± SD of the two groups was reported in the original article
[b]Median (interquartile range).
[c]Predicted 50th percentile male body weight from the age. When age was reported as mean ± SD or median (interquartile range), we reported body weight for mean age or median age.
[d]When vitamin C was given as g/d, dose was divided by reported body weight or predicted body weight.
[e]Did not receive any dose of vitamin C or placebo.
[f]The patients did not have any more convulsions or coma after 3–7 d, electroencephalogram and/or cerebrospinal fluid was completely normal on discharge.
[g]Unmatched mortality and mortality after propensity matching.
Dashes denote that the patients in the group did not receive placebo or vitamin C.

remains unclear and, in septic adult patients, highly controversial (6). To date, adverse events related to high-dose vitamin C in adults have been exceedingly rare. In a previous SR, 74 studies in adults were identified. These studies reported on a total of 2,801 participants who received a median dose of vitamin C of 455 mg/kg/d (interquartile range 260–925 mg/kg/d) (26). This systematic review found no evidence that high-dose vitamin C therapy was more harmful than placebo, including evidence from nine double-blind RCTs. In the adult population, however, the following rare or unique adverse events likely related to high-dose vitamin C (prevalence < 1/500) were reported: oxalate nephropathy, false glucometer readings incorrectly suggesting hyperglycemia, and hemolysis in glucose-6-phosphate dehydrogenase deficiency patients (26).

Our findings on the available literature of high-dose vitamin C therapy in neonatal and pediatric patients are aligned with those of the systematic review in adults (26). In particular, two double-blinded RCTs and one open-label RCT also reported no difference in adverse events between high-dose vitamin C and either placebo or control. Of note, the dose range reported in the pediatric studies (100–1,500 mg/kg/d) was comparable with the range reported in adult studies. The studies included in the present review included patients across the entire range of pediatric age groups, from very preterm infants enrolled within hours after birth to adolescents. Although our review represents the most comprehensive available evidence on vitamin C in children, the small sample size of the included studies warrants caution when interpreting the results. In particular, as we could only identify 194 pediatric patients who received high-dose vitamin C, the combined sample size may not have sufficient sensitivity to detect uncommon adverse events in children.

Implications of Study Findings

Our findings support the observations from adult studies, including high-quality RCTs in critically ill patients, that high-dose vitamin C is probably safe (26). Extrapolation of findings from adult studies to neonatal and pediatric age groups represents a common occurrence in pediatric medicine (41). The available high-quality data were based on only 194 neonates and children and thus do not allow to exclude that adverse events in children may occur at a higher rate compared with adults. However, within the limitations of such numbers, they imply that, if such adverse events do occur, they are likely uncommon. Finally, the lack of any unfavorable clinical outcomes in recent double-blind RCTs implies an additional degree of safety (31, 32, 37, 38). Similar to the adult population, however, our findings also do not provide robust evidence of efficacy in any of the conditions where high-dose vitamin C has been used in children so far. Of note, the design of optimal dosing for interventional drug trials in children faces unique challenges and often lags years behind adult trials, which may hinder the timely translation of potentially promising interventions in a highly vulnerable group (42, 43).

Strength and Limitations

To our knowledge, this is the first SR to report on the efficacy and possible harm related to parenteral high-dose vitamin C in neonates and children. High-dose vitamin C has been studied as metabolic resuscitation for adult septic shock with inconsistent results, and its efficacy for pediatric septic shock remains in under investigation (17). Our SR provides the most comprehensive report on its safety in children to date.

There are several limitations to our study. First, although we conducted a systematic search based on broad search criteria, only 12 articles reporting on 194 patients met eligibility criteria. Such limited numbers expose this study to a high chance of type II error in relation to both efficacy and harm; however, the findings are broadly consistent with the results of high-dose vitamin C studies in adults (26). Second, we included only English articles and excluded 36 non-English articles. However, excluding non-English studies does not appear to lead to systematic bias (44). Third, we arbitrarily defined high-dose vitamin C as greater than or equal to 75 mg/kg/d, which was the same dose assessed in an adult review. This threshold may have excluded studies reporting on adverse events in children treated with lower doses of parenteral vitamin C. However, this approach reflects current practice in adults with critical illness and is therefore relevant to current practice (16, 17). Fourth, no study specifically targeted patients in PICUs. Thus, our results provide no specific information on this treatment in critically ill children. However, our results provide the best available evidence of efficacy and harm for high-dose vitamin C in pediatric patients, an area of rapidly growing interest and forthcoming trials, and an area

of likely parental or family enquiry in the future. Fifth, because there was no information on kidney biopsies or autopsies, the included studies could not diagnose oxalate nephropathy, leading to additional uncertainty about this specific adverse effect. In this regard, in the adult high-dose vitamin C studies, all reported cases of oxalate nephropathy were diagnosed by kidney biopsies or autopsy (45–48). However, the mortality of pediatric patients is low and renal biopsies and autopsies rare (49). Finally, we did not assess the quality of the included studies other than describing the study design and could not perform a meta-analysis because of study and outcome heterogeneity.

CONCLUSIONS

In this SR, we assessed the literature on high-dose vitamin C in children. We found 12 studies reporting on 194 children ranging from preterm babies to adolescents. There was no ability to assess efficacy due to trial design and heterogeneity of outcomes and disease. However, there were no reported harmful effects related to parenteral high-dose vitamin C therapy or inferior clinical outcomes in the high-dose vitamin C group in double-blind RCTs. These findings, despite their clear limitations, both in isolation and in the context of evidence generated in adult patients, support a degree of safety for parenteral high-dose vitamin C. They also argue for the design and conduct of high-quality trials of parenteral high-dose vitamin C in critically ill children to systematically assess the role for high-dose vitamin C treatment in the PICU setting.

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1 *Department of Intensive Care, Austin Hospital, Heidelberg, VIC, Australia.*

2 *Australian and New Zealand Intensive Care Research Centre, Monash University, Melbourne, VIC, Australia.*

3 *Child Health Research Centre, The University of Queensland, Brisbane, QLD, Australia.*

4 *Pediatric Intensive Care Unit, Queensland Children's Hospital, South Brisbane, QLD, Australia.*

5 *Department of Intensive Care, Siriraj Hospital, Mahidol University, Bangkok, Thailand.*

6 *Department of Anaesthesia, Northern Health, Epping, VIC, Australia.*

7 *Department of Pharmacy, Queensland Children's Hospital, South Brisbane, QLD, Australia*

8 *Department of Intensive Care Medicine and Neonatology, and Children's Research Center, University Children's Hospital Zurich, University of Zurich, Zurich, Switzerland.*

9 *Department of Medicine and Radiology, Centre for Integrated Critical Care, University of Melbourne, Melbourne, VIC, Australia.*

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For information regarding this article, E-mail: Rinaldo.bellomo@austin.org.au

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Supplementary material 1. Detailed search strategies.

Database: Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Daily and Versions(R) <1946 to June 5, 2020>

Search Strategy:
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1 Ascorbic Acid/ (41096)
2 (ascor* or l-ascor* or acidylina* or adenex* or afj c or agrumina* or alle?corb* or antiscorb* or arcavit* c or arkovital* c or ascelat* or ascofar* or ascomed* or asconvita* or askorbin* or austrovit* c or bentavit* c or c crivit* or c ine or c level or c lisa or c long or c monovit* or c prana or c rivitin* or c sol or c tamin* or c tonic or c tron or c vescent or c vicotrat* or c?vimin* or c vit* or c-will or cantan or cantaxin or catavin c or ce arom or ce major or ce quin* or ce?vi?sol or ce vit* or cebetate or cebicure or cebion* or cecap or cecon* or cecorb* or cecrisina or cedon* or cedoxon* or cee-500 or ceevifil or cegiolan or celaskon* or celin* or cenetone or cenol* or cequinyl or cereon or cergona or cescorbat or cetami* or cetebe or ceterapion* or cetrinets or ceva?in* or cevex or cevibram or cevig* or cevilat or cevimin* or cevisol or cevit* or cewin or chewcee or chivibit c or ci drol or ciamin* or ciergin or cifilina or cipca or cisir or cital or citamino or cith or citoascorbina or citoxyl or citran or citravite or citritabs or citrovitamina or civigor or civitin* or co biagini or concemin or cortalex).mp. (68553)
3 (xylo?ascorb* or dagrascorb* or dagravit c or dancimin c or davitamon c or dayvital or delo c or difvitamin c or dumovit* c or erftamin* c or esuron or esurvit* or flavettes or godabion c or gregovite c or hicee or hybrin* or ido c or ikacee or inovitan c or irocevit or irocevite or jarexin* or keto hexuronic acid lactone or lacivit* or laroscorbine or leder?c or lemascorb or limcee or limo ce or liqui cee or magnorbin* or mega-c?a or myascorbin or natrascorb or nybadol or paa 500 or parkovit c or pharmascorb* or pharmatovit* c or planavit* c or plivit* c or pro-c or proscorbin* or redoxon or ribena or scorbacid* or scorbettes or scorbex or scorbin c or scorbitol or scorbumine or scottavit* c or secorbate or sevalin or sigmavit* c or sodascorb* or sweetcee or synum c or take-c or tanvimil-c or testascorb* or ucemine c or upsa?c or vi?ci sin or vi dom c or vi-c 500 or vicef or vicelat or vicetrin or vici monico or viciman or vicin or vicitina or vicon or viforcit* or viscorin* or vita-cedol orange or vitac or vitace or vitacee or vitaci* or vitamin* C or vitaplex c or vitapric or vitapur c or vitasan c or vitascorb* or vitelix c or vitocee or vorange or wandervit* c or witamina c or xitix or xon-ce).mp. (24927)
4 or/1-3 (77268)
5 Administration, Intravenous/ (7644)
6 infusions, parenteral/ or infusions, intravenous/ (79210)
7 (intravenous* or i?v? or infus* or inject* or intraport* or intravascul* or parenteral* or vein or venous*).mp. (2074773)
8 or/5-7 (2074773)
9 4 and 8 (6591)
10 exp animals/ not humans.sh. (4589476)
11 9 not 10 (3895)
12 limit to children (468)

Database: Embase Classic+Embase <1947 to June 5, 2020>
Search Strategy:
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1 Ascorbic Acid/ (98411)
2 (ascor* or l-ascor* or acidylina* or adenex* or afj c or agrumina* or alle?corb* or antiscorb* or arcavit* c or arkovital* c or ascelat* or ascofar* or ascomed* or asconvita* or askorbin* or austrovit* c or bentavit* c or c crivit* or c ine or c level or c lisa or c long or c monovit* or c prana or c rivitin* or c sol or c tamin* or c tonic or c tron or c vescent or c vicotrat* or c?vimin* or c vit* or c-will or cantan or cantaxin or catavin c or ce arom or ce major or ce quin* or ce?vi?sol or ce vit* or cebetate or cebicure or cebion* or cecap or cecon* or cecorb* or cecrisina or cedon* or cedoxon* or cee-500 or ceevifil or cegiolan or celaskon* or celin* or cenetone or cenol* or cequinyl or cereon or cergona or cescorbat or cetami* or cetebe or ceterapion* or cetrinets or ceva?in* or cevex or cevibram or cevig* or cevilat or cevimin* or cevisol or cevit* or cewin or chewcee or chivibit c or ci drol or ciamin* or ciergin or cifilina or cipca or cisir or cital or citamino or cith or citoascorbina or citoxyl or citran or citravite or citritabs or citrovitamina or civigor or civitin* or co biagini or concemin or cortalex).mp. (122362)

3 (xylo?ascorb* or dagrascorb* or dagravit c or dancimin c or davitamon c or dayvital or delo c or difvitamin c or dumovit* c or erftamin* c or esuron or esurvit* or flavettes or godabion c or gregovite c or hicee or hybrin* or ido c or ikacee or inovitan c or irocevit or irocevite or jarexin* or keto hexuronic acid lactone or lacivit* or laroscorbine or leder?c or lemascorb or limcee or limo ce or liqui cee or magnorbin* or mega-c?a or myascorbin or natrascorb or nybadol or paa 500 or parkovit c or pharmascorb* or pharmatovit* c or planavit* c or plivit* c or pro-c or proscorbin* or redoxon or ribena or scorbacid* or scorbettes or scorbex or scorbin c or scorbitol or scorbumine or scottavit* c or secorbate or sevalin or sigmavit* c or sodascorb* or sweetcee or synum c or take-c or tanvimil-c or testascorb* or ucemine c or upsa?c or vi?ci sin or vi dom c or vi-c 500 or vicef or vicelat or vicetrin or vici monico or viciman or vicin or vicitina or vicon or viforcit* or viscorin* or vita-cedol orange or vitac or vitace or vitacee or vitaci* or vitamin* C or vitaplex c or vitapric or vitapur c or vitasan c or vitascorb* or vitelix c or vitocee or vorange or wandervit* c or witamina c or xitix or xon-ce).mp. (32822)
4 or/1-3 (127933)
5 Administration, Intravenous/ (382730)
6 infusions, parenteral/ or infusions, intravenous/ (382325)
7 (intravenous* or i?v? or infus* or inject* or intraport* or intravascul* or parenteral* or vein or venous*).mp. (3527984)
8 or/5-7 (3527984)
9 exp animals/ not humans.sh. (26353065)
10 4 and 8 (14630)
11 10 not 9 (2842)
12 limit to children (272)

Database: EBM Reviews - Cochrane Database of Systematic Reviews <2005 to June 14, 2019>, EBM Reviews - ACP Journal Club <1991 to May 2019>, EBM Reviews - Database of Abstracts of Reviews of Effects <1st Quarter 2016>, EBM Reviews - Cochrane Clinical Answers <May 2019>, EBM Reviews - Cochrane Central Register of Controlled Trials <May 2019>, EBM Reviews - Cochrane Methodology Register <3rd Quarter 2012>, EBM Reviews - Health Technology Assessment <4th Quarter 2016>, EBM Reviews - NHS Economic Evaluation Database <1st Quarter 2016>
Search Strategy:
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1 Ascorbic Acid/ (2020)
2 (ascor* or l-ascor* or acidylina* or adenex* or afj c or agrumina* or alle?corb* or antiscorb* or arcavit* c or arkovital* c or ascelat* or ascofar* or ascomed* or asconvita* or askorbin* or austrovit* c or bentavit* c or c crivit* or c ine or c level or c lisa or c long or c monovit* or c prana or c rivitin* or c sol or c tamin* or c tonic or c tron or c vescent or c vicotrat* or c?vimin* or c vit* or c-will or cantan or cantaxin or catavin c or ce arom or ce major or ce quin* or ce?vi?sol or ce vit* or cebetate or cebicure or cebion* or cecap or cecon* or cecorb* or cecrisina or cedon* or cedoxon* or cee-500 or ceevifil or cegiolan or celaskon* or celin* or cenetone or cenol* or cequinyl or cereon or cergona or cescorbat or cetami* or cetebe or ceterapion* or cetrinets or ceva?in* or cevex or cevibram or cevig* or cevilat or cevimin* or cevisol or cevit* or cewin or chewcee or chivibit c or ci drol or ciamin* or ciergin or cifilina or cipca or cisir or cital or citamino or cith or citoascorbina or citoxyl or citran or citravite or citritabs or citrovitamina or civigor or civitin* or co biagini or concemin or cortalex).mp. (5438)
3 (xylo?ascorb* or dagrascorb* or dagravit c or dancimin c or davitamon c or dayvital or delo c or difvitamin c or dumovit* c or erftamin* c or esuron or esurvit* or flavettes or godabion c or gregovite c or hicee or hybrin* or ido c or ikacee or inovitan c or irocevit or irocevite or jarexin* or keto hexuronic acid lactone or lacivit* or laroscorbine or leder?c or lemascorb or limcee or limo ce or liqui cee or magnorbin* or mega-c?a or myascorbin or natrascorb or nybadol or paa 500 or parkovit c or pharmascorb* or pharmatovit* c or planavit* c or plivit* c or pro-c or proscorbin* or redoxon or ribena or scorbacid* or scorbettes or scorbex or scorbin c or scorbitol or scorbumine or scottavit* c or secorbate or sevalin or sigmavit* c or sodascorb* or sweetcee or synum c or take-c or tanvimil-c or testascorb* or ucemine c or upsa?c or vi?ci sin or vi dom c or vi-c 500 or vicef or vicelat or vicetrin or vici monico or viciman or vicin or vicitina or vicon or viforcit* or viscorin* or vita-cedol orange or vitac or vitace or vitacee or vitaci* or vitamin* C or vitaplex c or vitapric or vitapur c or vitasan c or vitascorb* or vitelix c or vitocee or vorange or wandervit* c or witamina c or xitix or xon-ce).mp. (3773)
4 or/1-3 (7089)
5 Administration, Intravenous/ (937)

6 infusions, parenteral/ or infusions, intravenous/ (11538)
7 (intravenous* or i?v? or infus* or inject* or intraport* or intravascul* or parenteral* or vein or venous*).mp. (280728)
8 or/5-7 (280728)
9 4 and 8 (1340)
10 exp animals/ not humans.sh. (44)
12 9 not 10 (1340)
13 exp adolescent/ or exp child/ or exp infant/ or (infant disease* or childhood disease*).ti,ab,kf. or (adolescen* or babies or baby or boy? or boyfriend or boyhood or child* or girl? or infant* or juvenil* or kid? or minors or minors* or neonat* or neonat* or newborn* or new-born* or paediatric* or peadiatric* or pediatric* or perinat* or preschool* or puber* or pubescen* or school* or teen* or toddler? or underage? or under-age? or youth*).ti,ab,kf. (565024)
14 11 and 12 (461)

Trial Registry: ClinicalTrials.gov

https://clinicaltrials.gov/
Date searched: June 5, 2020
Advanced Search >
Study Type: All Studies
Study Results: All Studies
Interventions: Vitamin C OR ascorbic acid OR ascorbate
Applied Filters: Child (Birth-17) (159)

Chapter 8: Scoping Review of glycocalyx biomarkers

Biomarkers

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ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/ibmk20

Glycocalyx damage biomarkers in healthy controls, abdominal surgery, and sepsis: a scoping review

Fumitaka Yanase , Thummaporn Naorungroj & Rinaldo Bellomo

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To link to this article: https://doi.org/10.1080/1354750X.2020.1787518

BIOMARKERS
2020, VOL. 25, NO. 6, 425–435
https://doi.org/10.1080/1354750X.2020.1787518

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REVIEW

Glycocalyx damage biomarkers in healthy controls, abdominal surgery, and sepsis: a scoping review

Fumitaka Yanase[a,b], Thummaporn Naorungroj[a,c] and Rinaldo Bellomo[a,b,d]

[a]Department of Intensive Care, Austin Hospital, Melbourne, Australia; [b]Australian and New Zealand Intensive Care Research Centre, Monash University School of Public Health and Preventive Medicine, Melbourne, Australia; [c]Department of Intensive Care, Siriraj Hospital, Mahidol University, Bangkok, Thailand; [d]Centre for Integrated Critical Care, Department of Medicine and Radiology, University of Melbourne, Melbourne, Australia

ABSTRACT

Objective: Despite wide interest in glycocalyx biomarkers, their values in healthy individuals, patients after abdominal surgery, and septic patients have been poorly understood.

Methods: We searched MEDLINE, CENTRAL and EMBASE for papers measured glycocalyx biomarkers in healthy individuals, patients after abdominal surgery and septic patients.

Results: We extracted 3948 titles and identified 58 eligible papers. Syndecan 1 was the most frequently measured biomarker (48 studies). Its mean or median value in healthy individuals varied to a biologically implausible degree, from 0.3 to 58.5 ng/ml, according to assay manufacturer. In post-operative patients, syndecan 1 levels increased after pancreatic surgery or liver surgery, however, they showed minor changes after hysterectomy or laparoscopic surgery. In septic patients, biomarker levels were higher than in healthy volunteers when using the same assay. However, six healthy volunteer studies reported higher syndecan 1 values than after pancreatic surgery and 24 healthy volunteer studies reported higher syndecan 1 values than the lowest syndecan 1 value in sepsis. Similar findings applied to other glycocalyx biomarkers.

Conclusion: Glycocalyx damage biomarkers values are essentially defined by syndecan 1. Syndecan 1 levels, however, are markedly affected by assay type and show biologically implausible values in normal, post-operative, or septic subjects.

ARTICLE HISTORY
Received 3 January 2020
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KEYWORDS
Glycocalyx; syndecan 1; healthy volunteer; abdominal surgery; sepsis

Introduction

The glycocalyx is a gel-like latticework that covers the internal wall of vascular endothelial cells. It is composed of glycosaminoglycans, glycoproteins, proteoglycans and adherent plasma proteins (Weinbaum et al. 2007). It is currently considered an important structure that helps maintain endothelial homeostasis by regulating vascular tone, initiating nitric oxide vasodilation, modulating vascular permeability by limiting fluid movement, regulating platelet and leukocyte aggregation, and adhesion to endothelial cells (Ince et al. 2016, Uchimido et al. 2019). When the glycocalyx is damaged, these functions are compromised, and hypotension, edema or microemboli may be increased (Pries et al. 2000, Colbert et al. 2016).

In healthy individuals, the thickness of the glycocalyx is estimated to be between 0.5 to 5.0 μm (Klitzman et al. 1979, Pries et al. 2000, Schmidt et al. 2012). When the glycocalyx is damaged, its components (syndecan, hyaluronan, heparan sulfate and chondroitin sulfate) break down, are released, and their levels in blood can be expected to increase. Unfortunately, there is no gold standard to detect the presence and degree of glycocalyx damage in humans. However, the above biomarkers are frequently measured and used to report and quantify probable glycocalyx damage in the clinical setting (Uchimido et al. 2019). Despite such widespread use, to the best of our knowledge, there has not been any scoping review of glycocalyx biomarkers in key populations. Thus, we do not know the normal value of glycocalyx markers among healthy individuals, the glycocalyx biomarker value before and after abdominal surgery and the value in septic patients. This makes assessment of future and current investigations in this field difficult to interpret.

Accordingly, we conducted a scoping review to identify studies of glycocalyx damage biomarker levels in healthy individuals to establish a 'normal reference range' and to assess changes in such marker among patients undergoing abdominal surgery or experiencing sepsis. Our primary aim was to test the hypothesis that a normal range for such biomarkers could be defined. Our secondary aim was to test the hypothesis that the magnitude of response of such biomarkers to surgery or sepsis could be defined and compared with normal values.

CONTACT Rinaldo Bellomo rinaldo.bellomo@austin.org.au Intensive Care Research, Austin Health, 145 Studley Road, Heidelberg, Victoria, 3084, Australia
Supplemental data for this article can be accessed here.

Clinical significance

- Syndecan 1 is by far the most frequently measured glycocalyx biomarkers among healthy volunteers, patients with abdominal surgery, and patients with sepsis.
- Syndecan 1 appears to increase according to the disease severity when measured by the same assay.
- When measured by different assay, its levels differ markedly and show biologically implausible variability (195-fold) in absolute values and a high degree of overlap among surgical and septic patients and normal volunteers.
- Similar findings apply to other glycocalyx biomarkers. Our study calls into question the accuracy of our current quantitative understating of glycocalyx biomarkers.

Material and methods

Study design

We performed a scoping review using the PRISMA extension method for Scoping Reviews (Tricco et al. 2018).

Registration and search strategy

The search strategy was developed with the assistance with clinical librarian in Austin hospital. We searched the Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE (Ovid) and Embase (Ovid) on 5th March 2020 using a list of keywords, medical subject heading (MeSH) terms, truncations and boolean operators. Detailed search syntax included is in Supplementary material 1. Authors' personal papers were also added for screening. All of the searched articles were screened for eligibility.

Inclusion and exclusion criteria

We included randomized controlled trials, prospective cohort studies and retrospective cohort studies that focussed on the value of glycocalyx markers (syndecan, heparan sulfate, hyaluronic acid and chondroitin sulfate) in healthy individuals, in patients who underwent abdominal surgery and in patients with sepsis. We only included English language papers (Morrison et al. 2012). The definition of healthy individuals was that of subjects without any medication or comorbidities. The definition of major abdominal surgery included any type of abdominal surgery (open surgery, laparoscopic surgery and transplant surgery of intra-abdominal organ). The definition of sepsis was that of sepsis as reported by the studies themselves focussing on any such cohort (Bone et al. 1992, Levy et al. 2003, Singer et al. 2016).

We excluded reviews, letters, commentaries, correspondence, case reports, conference abstracts, expert opinions or editorials. We also excluded animal study, *in vitro* or *ex vivo* studies, and studies that used other techniques (side stream dark field [SDF] imaging or measuring glycocalyx volumes) to evaluate glycocalyx damage.

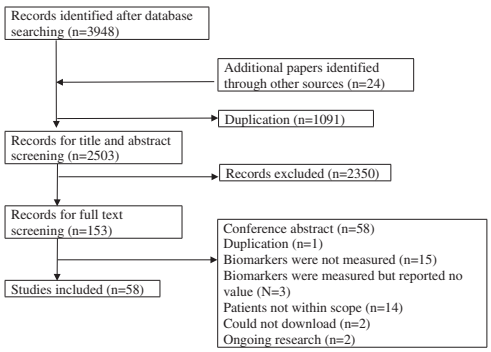

Figure 1. Flow diagram of the selection process for published reports.

Data collection

Two reviewers (FY and TN) independently assessed title and abstract for potential relevance. The full text of potentially relevant articles were assessed by the two reviewers. We resolved any disagreement through discussion, or, if required, we consulted a third reviewer (RB). For each study, we extracted year of publication, number of participants, participants' age, proportion of male, participants' body mass index (BMI), participants' body weight, participants' height, name of biomarkers, method of the biomarker measurement (company and assay kit name, when available) and each biomarker level. For abdominal surgery papers, we collected patient characteristics, inclusion criteria and a duration of surgery. In addition, we extracted biomarker levels according to each study timelines: before surgery (before skin incision), surgery completion (end of surgery), closest value to surgery completion (closest biomarker value within 24 h after the operation), closest value to 24 h (closest biomarker value measured following 24 h after the completion of surgery).

When sepsis papers reported the severity of the sepsis (sepsis, severe sepsis or septic shock), we reported the variables according to the severity. Moreover, we collected main infection source, disease severity (SOFA score and APACHE II score) and mortality at last follow up. We defined sampling time as 'not reported' if there was no precise information for sampling time; day 1 as any time from 0 to 24 h after the sepsis diagnosis; day 2 as any from 24 to 48 h after the diagnosis, and closest to day 3 as 48 h after the diagnosis, and collected bookmarkers data according to this time frame. When there were two or more data 48 h after the diagnosis, we collected the data closest to day 3. We reported each variable as reported in each study (mean ± standard deviation [SD], or median and interquartile range [IQR], or any other form of reporting). If only figure-based data was available, FY and TN independently estimated its mean or median value from the figure and reported the values after agreement.

Results

We extracted 3948 titles from the databases and performed full text screening for 153 papers. Of these, 58 studies from

Table 1. Patient characteristics for cohorts of healthy subject.

Author and Year	Patient No	Age (years)	Male (%)	BMI (kg/m^2)	Weight (kg)	Height (cm)	Industry funding
Albert (Albert et al. 2018) 2018	20	N/R	N/R	N/R	N/R	N/R	No
Anand (Anand et al. 2016) 2016	50	36.1 (23.7 – 45)	60	N/R	N/R	N/R	No
Astapenko (Astapenko et al. 2019) 2019	21	20.70 ± 0.78	48	23.52 ± 2.86	N/R	N/R	No
Bihari (Bihari et al. 2019) 2019	6	33 ± 4	100	N/R	80.8 ± 10.9	183.1 ± 6.8	No
Chaves (Chaves et al. 2018) 2018	25	38.5 ± 9.6	44	N/R	N/R	N/R	No
Dane (Dane et al. 2014) 2014	10	44.8 ± 10.3	100	25.6 ± 3.7	N/R	N/R	Yes
Hornstrup (Hornstrup et al. 2018) 2018	56	62 ± 3	46	N/R	N/R	N/R	No
Hulde (Hulde et al. 2018) 2018	16	28.29 ± 5.42	0	22.53 ± 2.12	65.16 ± 9.08	170.18 ± 4.97	No
	10	34.11 ± 6.93	100	22.87 ± 1.52	73.94 ± 5.95	180 ± 4.97	
Kim (Kim et al. 2015) 2015	20	36.5 ± 8.5	10	N/R	N/R	N/R	N/R
Larsen (Larsen et al. 2015) 2015	31	62 (60 – 64)	52	N/R	N/R	N/R	No
Lee (Lee et al. 2019) 2019	13	49.8 ± 7.4	100	23.5 ± 2.0	78.5 ± 8.2	N/R	No
Li (Li et al. 2018) 2018	35	41.15 ± 12.24	69	N/R	N/R	N/R	No
Majerczak (Majerczak et al. 2016) 2016	21	22.7 ± 0.3	100	23.3 ± 0.7	74.9 ± 2.9	179 ± 1	No
Majerczak (Majerczak et al. 2017) 2017	10	22.9 ± 1.4	100	23.3 ± 2.6	73.4 ± 11.5	177 ± 6	No
Miranda (Miranda et al. 2016) 2016	24	34 ± 7	58	N/R	N/R	N/R	N/R
Naumann (Naumann et al. 2018a) 2018	19	N/R	N/R	N/R	N/R	N/R	No
Naumann (Naumann et al. 2018b) 2018	17	N/R	N/R	N/R	N/R	N/R	No
Nieuwdorp (Nieuwdorp et al. 2006a) 2006	7	52 ± 11	N/R	24 ± 4	N/R	N/R	No
Nieuwdorp (Nieuwdorp et al. 2006b) 2006	10	25.3 ± 2.6	100	22.5 ± 1.4	N/R	N/R	No
Nieuwdorp (Nieuwdorp et al. 2007) 2007	99	34.9 ± 16.4	44	23.3 ± 3.6	N/R	N/R	No
Oda (Oda et al. 2019) 2019	78	46 (42 – 52)	53	N/R	N/R	N/R	No
Osuka (Osuka et al. 2018) 2018	12	38 (28.5 – 44)	41.7	N/R	N/R	N/R	No
Pranskunas (Pranskunas et al. 2018) 2018	20	N/R	N/R	N/R	N/R	N/R	No
Rahbar (Rahbar et al. 2015) 2015	5	N/R	N/R	N/R	N/R	N/R	No
Saragih (Saragih et al. 2018) 2018	30	1.9 (0.2 – 2.9)*	40	N/R	N/R	N/R	N/R
Sallisalmi (Sallisalmi et al. 2012) 2012	20	45 (38 – 49.5)	35	N/R	67 (60 – 78)	169 (163 – 175)	No
Salmito (Salmito et al. 2015) 2015	25	38.5 ± 9.6	44	N/R	N/R	N/R	N/R
Sapp (Sapp et al. 2019) 2019	10	22 ± 2	N/R	24 ± 3	N/R	N/R	No
Schiefer (Schiefer et al. 2015) 2015	10	N/R	N/R	N/R	N/R	N/R	No
Schmidt (Schmidt et al. 2014) 2014	4	N/R	50	N/R	N/R	N/R	No
Steppan (Steppan et al. 2011) 2011	18	34.5 ± 8.6	55.6	N/R	N/R	N/R	N/R
Vlahu (Vlahu et al. 2012) 2012	21	44.1 ± 14.1	57	22.6 ± 2.5	N/R	N/R	No
Yeo (Yeo et al. 2019) 2019	29	27 (18 – 35)*	64	N/R	N/R	N/R	No

N/R; not reported.
All values were reported as mean ± standard deviation or median (interquartile range).
*Values were reported as median (range).

2006 to 2020 met our inclusion criteria (Figure 1). In total, 3580 patients were measured seven biomarkers (syndecan 1, syntecan 2, sydecan 3, syndecan 4, hyaluronan, heparan sulfate and chondroitin sulfate). However, Syndecan 1 was by far the most frequently measured biomarker in 48 studies (83%) and 3317 patients (93%), and hyaluronan was the second most frequently measured biomarker in 17 studies (29%).

The characteristics of the studies reporting biomarker values in healthy individuals are shown in Table 1 (Nieuwdorp et al. 2006a, 2006b, Nieuwdorp et al. 2007, Steppan et al. 2011, Sallisalmi et al. 2012, Vlahu et al. 2012, Dane et al. 2014, Schmidt et al. 2014, Rahbar et al. 2015, Salmito et al. 2015, Schiefer et al. 2015, Larsen et al. 2015, Kim et al. 2015, Anand et al. 2016, Majerczak et al. 2016, Miranda et al. 2016, Majerczak et al. 2017, Albert et al. 2018, Chaves et al. 2018, Hornstrup et al. 2018, Hulde et al. 2018, Li et al. 2018, Naumann et al. 2018, Naumann et al. 2018, Osuka et al. 2018, Pranskunas et al. 2018, Saragih et al. 2018, Astapenko et al. 2019, Bihari et al. 2019, Lee et al. 2019, Oda et al. 2019, Sapp et al. 2019, Yeo et al. 2019). In total, 802 patients were included in 33 studies. The number of enrolled subject in each study varied from 4 to 99. Their mean or median ages varied from 20.7 to 62 and one paper reported paediatric volunteers (1.9 year old). Out of 33 studies, syndecan 1 was the most frequently measured biomarker (27 studies [82%] and 664 patients [83%]) while hyaluronan was measured in 10 papers (30%) (Table 2 and Supplementary Table 1). Heparan sulfate was measured in six studies (18%) and chondroitin sulfate was measured in two studies (6%).

According to the assay used, in studies of syndecan 1 in healthy individuals, its overall median (range) of all studies' mean or median values was 22.65 (0.3 to 58.5) ng/ml (a 195-fold difference according to the measurement techniques) (Table 2). An assay kit from Abcam or Diaclone company was the most widely used (9 studies [27%]). The mean or median syndecan value ranged from 1.74 to 50 ng/ml (a 29-fold difference) in the Abcam kit and 10.6 to 49.8 ng/ml (a 4.7-fold difference) in the Diaclone kit. Also, the median (range) of all studies' mean or median hyaluronan values was 65 (16.8 to 782) ng/ml (47-fold difference), that of heparan sulfate was 150 (133.9 to 7000) ng/ml (52-fold difference) and the range of chondroitin sulfate varied from 22.9 ng/ml to 24000 ng/ml (1048-fold difference) according to the measurement technique (Supplementary Table 1).

There were eight papers in abdominal surgery patients (284 patients) and three paper focussing on liver transplant patients (78 patients) (Table 3) (Steppan et al. 2011, Johansson et al. 2014, Schiefer et al. 2015, Johansson et al. 2017, Nemme et al. 2017, Belavic et al. 2018, Holzmann et al. 2018, Passov et al.

Table 2. Syndecan 1 value among healthy individual.

Author and Year	Method	Syndecan1 (ng/ml)
Albert (Albert et al. 2018) 2018	Human Syndecan-1 ELISA Kit (CD138); cat: ab46506; Abcam plc., San Francisco, CA, USA	24.8 (21.5 – 30.6)
Chaves (Chaves et al. 2018) 2018	Human Syndecan 1 ELISA kit cat#ab46506, Abcam, Cambridge, MA, USA	28.2 ± 9.8
Hornstrup (Hornstrup et al. 2018) 2018	Abcam plc, Cambridge, United Kingdom	9.3 (7.9 – 17.9)
Miranda (Miranda et al. 2016) 2016	ELISA, Abcam®, Cambridge, UK	42 (27 – 80)
Naumann (Naumann et al. 2018) 2018 A	Abcam, ab46506, Cambridge, Mass	30 (20 – 44)
Naumann (Naumann et al. 2018) 2018 B	Abcam; product code ab46506, Cambridge, MA	30 (20 – 44)
Osuka (Osuka et al. 2018) 2018	ab46506-Syndecan-1 Human ELISA Kit; Abcam	1.74 (0 – 5.5)
Sallisalmi (Sallisalmi et al. 2012) 2012	Abcam plc, Cambridge, UK	50[§]
Salmito (Salmito et al. 2015) 2015	Abcam	28.2 ± 9.8
Pranskunas (Pranskunas et al. 2018) 2018	Human Syndecan-1(CD138) ELISA kit, BioVendor, Brno, Czech Republic	8.2 (2.4 – 15.1)
Kim (Kim et al. 2015) 2015	Cell Sciences Inc.	18.5 (14.5 – 27.6)
Anand (Anand et al. 2016) 2016	ELISA, Diaclone; Besancom Cedex, France	28.15 (7.47 – 45.7)
Dane (Dane et al. 2014) 2014	ELISA, Diaclone Research, Besancon, France	49.8 ± 17.4
Hulde (Hulde et al. 2018) 2018	Diaclone SAS, Besanc¸on, France	11.1 ± 2.4 (ovulation phase)[†], 12.6 ± 2.3(luteal phase)[†], 12* (follicular phase)[†], 19* (male group)
Majerczak (Majerczak et al. 2016) 2016	Diaclone CD138, No 950.640.096, Diaclone Research, Besancon, France	39.5 ± 3.4
Majerczak (Majerczak et al. 2017) 2017	Diaclone CD138, No 950.640.096, Diaclone Research, Besancon, France	48*
Oda (Oda et al. 2019) 2019	950.640.192, Diaclone, Besancon, Cedex, France	19.3 (13.7 – 27.3)
Schiefer (Schiefer et al. 2015) 2015	Diaclone Research; Besancon, France	10.6 ± 9.4
Steppan (Steppan et al. 2011) 2011	Diaclone; Besancon Cedex, France	20.5 ± 5.05
Vlahu (Vlahu et al. 2012) 2012	Diaclone; Gen-Probe Inc., CA	27.5 (18.9 – 33.7)
Larsen (Larsen et al. 2015) 2015	Nordic Biosite, Copenhagen, Denmark	10.7 (9 – 16)
Li (Li et al. 2018) 2018	Quantikine, XiTang, Inc., Shanghai, China	0.819 (0.260)[‡]
Astapenko (Astapenko et al. 2019) 2019	RayBiotech, Norcross, Georgia, USA, Human Syndecan ELISA kit	0.3 (0.23 – 0.39)
Sapp (Sapp et al. 2019) 2019	RayBiotech, Norcross, GA	2.3, 2.2 (measured two times from the same patients)
Bihari (Bihari et al. 2019) 2019	R&D Systems, Minneapolis, MN	1.19 ± 0.66, 0.63 ± 0.59, 0.58 ± 0.34, 0.71 ± 0.77 (measured four times from the same patients)
Saragih (Saragih et al. 2018) 2018	N/R	27.7 ± 2.24
Yeo (Yeo et al. 2019) 2019	N/R	58.5 (40.8 – 76.2)[‖]

N/R; not reported.
All values are mean ± standard deviation, median (interquartile range) or format used in original study.
*When only figure available, estimated mean value was reported in the table.
[†]Values in the female group were reported according to menstruation cycle.
[‡]Reported as median (range from 25th quantile to 75th quantile).
[§]When only figure available, estimated median value was reported in the table.
[‖]Reported as mean (95% confidence interval).

2019, Kaneko et al. 2020, Li et al. 2020, Nemme et al. 2020, Schiefer et al. 2020). The number of the patients in each group varied from 7 to 55, and mean or median age varied from 46 to 68. All of the surgical studies (100%) measured syndecan 1 in 362 patients (100%), two studies (20%) measured hyaluronan and five studies (50%) measured heparan sulfate. Median (range) of syndecan 1 studies' mean or median baseline values was 18.5 (4 to 39.46) ng/ml and its value after surgery was 21.35 (4.3 to 60) ng/ml (Table 4). In prolonged (6 h) pancreas surgery, the postoperative syndecan 1 value increased from 20 ng/ml to 30 ng/ml. (Holzmann et al. 2018) However, in short (within 90 min) hysterectomy, mean syndecan 1 value decreased in the two groups and only one group increased after the surgery (Nemme et al. 2017, Nemme et al. 2020). Except for the above hysterectomy papers, all abdominal surgery studies showed slight increases in other biomarker levels (hyaluronan and heparan sulfate) after surgery (Supplementary Table 2 and 3). Three studies reported syndecan 1 level in liver transplant patients with increased mean values after liver reperfusion (Supplementary Table 4). And when measured from different assay, six healthy volunteer studies reported higher syndecan 1 values than that of after pancreatic surgery.

We identified 20 studies with data in 2416 sepsis patients (Table 5) (Nelson et al. 2008, Steppan et al. 2011, Sallisalmi et al. 2012, Yagmur et al. 2012, Ostrowski et al. 2013, Donati et al. 2014, Johansson et al. 2014, Nelson et al. 2014, Ostrowski et al. 2015, Ostrowski et al. 2015, Anand et al. 2016, Puskarich et al. 2016, Murphy et al. 2017, Nelson et al. 2017, Smart et al. 2017, Wu et al. 2017, Saragih et al. 2018, Hippensteel et al. 2019, Inkinen et al. 2019, Smart et al. 2019). They included from 10 to 619 patients in each group and their mean or median age was from 41 to 74 years, and mortality at last follow up ranged from 3% to 79%. Eighteen studies (90%) measured syndecan 1 in 2291 patients (95%) and six studies (30%) measured hyaluronan (Table 6 and Supplementary Table 5). Other biomarkers (heparan sulfate, syndecan 2, syndecan 3, syndecan 4 and chondroitin sulfate) were measured in four or less studies (Supplementary Table 6 and 7).

In sepsis papers, the median (range) of the studies' mean or median syndecan 1 levels was 103 (1.4 to 800) ng/ml and hyaluronan was 219 (4.8 to 1000) ng/ml on day 1 (Table 6 and Supplementary Table 5) and an assay kit from Diaclone was the most popular (10 studies [56%]). Syndecan 1 and hyaluronan appeared to increase according to sepsis severity

Table 3. Characteristics of abdominal surgery studies.

Author and Year	Number of patient	Age (years)	Male (%)	BMI (kg/m^2)	Inclusion criteria	Duration of surgery (h)	Patient characteristics	Industry funding
Belavić (Belavic et al. 2018) 2018	30 30 30	48 (39.8 – 61.5) 61 (40.8 – 70) 56.5 (41 – 63.5)	27 30 23	29 (27 – 31.6) 28 (25.1 – 33.0) 28 (26.4 – 32.7)	Laparoscopic cholecystectomy for gallstones. ASA class I and II.	1.0 (0.8 – 1.1) 1.0 (0.8 – 1.3) 0.9 (0.8 – 1.2)	Restrictive fluid balance Low liberal fluid balance High liberal fluid balance	N/R
Holzmann (Holzmann et al. 2018) 2018	55 12 43	61 (51 – 70) 64 (57 – 73) 59 (51 – 70)	67 67 67	N/R N/R N/R	Pancreaticoduodenectomy ($n = 31$) and oncological abdominal surgery ($n = 24$) as main surgeries.	6.0 (5.0 – 7.5) 7.3 (5.6 – 9.4) 6.0 (5.0 – 7.5)	All patients Sepsis after the operation Without sepsis after the operation	None
Johansson (Johansson et al. 2017) 2017	8	68 (63 – 71)	75	25 (24 – 27)	Age >18. Pancreaticoduodenectomy	N/R	Usual care	No
Kaneko (Kaneko et al. 2020) 2020	26 26	67 (60 – 74) 67 (62 – 72)	N/R N/R	23.1 ± 4.3 22.3 ± 3.8	Scheduled major abdominal surgery for cancer. Normal cardiac, lung and renal function without diabetes mellitus or liver cirrhosis.	N/R N/R	Placebo infusion Phenylephrine 0.5 μg/kg/min infusion	No
Li (Li et al. 2020) 2020	20	47.0 ± 7.9	80	22.9 ± 3.2	Elective partial hepatectomy for hepatocellular carcinoma.	3.2 ± 1.1	Hydroxyethyl Starch (15 mL/kg) loading after anaesthesia	No
Nemme (Nemme et al. 2017) 2017	7	N/R	0	N/R	Age 25 – 55 Open hysterectomy Expected operating time <90 min No chronic cardiopulmonary or renal diseases Expected bleeding <500 ml	N/R	N/R	No
Nemme (Nemme et al. 2020) 2020	13 11	47 ± 5 46 ± 4	0 0	N/R N/R	Age 25–55, without renal or cardiopulmonary disease. Hysterectomy. Expected operating time of <90 min, blood loss <500 ml.	N/R N/R	Sevoflurane Propofol	No
Passov (Passov et al. 2019) 2019	10	49 (28 – 66)*	90	N/R	Liver transplant surgery for cholangiocarcinoma	N/R	N/R	No
Schiefer (Schiefer et al. 2015) 2015	30	54 ± 11	70	N/R	Liver transplant surgery	N/R	N/R	No
Schiefer (Schiefer et al. 2020) 2020	38	57 ± 9	79	N/R	Liver transplant surgery	N/R	N/R	No
Steppan (Steppan et al. 2011) 2011	28	62.9 ± 12.7	53.6	N/R	Abdominal surgery (mainly pancreas).	N/R	N/R	N/R

N/R; not reported.
All values were reported as mean ± standard deviation or median (interquartile range).
*Values were reported as median (range).

Table 4. Syndecan 1 values (ng/ml) after the surgery.

Author and Year	Method	Patient characteristics	Before surgery†	Surgery completion	Closest value to surgery completion‡	Closest value to 24 hours§
Belavić (Belavic et al. 2018) 2018	ELISA, aBclonal, Woburn, MA, USA	Restrictive fluid balance	4.1 (3.6 – 4.8)	4.3 (3.4 – 6.2)	5.4 (5.0 – 5.8)	N/R
		Low liberal fluid balance	4 (3.1 – 4.7)	5*	6.1 (4.7 – 6.8)	N/R
		High liberal fluid balance	4.1 (3.4 – 4.5)	7.4 (5.1 – 8.8)	7.9 (6.3 – 9.7)	N/R
Li (Li et al. 2020) 2020	Cell Sciences, Canton, MA	Hydroxyethyl Starch (15 ml/kg) loading after anaesthesia	39.46 ± 20.2	46.84 ± 22.33	N/R	N/R
Kaneko (Kaneko et al. 2020) 2020	ELISA, Diaclone	Placebo infusion	16.9 (11.5 – 29.3)	N/R	N/R	N/R
		Phenylephrine infusion	18.5 (12.0 – 28.6)	N/R	N/R	N/R
Holzmann (Holzmann et al. 2018) 2018	ELISA kit (Diaclone, Besancon, France)	All patient	20*	30* (median)	N/R	30*
		Sepsis after the operation	20*	38.5 (23.3 – 126.5)	N/R	16.5 (0.0 – 32.5)
		Without sepsis after the operation	20*	23.0 (9.0 – 91.0)	N/R	90.0 (34.0 – 292.0)
Nemme (Nemme et al. 2017) 2017	Human CD138/ syndecan 1 (Diaclone, France)	Open hysterectomy	21.0 ± 3.6	19.7 ± 5.1	N/R	N/R
Nemme (Nemme et al. 2020) 2020	Diaclone Research, Besancon, France	Sevoflurane	18*	16*	N/R	N/R
		Propofol	14*	16*	N/R	N/R
Steppan (Steppan et al. 2011) 2011	Diaclone; Besancon Cedex, France	Major abdominal surgery	N/R	50.5 ± 46.9	59.6 ± 59.8	85.6 ± 131
Johansson (Johansson et al. 2017) 2017	Nordic Biosite, Copenhagen, Denmark					

Pancreaticoduodenectomy30*60*75*N/RN/R; not reported.
All syndecan 1 values were reported as mean ± standard deviation, median (interquartile range) or other formats that original paper used.
*When only figure was available, estimated median values were reported in the table.
†When syndecan 1 value before anaesthesia induction and that of after induction of aesthesia were available, syndecan 1 value before anaesthesia induction was reported.
‡Closest syndecan 1 value within 24 h after the operation.
§Closest syndecan 1 value measured following 24 h after the completion of surgery.

in two studies (Donati et al. 2014, Ostrowski et al. 2015). For example, median (IQR) vales were 85.78 (40.16 to 141.2) ng/ml and 63.2 (52 to 92) ng/ml in the sepsis group and 653.5 (338.93 to 1430.23) and 423 (183.3 to 686) ng/ml in the septic shock group when measured in the same paper using the same techniques (Anand et al. 2016). However, one study reported that syndecan 1 value in sepsis patients receiving with noradrenaline was lower than those without noradrenaline (Johansson et al. 2014).

The studies that compared biomarker levels between healthy individuals and sepsis patients using the same techniques reported that all of the biomarkers increased in sepsis patients compared to healthy volunteers (Anand et al. 2016, Steppan et al. 2011). For example, increases were 3 to 4-fold with sepsis and 20-fold with septic shock with similar changes in hyaluronan (Anand et al. 2016). However, when measured from different techniques, 24 studies reported higher syndecan 1 values in normal subjects than the lowest mean syndecan 1 value in sepsis patients (Smart et al. 2019). Moreover, 10 studies reported higher hyaluronan mean or median values in healthy individuals than the lowest median hyaluronan value in sepsis patients (Anand et al. 2016).

Discussion

Key findings

We identified 58 studies, which measured glycocalyx damage biomarkers in healthy subjects, patients undergoing abdominal surgery, or sepsis. We found that syndecan 1 was the most frequently measured biomarker. We also found a 195-fold syndecan 1 value variation in healthy volunteers according to the assay used. And even when investigators used the same company's assay kit in healthy volunteers, there was still a 29-fold difference syndecan 1 levels. Biomarkers increased only slightly after prolonged surgery. In sepsis patients, when measured from the same technique, biomarker values were much higher than in healthy volunteers and increased according to illness severity. When syndecan 1 was measured by different assays, however, six studies reported higher syndecan 1 value in healthy individuals than after pancreatic surgery and 24 studies reported higher syndecan 1 values than the lowest mean syndecan 1 value in sepsis patients.

Relationship to previous studies

In abdominal surgery patients, we found that glycocalyx biomarkers showed minor changes in short and/or laparoscopic surgery, and they increased mildly after long, open abdominal surgery. This might reflect the fact that inflammation markers were lower in short and laparoscopic surgery than long and open surgery (Jacobi 1998, Fretland et al. 2015). However, there were only 11 studies of abdominal surgery and these findings lack robustness. Of concern, six studies reported higher syndecan 1 value in healthy individuals than after pancreatic surgery.

Table 5. Characteristics of sepsis studies.

Author and Year	Severity of sepsis	Number of patient	Age (years)	Male (%)	Main infection source	SOFA score	APACHE II score	Mortality at last follow up (%)		Industry Funding
Anand (Anand et al. 2016) 2016	Sepsis	15	56 (36 – 67)	53	N/R	6 (4 – 8)	10 (8 – 12)	90 days	6.66	No
	Severe sepsis	45	56 (46 – 63)	60	N/R	8.5 (5 – 11)	20 (14.5 – 24)	90 days	13.3	
	Septic shock	90	56 (44.5 – 65)	62	N/R	12 (10 – 14)	25 (20.75 – 30)	90 days	37.7	
Donati (Donati et al. 2014) 2014	Sepsis, severer sepsis, septic shock	10	70 (65 – 72)	50	Mainly lung	8 (5 – 12)	N/R		N/R	N/R
	Sepsis, severer sepsis, septic shock	10	74 (64 – 79)	70	Mainly lung	5 (3 – 7)	N/R		N/R	
Hippensteel (Hippensteel et al. 2019) 2019	Septic shock	56	59.4 ± 15.3	54	N/R	7.0 ± 4.1	N/R		N/R	No
	Sepsis, severer sepsis, septic shock	100	61.4 ± 2.7	66	N/R	3.1 ± 2.9	N/R		N/R	
Inkinen (Inkinen et al. 2019) 2019	Sepsis	619	66 (55 – 75)	64	Mainly lung	8 (6 – 10)	N/R	90 days	29.1	No
Johansson (Johansson et al. 2014) 2014	Sepsis without noradrenaline	53	65 (55 – 73)	66	Mainly lung	5 (5 – 7)	N/R	90 days	45	No
	Sepsis with noradrenaline	14	72 (69 – 77)	36	Mainly lung	6 (4 – 8)	N/R	90 days	79	
Murphy (Murphy et al. 2017) 2017	Non-pulmonary sepsis	127	56 ± 15	50	Mainly abdominal	N/R	34 ± 6	Hospital	38	No
	Pulmonary sepsis	135	56 ± 17	55	Only lung	N/R	33 ± 6	Hospital	34	
Nelson (Nelson et al. 2008) 2008	Septic shock	18	65 (28 – 87)*	33	N/R	14 (5 – 18)*	N/R	90 days	45	No
Nelson (Nelson et al. 2014) 2014	Septic shock	24	66 (45 – 86)*	29	Mainly lung	3.5 (1 – 4)*	N/R	90 days	38	No
Nelson (Nelson et al. 2017) 2017	Sepsis	73	N/R	N/R	N/R	N/R	N/R		N/R	No
Ostrowski (Ostrowski et al. 2013) 2013	Severe sepsis, septic shock	20	59 ± 14	85	Mainly soft tissue	9 ± 4	23 ± 9	90 days	35	No
Ostrowski (Ostrowski et al. 2015a) 2015	Sepsis	95	61 (46 – 73)	49	Mainly lung	1 (1 – 2)	N/R	90 days	6	N/R
	Severe sepsis	100	68 (50 – 80)	59	Mainly lung	3 (2 – 4)	N/R	90 days	13	
	Septic shock	13	66 (57 – 70)	23	Mainly lung	4 (3 – 5)	N/R	90 days	15	
Ostrowski (Ostrowski et al. 2015b) 2015	Severe sepsis, septic shock	184	67 (59 – 75)	55	N/R	8 (6 – 10)	N/R	1 year	61	Yes
Puskarich (Puskarich et al. 2016) 2016	Sepsis	175	61 (48 – 71)	53	Mainly lung	7 (4 – 9)	N/R	Hospital	21	No
Sallisalmi (Sallisalmi et al. 2012) 2012	Septic shock	20	58 (48 – 65)	65	Mainly lung	13 (11 – 14)	24.5 (21.5 – 29.8)	28 days	15	No
Saragih (Saragih et al. 2018) 2018	Sepsis	49		65.3	Mainly lung	N/R	N/R			N/R
Smart (Smart et al. 2017) 2017	Sepsis	86	59 (52 – 66)	59	Mainly lung	4 (3 – 6)	N/R	30 days	19	None
Smart (Smart et al. 2019) 2019	Sepsis	31	45 (39–52)	58	Mainly lung and urinary tract	0 (0–1)	N/R	Hospital	3	No
	Sepsis	34	41 (35 – 47)	62		0 (0 – 1)	N/R		3	
Steppan (Steppan et al. 2011) 2011	Severe sepsis, septic shock	104	64.9 ± 12.4	55.8	Mainly lung	N/R	N/R	28 days	52.9	N/R
Wu (Wu et al. 2017) 2017	Severe sepsis	15	65.47 ± 10.20	73	N/R	N/R	N/R	ICU	26.7	No
Yagmur (Yagmur et al. 2012) 2012	Sepsis	101	66 (21 – 90)*	59	Mainly lung	9 (0 – 19)*	22 (4 – 40)*	ICU	28	No

N/R; not reported, APACHE; Acute physiology and chronic health evaluation, ICU; Intensive care unit, SOFA; Sequential organ failure assessment.
Values were reported as mean ± standard deviation, median (interquartile range) or as in the original paper.*Values were reported as median (range).

Table 6. Syndecan 1 value (ng/ml) in sepsis studies.

Author and Year	Method	Severity of sepsis	Sampling time N/R*	Day 1 (0–24 h)	Day 2 (24–48 h)	Closest value to day 3	
Hippensteel (Hippensteel et al. 2019) 2019	ab46506, Abcam, Cambridge, MA, USA	Septic shock	N/R	118 (113 – 341)	N/R		N/R
		Sepsis, severe sepsis, septic shock	N/R	283 (115 – 584)	N/R		N/R
Murphy (Murphy et al. 2017) 2017	Syndecan-1 Item No. ab46506, Abcam, Cambridge, MA, USA	Non-pulmonary sepsis	N/R	N/R	180*		N/R
		Pulmonary sepsis	N/R	N/R	150*		N/R
Puskarich (Puskarich et al. 2016) 2016	ELISA, Abcam, Cambridge MA	Sepsis	152 (49 – 345)	N/R	N/R		N/R
Sallisalmi (Sallisalmi et al. 2012) 2012	Abcam plc, Cambridge, UK	Septic shock	N/R	800*	N/R	Day 4	600†
Wu (Wu et al. 2017) 2017	Human syndecan-1/ CD138 (SDC-1) ELISA Kit, Cusabio, Wuhan, China	Severe sepsis	N/R	82.2047 ± 53.45	116.99 ± 121.22	Day 3	137.64 ± 182.36
Anand (Anand et al. 2016) 2016	ELISA, Diaclone; Besancom Cedex, France	Sepsis	N/R	85.78 (40.1 – 141.2)	N/R	Day 3	100.7 (54.35 – 142.3)
		Severe sepsis	N/R	342.1 (130 – 568.1)	N/R		342.2 (123.2 – 612.25)
		Septic shock	N/R	653.5 (338.9 – 1430.2)	N/R		654.26 (357.1 – 1673)
Donati (Donati et al. 2014) 2014	Human sCD138/ Syndecan-1 enzyme-linked immunosorbent assay [ELISA] Gen-Probe Diaclone SAS, Besancon, France	Sepsis, severer sepsis, septic shock	219.4 (84.5 – 361.5)	N/R	N/R		N/R
		Sepsis, severer sepsis, septic shock	100.0 (5.4 – 378.4)	N/R	N/R		N/R
Inkinen (Inkinen et al. 2019) 2019	Human CD138 ELISA kit, Diaclone, Besancon, France	Sepsis	N/R	109.00 (62.30 – 215.40)	N/R		N/R
Johansson (Johansson et al. 2014) 2014	Diaclone SAS, Besancon, France	Sepsis without noradrenaline	N/R	76 (43 – 235)	N/R		N/R
		Sepsis with noradrenaline	N/R	44 (37 – 79)	N/R		N/R
Nelson (Nelson et al. 2008) 2008	Diaclone, Ta¨by, Sweden	Septic shock	N/R	246 (180 – 496)§	N/R		N/R
Nelson (Nelson et al. 2017) 2017	ELISA, Cat No 950.640.192, Diaclone, Täby, Sweden	Sepsis	N/R	207 (71 – 456)	N/R		N/R
Ostrowski (Ostrowski et al. 2013) 2013	Diaclone SAS, Besancon, France	Severe sepsis, septic shock	172 ± 102	N/R	N/R		N/R
Ostrowski (Ostrowski et al. 2015) 2015 A	Diaclone; Nordic Biosite, Copenhagen, Denmark	Sepsis	31 (22 – 50)	N/R	N/R		N/R
		Severe sepsis	61 (35 – 95)	N/R	N/R		N/R
		Septic shock	61 (39 – 119)	N/R	N/R		N/R
Ostrowski (Ostrowski et al. 2015) 2015 B	Diaclone SAS, Besancon, France	Sever sepsis, septic shock	N/R	97 (51 – 204)	N/R		N/R
Steppan (Steppan et al. 2011) 2011	Diaclone; Besancon Cedex, France	Severe sepsis, septic shock	N/R	160 ± 109	161 ± 99.4	Day 3	165 ± 86.5
Smart (Smart et al. 2017) 2017	ELISA kits (R&D Systems, Minneapolis, MN)	Sepsis	N/R	3.002 (2.345 – 3.842)‡	3.592 (2.757 – 4.681)‡		N/R
Smart (Smart et al. 2019) 2019	R&D Systems, Minneapolis, MN, USA	Sepsis	N/R	1.5 (1.2 – 1.9)	N/R		N/R
		Sepsis	N/R	1.4 (1.1 – 1.8)	N/R		N/R
Saragih (Saragih et al. 2018) 2018	N/R	Sepsis	N/R	83.40 (10.10 – 2257.91)§	85†		90†

N/R; not reported.
All values were reported as mean ± standard deviation, median (interquartile range) or other formats that original paper used.
*Timing of blood sampling was not reported.
†When only figure was available, estimated median value was reported.
‡Values were reported as Mean (95% confidence interval).
§Values were reported as median (range).

Raised glycocalyx damage biomarker values in sepsis patients have been related to mortality or disease severity (Donati et al. 2014, Anand et al. 2016). However, our data suggests that absolute biomarker levels are unreliable and differ markedly according to the assay used (Belavic et al. 2018, Li et al. 2018). Thus, while changes within a given population and using the same assay can robustly identify increases in biomarkers, they cannot reliably or accurately describe the actual value of such biomarkers.

Study implications

Our findings imply that the value of glycocalyx biomarkers in healthy volunteers and in patients varies markedly according to the assay used. Thus, absolute values may be of limited value because lower syndecan 1 or hyaluronan values have been reported in abdominal surgery patients or sepsis patients than in healthy subjects (Anand et al. 2016, Smart et al. 2017, Li et al. 2018). Finally, given the inter-assay variability in absolute values, our findings imply that, for future research, researcher may be better to use same assay kit and to compare changes from their baseline values.

Strengths and limitations

To our knowledge, this is the first systematically conducted scoping review of glycocalyx biomarkers in healthy individuals, perioperative patients and septic patients. A previous study reviewed glycocalyx biomarker levels in sepsis; however, it did not report a systematic search strategy or data on patient groups other than those with sepsis (Uchimido et al. 2019). This allowed us to access a comprehensive data set and provides the most comprehensive quantitative assessment of such biomarkers.

Our review has some limitations. First, we did not assess other glycocalyx measurement techniques (SDF imaging or orthogonal phase spectrometry) (Rovas et al. 2019). However, such approaches cannot be easily applied to assess sizeable cohorts patients and lack inter-observer reliability (Rovas et al. 2019, Valerio et al. 2019). Second, due to the limitations of the data available, we could not conduct a meta-analysis and could not provide pooled estimated biomarker levels and risk of bias assessment. Because our data showed diverse ranges of normal biomarker levels using different assays, we could not conduct an accurate analysis to estimate correct normal values in healthy subjects. Third, most of the papers did not mention sample processing, which might affect the results. The coefficient of variation of syndecan 1 assay kits was reported at 10.2% (inter-assay) and 6.2% (intra-assay) for Abcam kits, and 6.2% or less than 10% (inter-assay) for Diaclone kits (Hornstrup et al. 2018, Hahn et al. 2019, Lee et al. 2019, Nemme et al. 2020). However, our data show that even when the same company kit was used in healthy volunteers, there were wide discrepancies in syndecan 1 values. Finally, we excluded studies not in the English language. However, there is no evidence of a bias in systematic reviews and meta-analysis from excluding non-English language studies (Morrison et al. 2012).

Conclusions

This systematically conducted scoping review identified 58 studies reporting the values glycocalyx damage markers in healthy volunteers, patients who underwent abdominal surgery and septic patients. Our study showed biologically implausible variability in absolute values according to the assay used to measure such biomarkers and overlap among these groups of patients and normal volunteers. These observations imply that absolute values cannot be used to describe normal reference values and that different studies cannot be compared unless identical assays are used. Finally, they suggest that using the same assay, it may not be possible to have an accurate description of absolute values, but it may be possible to report on directional change and its percentage decrease or increase from baseline.

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ORCID

Fumitaka Yanase http://orcid.org/0000-0003-3859-3537

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Supplementary material 1. Search strategy.

Cochrane Central Register of Controlled Trials (CENTRAL)
#1 MeSH descriptor: [Glycocalyx] explode all trees
#2 (glycocalyx or glycocalix):ti,ab,kw
#3 #1 or #2
#4 (damage* or protect* or restor* or shed* or integrity or flaking or altered or loss or injur* or degrad*):ti,ab,kw
#5 #3 and #4

MEDLINE (Ovid)
1. Glycocalyx/
2. (glycocalyx or glycocalix).mp
3. 1 OR 2
4. (damage* or protect* or restor* or shed* or integrity or flaking or altered or loss or injur* or degrad*).mp
5. 3 AND 4

Embase (Ovid)
1. Glycocalyx/
2. (glycocalyx or glycocalix).mp
3. 1 OR 2
4. (damage* or protect* or restor* or shed* or integrity or flaking or altered or loss or injur* or degrad*).mp
5. 3 AND 4

Supplementary Table 1. Hyaluronan, heparan sulfate and chondroitin sulfate values among healthy individuals.

Author and Year	Method	Hyaluronan (ng/ml)	Method	Heparan sulfate (ng/ml)	Method	Chondroitin sulfate (ng/ml)
Anand(1) 2016	ELISA, TECO medical; Switzerland	33.1 (17 - 53)		N/R		N/R
Hulde(2) 2018	Echelon Biosciences Inc., Salt Lake City, UT, USA	663 ± 35 (ovulation phase), 7 82 ± 55 (luteal phase), 700* (follicular phase), 630* (male group)	Fa. Cusabio Art.Nr.: CSB-E09585h	150* (ovulation phase), 150* (luteal phase), 150* (follicular phase), 150* (male group)		N/R
Majerczak(3) 2016	Corgenix, Inc., Broomfield, Colorado, USA	40.4 ± 3.5	Seikagaku Corp., Tokyo, Japan	4910 ± 1090		N/R
Majerczak(4) 2017	Corgenix, Inc., Broomfield, Colorado, USA	45*	Seikagaku Corp., Tokyo, Japan	7000*		N/R
Nieuwdorp(5) 2006 A	Echelon Biosciences, Salt Lake City, UT	65 ± 8		N/R		N/R
Nieuwdorp(6) 2006 B	Echelon Biosciences, Salt Lake City, UT	70 ± 6		N/R		N/R
Nieuwdorp(7) 2007	Echelon Biosciences, Salt Lake City, UT, USA	60 ± 18		N/R		N/R
Rahbar(8) 2015	R&D Systems, Cat. No. DHYAL0, Minneapolis, MN	627.6 (484.1 - 753.1)	Biotang, Cat. No. HU8718, Lexington, MA	133.9 (130.5 - 138.3)	Biotang, Cat. No. HU8720, Lexington, MA	22.9 (22.5 - 23.3)
Schmidt(9) 2014	Not commercial kit	600†	Genway Biotech, San Diego, CA	150†	Not commercial kit	24000†
Steppan(10) 2011		N/R	Seikagaku Corp., Tokyo, Japan	1960 ± 1210		N/R
Vlahu(11) 2012	Corgenix Inc., Broomfield, CO	16.8 (6.4 - 29.5)		N/R		N/R

N/R; not reported.
All values were reported as mean ± standard deviation, median (interquartile range) or other formats that original paper used.
*: When only figure was available, estimated mean value was reported in the table.
†:When only figure was available, estimated median value was reported in the table.

Supplementary Table 2. Hyaluronan value (ng/ml) after surgery

Author and Year	Method	Patient character	Before surgery†	Surgery completion	Closest value to surgery completion‡	Closest value to 24 hours§
Belavić(12) 2018	ELISA, aBclonal, Woburn, MA, USA	Restrictive fluid balance	300*	317 (253 - 366)	325*	N/R
		Low liberal fluid balance	308 (226 - 360)	357 (305 - 395)	350 (319 - 403)	N/R
		High liberal fluid balance	336 (263 - 358)	393 (342 - 463)	350*	N/R
Nemme(13) 2017	Hyaluronan immunoassay (R&D Systems, Inc., MN, USA)	Open hysterectomy	38.0 ± 6.9	27.7 ± 5.3	N/R	N/R

All values were reported as mean ± standard deviation, median (interquartile range) or other formats from original paper.
*: When only figure was available, estimated median values were reported in the table.
†:When hyaluronan value before anesthesia induction and that of after induction of aesthesia were available, hyaluronan value before anesthesia induction was reported.
‡: Closest hyaluronan value within 24 hours after the operation
§: Closest hyaluronan value measured following 24 hours after the completion of surgery

Supplementary Table 3. Heparan sulphate value (ng/ml) after the surgery

Author and Year	Method	Patient character	Before surgery†	Surgery completion	Closest value to surgery completion‡	Closest value to 24 hours§
Belavić(12) 2018	ELISA, aBclonal, Woburn, MA, USA	Restrictive fluid balance	11.7 (10 - 13.2)	12.4 (11.5 - 14.4)	12*	N/R
		Low liberal fluid balance	11*	13*	12*	N/R
		High liberal fluid balance	11*	12*	12*	N/R
Li(14) 2020	ARG81249, Taiwan	Hydroxyethyl Starch (15 mL/kg) loading after anesthesia	0.1 ‖	0.2 ‖	N/R	N/R
Nemme(13) 2017	AMS.E-EL-H2364 (Ams- bio, Abingdon, UK)	Open hysterectomy	3400 ± 900	5500 ± 800	N/R	N/R
Nemme(15) 2019	Seikagaku Corp, Tokyo, Japan	Sevoflurane	6*	15*	N/R	N/R
		Propofol	5*	26*	N/R	N/R
Steppan(10) 2011	ELISA (Seikagaku Corp., Tokyo, Japan)	Major abdominal surgery	N/R	7960 ± 3260	8480 ± 3330	8490 ± 3460

All values were reported as mean ± standard deviation, median (interquartile range) or other formats that original paper used.
*: When only figure was available, estimated median values were reported in the table.
†:When heparan sulphate value before anesthesia induction and that of after induction of aesthesia were available, heparan sulphate value before anesthesia induction was reported.
‡: Closest heparan sulphate value within 24 hours after the operation
§: Closest heparan sulphate value measured following 24 hours after the completion of surgery
‖: Unit was U/L.

Supplementary Table 4. Syndecan 1 value (ng/ml) after liver transplant

Author and Year	Method	Before surgery	Before IVC clamping	After liver reperfusion	End of surgery	24 hours after liver reperfusion
Passov(Passov, et al., 2019) 2019	Diaclone SAS, Besancon Cedex, France	50*	N/R	1600*	N/R	N/R
Schiefer(16) 2015	Diaclone Research; Besancon, France	74.3 ± 59.9	83.5 ± 53.3	312.6 ± 114.8	291.8 ± 117.5	279.7 ± 138.3
Schiefer(17) 2020	Diaclone Research; Besancon, France	191 (103 - 303)			3293 (2763 - 3941)	1017 (633 - 2095)

All values were reported as mean ± standard deviation.

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*: When only figure was available, estimated median values were reported in the table.

Supplementary Table 5. Hyaluronan value (ng/ml) among sepsis patients.

Author and Year	Method	Severity of sepsis	Sampling time N/R	Day 1 (0 - 24 hours)	Day 2 (24 - 48 hours)	Closest value to day 3	
Anand(1) 2016	ELISA, TECO medical; Switzerland	Sepsis	N/R	63.2 (52 - 92)	N/R	Day 3	97 (63.2 - 147)
		Severe sepsis	N/R	324.1 (139.2 - 467.4)	N/R		282.2 (162.2 - 398.2)
		Septic shock	N/R	423 (183.3 - 686)	N/R		342 (147 - 651)
Donati(18) 2014	Hyaluronic Acid Quantitative Test kit; Corgenix, Inc., Broomfield, CO, USA	Sepsis, severer sepsis, septic shock	211.9 (75 - 423.7)	N/R	N/R		N/R
		Sepsis, severer sepsis, septic shock	275.7 (109.3 - 450.7)	N/R	N/R		N/R
Nelson(19) 2014	Not commercial kit	Septic shock		1000 ‡			
Smart(20) 2017	R&D Systems, Minneapolis, MN	Sepsis	N/R	114 (80 - 163)*	N/R		N/R
Smart(21) 2019	R&D Systems, Minneapolis, MN, USA	Sepsis	N/R	5.9 (3.6 - 9.6)	N/R		N/R
		Sepsis	N/R	4.8 (2.5 - 9.1)	N/R		N/R
Yagmur(22) 2012	Automated latex agglutination assay (WAKO, Osaka, Japan)	Sepsis	N/R	344 (0 - 2662)†	N/R	Day 3	253 (23 - 2662)†

All values were reported as mean ± standard deviation, median (interquartile range) or other formats that original paper used.
*: Values were reported as mean (95% confidence interval)
†: Values were reported as median (range)
‡: When only figure was available, estimated median value was reported.

Supplementary Table 6. Heparan sulfate value (ng/ml) among sepsis patients.

Author and Year	Method	Severity of sepsis	Sampling time N/R	Day 1 (0 - 24 hours)	Day 2 (24 - 48 hours)	Closest value to day 3	
Donati(18) 2014	Human heparan sulfate HS ELISA Kit; Cusabio Biotech Co., Ltd., Wuhan, Hubei Province 430206, China	Sepsis, severer sepsis, septic shock	23.3 (19.7 - 39.5)	N/R	N/R		N/R
		Sepsis, severer sepsis, septic shock	63.1 (40.5 - 96.0)	N/R	N/R		N/R
Hippensteel(23) 2019	N/R	Septic shock	N/R	150*	N/R		N/R
		Sepsis, severer sepsis, septic shock	N/R	200*	N/R		N/R
Nelson(19) 2014	Cat. no. 280564-1, AMS Biotechnology, UK	Septic shock	N/R	30000*	N/R		N/R
Steppan(10) 2011	Seikagaku Corp., Tokyo, Japan	Severe sepsis, septic shock	N/R	3230 ± 2430	5680 ± 2430	Day 3	3450 ± 1860

All values were reported as mean ± standard deviation, median (interquartile range) or other formats that original paper used.
*: When only figure was available, estimated median value was reported.

Supplementary Table 7. Chondroitin sulfate value (ng/ml) among sepsis patients.

Author and Year	Method	Severity of sepsis	Sampling time N/R	Day 1 (0 - 24 hours)	Day 2 (24 - 48 hours)	Closest value to day 3
Nelson(19) 2014	Not commercial kit	Septic shock	N/R	6000*	N/R	N/R

*: When only figure was available, estimated median value was reported.

Chapter 9: Albumin and Dexamethasone for glycocalyx in abdominal surgery

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Basic Science

ORIGINAL CLINICAL RESEARCH REPORT

A Randomized, Multicenter, Open-Label, Blinded End Point, Phase 2, Feasibility, Efficacy, and Safety Trial of Preoperative Microvascular Protection in Patients Undergoing Major Abdominal Surgery

Fumitaka Yanase, MD,*† Shervin H. Tosif, MBBS, MPH,‡ Leonid Churilov, BSc, PhD,§ Ken Yee, MD,‖ Rinaldo Bellomo, MD, PhD,*† Kerry Gunn, MD,‖ Chang Kim, MD,‖ Camilla Krizhanovskii, PhD,¶# Robert G. Hahn, MD, PhD,** Bernhard Riedel, MD, MBA, PhD,††‡‡‡ and Laurence Weinberg, MD‡‡‡

BACKGROUND: The endothelial glycocalyx, a carbohydrate-rich layer coating all endothelial surfaces, plays a fundamental role in the function of microcirculation. The primary aim of this study was to evaluate the feasibility of using dexamethasone and albumin to protect the endothelial glycocalyx in patients undergoing abdominal surgery. Secondary and exploratory outcomes included efficacy and safety.

METHODS: We conducted a multicenter, open-label, blinded end point, phase 2, randomized trial. Patients undergoing colorectal, pancreas, or liver surgery were recruited and randomized to receive either intravenous dexamethasone (16 mg) and 20% albumin (100 mL) at induction of anesthesia, then 200 mL of 20% albumin with each subsequent 1000 mL of crystalloid administered (dexamethasone and albumin [Dex-Alb] group), or crystalloid fluid only with no dexamethasone (control group). Feasibility end points included patient recruitment and retention, consent rate, and successful study drug administration. The primary efficacy end point was the measurement of plasma syndecan-1 level on postoperative day (POD) 1, and secondary end points were heparan sulfate levels and inflammatory markers measured at 4 perioperative timepoints. Safety end points included errors in administration of the intervention, hyperglycemia, occurrence of postoperative complications, and patient retention.

RESULTS: Seventy-two patients were randomized. All feasibility end points were achievable. There were no statistically significant differences observed in median (interquartile range) syndecan-1 levels on POD 1 (39 ng·mL^{-1} [20–97] in the Dex-Alb group versus 41 ng·mL^{-1} [19–84] in the control group; difference in medians −2.1, 95% confidence interval [CI], −13 to 8.6; $P = .69$). The Dex-Alb group had lower POD 1 heparan sulfate levels (319 ng·mL^{-1} [161–717] in the Dex-Alb group versus 1422 [670–2430] ng·mL^{-1} in the control group; difference in medians −1085, 95% CI, −1779 to −391) and C-reactive protein (CRP) levels on POD 1 (48 [29–77] mg·L^{-1} in the Dex-Alb group versus 85 mg·L^{-1} [49–133] in the control group; difference in medians −48, 95% CI, −75 to −21). Fewer patients had one or more postoperative complication in the Dex-Alb group than in the control group (6 [17%] vs 18 patients [50%]; odds ratio = 0.2, 95% CI, 0.06–0.6).

CONCLUSIONS: Intravenous dexamethasone and albumin administration was feasible but did not reduce syndecan-1 on POD 1 in patients undergoing abdominal surgery. Given the clinically important CIs observed between the groups for heparan sulfate, CRP, and postoperative complications, a larger trial assessing the associations between dexamethasone and albumin administration and these outcomes is warranted. (Anesth Analg XXX;XXX:00–00)

From the *Department of Intensive Care, Austin Hospital, Melbourne, Australia; †Australian and New Zealand Intensive Care Research Centre (ANZIC-RC), School of Public Health and Preventive Medicine, Monash University, Melbourne, Australia; ‡Department of Anaesthesia, Austin Hospital, Melbourne, Australia; §Department of Medicine (Austin Health) & Melbourne Brain Centre at Royal Melbourne Hospital, Melbourne Medical School Faculty of Medicine, The University of Melbourne, Melbourne, Australia; ‖Department of Anaesthesia, Auckland City Hospital, Auckland, New Zealand; ¶Department of Research, Södertälje Hospital, Södertälje, Sweden; #Department of Molecular Medicine and Surgery, Karolinska Institute, Solna, Sweden; **Karolinska Institute at Danderyd's Hospital (KIDS), Stockholm, Sweden; ††Department of Anaesthesia, Perioperative and Pain Medicine, Peter MacCallum Cancer Centre; and the Sir Peter MacCallum Department of Oncology, University of Melbourne, Melbourne, Australia; and ‡‡‡Department of Critical Care, University of Melbourne, Melbourne, Australia.

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Address correspondence to Laurence Weinberg, MD, Department of Anaesthesia, Austin Hospital, Studley Rd, Heidelberg, VIC 3084, Australia. Address e-mail to laurence.weinberg@austin.org.au.

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KEY POINTS

- **Question:** Does dexamethasone and albumin protect the endothelial glycocalyx in patients undergoing abdominal surgery?
- **Findings:** The intervention did not reduce syndecan-1 levels but did reduce heparan sulfate, C-reactive protein, and postoperative complications without any adverse event.
- **Meaning:** Dexamethasone and albumin was safe, and a further phase 2b/3 trial is feasible.

GLOSSARY

ANCOVA = analysis of covariance; **ASA** = American Society of Anesthesiologists; **CI** = confidence interval; **CONSORT** = Consolidated Standards of Reporting Trials; **CRP** = C-reactive protein; **CVD** = Clavien-Dindo Grade; **DAH-30** = days alive and out of hospital at 30 days; **DASI** = Duke Activity Status Index; **Dex-Alb group** = dexamethasone and albumin group; **EGL** = endothelial glycocalyx layer; **ELISA** = enzyme-linked immunosorbent assay; **GAG** = glycosaminoglycan; **ICU** = intensive care unit; **IQR** = interquartile range; **IV** = intravenous; **NA** = not applicable; **POD** = postoperative day; **REDCap** = Research Electronic Data Capture; **ROC** = receiver operator curve; **S1P** = sphingosine-1-phosphate; **WCC** = white blood cell count

The endothelial glycocalyx layer (EGL) is a carbohydrate-rich layer lining the surface of the vascular endothelium. It consists of a core of endothelial membrane–bound proteoglycans and glycoproteins, to which glycosaminoglycan (GAG) side chains are attached. In turn, these enable albumin, antithrombin III, thrombomodulin, and other molecules to bind and modulate EGL permeability.[1–3] The structure and functions of the EGL are summarized in Supplemental Digital Content, Figure 1, http://links.lww.com/AA/D607. Although strategies that normalize macrocirculation have improved perioperative outcomes,[4] little attention has been given to strategies that protect the EGL.

EGL injury is associated with shedding of its components, resulting in increased plasma concentrations of EGL constituents such as syndecan-1 and heparan sulfate. Syndecan-1 is a single-pass transmembrane proteoglycan that can bind up to 5 GAGs, the most common of which is heparan sulfate.[5] In animal models, shedding of syndecan-1 and heparan sulfate has been reported in response to hyperglycemia,[6] hemorrhagic shock,[7] and ischemia-reperfusion injury.[8] Shedding of the endothelial glycocalyx in humans has also been demonstrated with hypervolemia[9] and during cardiac surgery.[10,11] In the setting of sepsis following abdominal surgery, elevated endothelial glycocalyx breakdown markers were associated with worse outcomes.[12] Therefore, interventions to preserve microvascular function during surgery have been proposed as a potential target for reducing postoperative complications and improving postoperative mortality.[13]

Corticosteroids and albumin have been shown to reduce endothelial glycocalyx damage in animal models of ischemia-reperfusion injury. Corticosteroids reduce the production of inflammatory cytokines that have a disruptive effect on the EGL,[14] while albumin appears to stabilize the EGL through electrostatic interactions with GAGs or sphingosine-1-phosphate (S1P).[15] In addition, both agents are readily available to most clinicians and are relatively free of side effects. In Australia, albumin is provided free of charge to clinicians by the National Blood Authority but can carry significant expense in other countries.

However, it is unknown whether intravenous (IV) dexamethasone and albumin can be used to protect the EGL in patients undergoing major abdominal surgery. Therefore, we conducted a multicenter, randomized, open-label, blinded end point, phase 2 trial to investigate the feasibility, safety, biological, and physiological effects of dexamethasone and albumin in patients undergoing major abdominal surgery. We hypothesized that, in these patients, administering dexamethasone and albumin would reduce markers of EGL shedding (syndecan-1 and heparan sulfate) compared to a control group that did not receive these interventions. The combined treatment was adopted to assess the feasibility of the protocol and efficacy of the intervention in anticipation of a future phase 2b/3 randomized trial.

METHODS

The trial was approved by the Austin Hospital Ethics Committee (no. 17/Austin/397), Melbourne, Australia, on November 22, 2017, and conducted across 2 Australian hospitals and 1 New Zealand hospital. Before patient enrollment, the trial was registered at the Australian New Zealand Clinical Trial Registry (ACTRN12618000361202, principal investigator: Shervin Tosif, date of registration: April 9, 2018) and is reported according to the Consolidated Standards of Reporting Trials (CONSORT) guidelines.[16] Written informed consent was obtained from all participants.

This was an investigator-initiated, multicenter, randomized, open-label, blinded end point trial comparing the effect of dexamethasone and albumin

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coadministration on plasma markers of EGL breakdown, compared with standard management in patients undergoing major abdominal surgery.

We selected 3 types of major abdominal surgery associated with significant perioperative stress response a priori, namely, pancreas, colorectal, and liver resection surgeries.[17–19] We included adult patients (≥18 years of age) requiring arterial cannulation for continuous hemodynamic monitoring, with an expected hospital stay of at least 1 postoperative night. Exclusion criteria were an American Society of Anesthesiologists (ASA) physical status score of V, pregnancy or breastfeeding, poorly controlled diabetes mellitus (hemoglobin A1c >9.0% or hemoglobin A1c >75 mmol·mol^{-1}), allergy to albumin or dexamethasone, chronic steroid use defined as prednisolone 10 mg per day or equivalent for greater than 1 week in the preceding 3 months, immunosuppressive drugs, and any surgical procedure within the preceding 2 months or expected within the subsequent 30 days.

We randomly assigned patients (1:1) by stratified randomization to control group or dexamethasone and albumin group (Dex-Alb group). Stratified randomization was performed using a permuted block random allocation algorithm to allocate equal numbers of patients to the Dex-Alb group or control group within each surgery type (liver, pancreas, bowel resection). After patients provided informed consent, randomization was performed by qualified research staff, who were independent from the clinical team, using the centralized Research Electronic Data Capture (REDCap) electronic system with a concealed allocation sequence.[20] REDCap electronic data capture tools are hosted at the University of Melbourne. REDCap is a secure, web-based software platform designed to support data capture for research studies.

Patients in the Dex-Alb group received 16 mg IV dexamethasone and 100 mL of 20% IV albumin solution over 30 minutes before skin incision, with ongoing fluid therapy that comprised a balanced crystalloid (Plasmalyte; Plasmalyte 148, Baxter Healthcare or Hartmann's solution; Compound Sodium Lactate, Baxter Healthcare) containing 200 mL of 20% albumin within every 1000 mL of crystalloid infused (a total of 1200 mL, 3.3% albumin solution equivalent). The physiochemical properties of the fluid solutions are summarized in Supplemental Digital Content, Table 1, http://links.lww.com/AA/D607.

Patients in the control group did not receive any IV steroid or albumin during their surgery. They received 100 mL of a physiological, balanced salt solution (Plasmalyte or Hartmann's solution) over 30 minutes before skin incision. For maintenance and resuscitation, they received the same balanced salt solution. Use of albumin was allowed in the control group only if it was considered critical to the patient's hemodynamic state.

All patients underwent preoperative optimization of hemoglobin according to the National Blood Authority Patient Blood Management guidelines,[21] and glycemic optimization according to the Australian Diabetes Society guidelines.[22] Perioperative anesthesia and surgical care were managed according to standard institutional practices. Hemodynamic management, including use of vasoactive medications and fluid therapy, was guided by advanced hemodynamic monitoring (EV1000 clinical platform, Edwards Lifesciences) that included the measurements of stroke volume variation, stroke volume, and cardiac output. All patients received lung-protective ventilation. Postoperative care was managed by the surgical/intensive care unit (ICU) teams using existing departmental enhanced postsurgery recovery protocols. The use of postoperative fluid and blood was at the discretion of the surgical/ICU team.

We collected patients' blood (serum and plasma) at 5 time points: before induction of anesthesia, at the end of surgery, on the evening of the day of surgery (postoperative day [POD] 0 night), and on POD 1 and POD 2. All samples were centrifuged at 3000 rpm for 10 minutes, separated, and stored at −80 °C. Routine perioperative blood samples (white blood cell count [WCC], C-reactive protein [CRP], or blood glucose) were measured by central laboratory analyzers at each trial site.

Study End Points

Feasibility end points included patient recruitment and retention, consent rate, and successful study drug administration. Efficacy end points included plasma syndecan-1 levels, heparan sulfate levels, and inflammatory biomarkers. We defined the primary efficacy outcome as plasma syndecan-1 level at POD 1. Syndecan-1 is the biomarker most commonly used to assess EGL damage, with peak levels reached within the first 24 hours postoperatively.[23,24] Secondary efficacy outcomes included plasma syndecan-1 at the end of surgery, POD 0 night and POD 2, heparan sulfate levels at the end of surgery, POD 0 night, POD1 and POD2, and inflammatory markers (CRP, WCC, and neutrophil/lymphocyte ratio). Safety outcomes included ICU admissions, peak glucose level within the first 24 postoperative hours, presence of one or more postoperative complications at POD 3, days alive and out of hospital at 30 days (DAH-30), and in-hospital mortality. DAH-30 was calculated as 0 when the patient died during their hospital stay. We defined a complication as any deviation from the normal expected postoperative course. Postoperative complications were identified from the conclusion of surgery up to POD 3 using the Postoperative

LWW	4 Color Fig(s):0	07/10/21	15:33	Art: AA-D-21-00011

Morbidity Survey.[25] This is a validated composite outcome measure that assesses the presence or absence of complications within 9 predefined domains. Severity of complications was graded by the Clavien-Dindo Grade (CVD) classification.[26]

Biomarker analysis was performed using commercially available enzyme-linked immunosorbent assay (ELISA) kits according to manufacturer and laboratory protocols. Syndecan-1 was evaluated with human CD138/syndecan-1 (Diaclone) and heparan sulfate with AMS.EH4010 (Amsbio), with coefficient of variation values of 6.2% and <10%, respectively.

Statistical Analysis

We used Stata version 15 (Stata Corp) and R 3.5.2 (The R Foundation for Statistical Computing). Data were coded with numerical values, and variable names were encrypted to blind their characteristics to the statistician. Baseline data were presented as median (interquartile range [IQR]) for continuous variables or as number of cases (percentage) for categorical variables. Continuous variables were compared by Mann-Whitney *U* test, and categorical data were compared by Fisher exact test between the 2 groups. Our predefined primary outcome analysis method was to use a 1-way analysis of covariance (ANCOVA) model with plasma syndecan-1 levels at POD 1 as the dependent variable, treatment group as a factor with 2 levels (control group or Dex-Alb group), and surgery type (colorectal, pancreas, or liver resection) and baseline syndecan-1 level as covariates. However, syndecan-1 data were found to be skewed with no appropriate transformations identified; thus, a quantile regression model was used to assess the difference in medians, with the treatment group as a factor with 2 levels (control group or Dex-Alb group) and surgery type (colorectal, pancreas, or liver resection) and baseline syndecan-1 level as covariates.[27] Accordingly, we prespecified the comparison using median regression as the main approach due to the nature of the outcomes we investigated. Considering the nonparametric nature of the relevant distributions, we provide additional information about the differences across the whole distribution. We have used 25th and 75th percentiles as the common reported metrics.

For longitudinal analyses of syndecan-1 and heparan sulfate over 5 time points (baseline, end of surgery, POD 0 night, POD 1, and POD 2), we used a clustered quantile regression model with individual postoperative time points as categorical variables, adjusted by type of surgery, with individual patients as clusters. Time-by-treatment group interactions were investigated by including relevant multiplicative terms in respective models. For other secondary, exploratory, and safety outcomes, continuous outcomes without baseline values (eg, days out of hospital or peak glucose) were analyzed using a quantile regression model adjusted by type of surgery, comparing the difference in median values. Continuous outcomes with baseline values (eg, syndecan 1 and heparan sulfate at each time point, CRP, WCC, neutrophil/lymphocyte ratio), we used a quantile regression model adjusted by type of surgery and by preoperative baseline values, comparing the difference in median values. For binary outcomes (ICU admission, presence of one or more postoperative complications, and in-hospital mortality), we applied the "Firth" logistic regression, which is particularly suitable for modeling rare outcomes. The threshold for statistical significance was set at 2-sided $P = .05$. All secondary and exploratory end points are reported as point estimates of treatment effects with 95% confidence intervals (CIs).[28]

Sample Size Justification

Clinically significant changes in syndecan-1 levels are not known. In addition, syndecan-1 levels vary significantly in healthy patients according to the assay used.[23] Sample size was estimated using an assumed 2-tailed type I error threshold of 0.05 and a power of 0.8. A total sample size of 72 patients equally distributed (36:36) between the Dex-Alb and control groups was estimated as necessary to observe a large standardized between-group difference (Cohen's $d \geq 0.7$) in the primary outcome. This study was designed to estimate sample size for a future phase 2b/3 trial.

RESULTS

Feasibility End Points

We identified 134 patients from July 2018 to January 2020 who met the inclusion criteria; we excluded 62 patients. The commonest reason for exclusion was unavailability of research staff to recruit the participants (25 patients [19%]). Five patients who underwent liver surgery were excluded because their planned surgery was aborted due to disseminated and inoperable disease. In total, 72 patients were successfully randomized, and 100% of patients received the allocated intervention (Figure 1).

Participant Characteristics

Baseline patient characteristics are summarized in Table 1. The median (IQR) age was 63 years (54–70) in the Dex-Alb group and 62 years (53–71) in the control group. Intraoperative data are presented in Table 2. Propofol-based total IV was the most commonly used anesthesia in the 2 groups. More than half of the patients in both groups also received regional anesthesia. Four patients (11%) in each group received a blood transfusion. The median total fluid volume during anesthesia was 6.3 $mL \cdot kg^{-1} \cdot h^{-1}$

LWW | 4 Color Fig(s):0 | 07/10/21 | 15:33 | Art: AA-D-21-00011

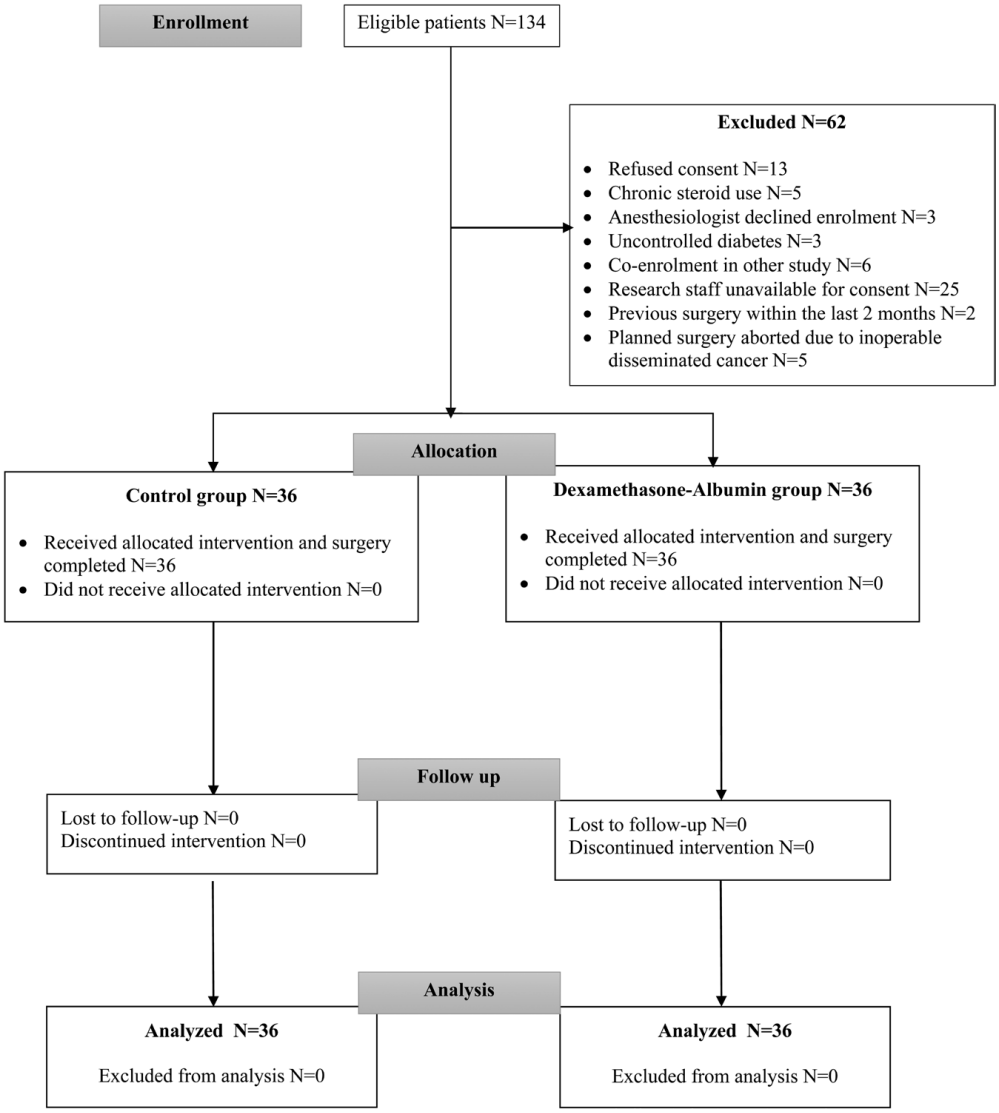

Figure 1. CONSORT diagram. CONSORT indicates Consolidated Standards of Reporting Trials.

(4.8–8.6) in the Dex-Alb group and 8.9 mL·kg^{-1}·h^{-1} (7.8–10) in the control group.

The use of a nonstudy fluid, defined as the use of any 4% albumin or additional 20% albumin in the Dex-Alb group, or the use of any 4% or 20% albumin in the control group, was observed in 3 patients (8.6%) in the Dex-Alb group and 5 patients (14%) in the control group. There were no violations in the delivery of trial protocol.

Primary Efficacy Outcome

Median syndecan-1 levels on POD 1 were 39 ng·mL^{-1} (20–97) in the Dex-Alb group and 41 ng·mL^{-1} (19–84) in the control group (difference in median −2.1 ng·mL^{-1}, 95% CI, −13 to 8.6; $P = .69$). When comparing the 25th and 75th percentile values, no statistically significant differences were observed in syndecan-1 values between the 2 groups in either of these quartiles

LWW	4 Color Fig(s):0	07/10/21	15:33	Art: AA-D-21-00011

Table 1. Baseline Patient Characteristics

Characteristic	Dex-Alb group n = 36	Control group n = 36	Standardized mean difference
Male (%)	16 (44)	21 (58)	0.281
Age (y)	63 (54–70)	62 (53–71)	0.118
Body mass index (kg·m^{-2})	27 (24–29)	26 (22–31)	0.042
ASA physical status (%)			0.256
I	1 (2.8)	1 (2.8)	
II	14 (39)	16 (44)	
III	20 (56)	19 (53)	
IV	1 (2.8)	0 (0.0)	
DASI (points)	51 (32–58)	51 (39–58)	0.141
Comorbidities			
Current smoker (%)	5 (14)	4 (11)	0.084
Diabetes (%)	9 (25)	5 (14)	0.284
Cardiovascular disease (%)	0 (0.0)	2 (5.6)	0.343
Cerebrovascular disease (%)	0 (0.0)	1 (2.8)	0.239
Respiratory disease (%)	5 (14)	2 (5.6)	0.284
Kidney disease (%)	0 (0.0)	0 (0.0)	<0.001
Metastatic disease (%)	7 (19)	7 (19)	<0.001
Statin use (%)	9 (25)	7 (20)	0.134
Baseline clinical laboratory values			
Hemoglobin (g·L^{-1})	138 (129–148)	134 (123–143)	0.278
White blood cell count (10^{9} L^{-1})	7.3 (5.6–9.0)	7.4 (5.2–9.1)	0.147
Neutrophil/lymphocyte ratio	2.7 (1.9–3.3)	2.7 (1.9–4.6)	0.308
Platelets (10^{9} L^{-1})	285 (238–315)	272 (204–358)	0.204
Urea (mmol·L^{-1})	5.1 (4.0–6.4)	5.2 (4.3–6.4)	0.266
Creatinine (μmol·L^{-1})	65 (57–77)	69 (62–75)	0.067
Total bilirubin (μmol·L^{-1})	6.0 (5.0–9.8)	7.0 (5.0–12.0)	0.413
Albumin (g·L^{-1})	38 (36–40)	37 (35–41)	0.001
C reactive protein (mg·L^{-1})	2.5 (1.3–6.3)	4.6 (2.2–11)	0.266
Baseline biomarker values			
Syndecan-1: baseline (ng·mL^{-1})	27 (20–42)	22 (13–44)	0.026
Heparan sulfate: baseline (ng·mL^{-1})	308 (233–512)	258 (199–606)	0.009

Abbreviations: ASA, American Society of Anesthesiologists; DASI, Duke Activity Status Index; Dex-Alb, dexamethasone and albumin.

(Supplemental Digital Content, Table 2, http://links.lww.com/AA/D607).

Secondary and Exploratory Efficacy Outcomes

A time by treatment interaction was not statistically significant between the groups with syndecan-1 (P = .99); however, it was statistically significant with heparan sulfate (P < .001). The key secondary outcomes are presented in Table 3 and Supplemental Digital Content, Table 2, http://links.lww.com/AA/D607. Median syndecan-1 and median heparan sulfate values in the control group peaked on the evening of surgery (Figure 2). Median heparan sulfate levels were lower in the Dex-Alb group on the evening of surgery (difference in medians −1455 ng·mL^{-1}; 95% CI, −2737 to −172) and at POD 1 (difference in medians −1085 ng·mL^{-1}; 95% CI, −1779 to −391). No differences in syndecan-1 levels were identified at any other time points. The time course of perioperative EGL biomarkers and inflammatory markers is shown in Figures 2 and 3, and Supplemental Digital Content, Figures 2 and 3, http://links.lww.com/AA/D607. Median CRP on POD 1 was 48 mg·L^{-1} (29–77) in the Dex-Alb group and 85 mg·L^{-1} (49–133) in the control group (difference in medians −48 mg·L^{-1}; 95% CI, −75 to −21; Figure 3; Table 3).

Safety End Points

Fewer patients had one or more postoperative complication in the Dex-Alb group than in the control group (6 patients [17%] in the Dex-Alb group versus 18 patients [50%] in the control group; odds ratio 0.2; 95% CI, 0.06–0.6; Supplemental Digital Content, Table 4, http://links.lww.com/AA/D607). Peak blood glucose in the first 24 hours after surgery was not higher in the Dex-Alb group (10 mmol·L^{-1} [8.2–12]) than in the control group (9.0 mmol·L^{-1} [7.3–10]). One patient (2.8%) in the Dex-Alb group developed a postoperative pulmonary complications versus 8 patients (22%) in the control group. Six patients (75%) with pulmonary complication in the control group had an increased requirement for oxygen. The severity of the complications is reported in Supplemental Digital Content, Table 5, http://links.lww.com/AA/D607. One patient in the Dex-Alb group died from hepatic artery thrombosis and multiorgan failure after surgery; this patient accounted for all grade IVb complications in the control group.

Subgroup Analysis

The perioperative time course for plasma syndecan-1 and heparan sulfate levels in the 3 subgroups of abdominal surgery is presented in Supplemental

Table 2. Intraoperative Data

Variable	Dex-Alb group n = 36	Control group n = 36	*P*
Maintenance anesthetic drug			
Propofol (%)	21 (58)	25 (69)	.56
Sevoflurane (%)	11 (31)	7 (19)	
Desflurane (%)	4 (11)	4 (11)	
Regional anesthesia			
Regional anesthesia use (%)	28 (78)	28 (78)	>.99
Epidural (%)	0 (0.0)	1 (2.8)	>.99
Spinal (%)	23 (64)	24 (67)	
Transverse abdominis plane block (%)	4 (11)	3 (8.3)	
Local anesthetic infiltration (%)	1 (2.8)	0 (0.0)	
Intraoperative intravenous lidocaine infusion (%)	23 (64)	26 (72)	.61
Lidocaine infusion dose[a] (mg)	300 (290–400)	335 (213–400)	.74
Fluid administration			
Target mean arterial pressure (mm Hg)	70 (65–70)	70 (65–71)	.10
Total fluid volume infused[b] (mL·kg^{-1}·h^{-1})	6.3 (4.8–8.6)	8.9 (7.8–10.4)	.25
Crystalloid (mL)	1500 (938–2000)	2000 (1500–3000)	.088
Albumin 20% (mL)	400 (300–500)	0.0 (0.0–0.0)	<.001
Blood products, patients (%)	4 (11)	4 (11)	>.99
Nonstudy fluid use[c] (%)	3 (8.3)	5 (14)	.71
Other			
Open surgery (%)	31 (86)	29 (81)	.75
Laparoscopic surgery (%)	5 (14)	7 (19)	
Estimated blood loss (mL)	100 (50–300)	200 (50–500)	.35
Anesthesia duration (h)	5.2 (4.5–6.3)	5.7 (4.0–7.1)	.74

Abbreviation: Dex-Alb, dexamethasone and albumin.
[a]Median (interquartile range) dose in patients receiving lidocaine during surgery.
[b]Sum of crystalloid, albumin, and transfusion volumes divided by anesthesia duration.
[c]Use of 4% or 20% albumin in the control group or use of 4% albumin or extra 20% albumin in the Dex-Alb group.

Digital Content, Figures 4 and 5, http://links.lww.com/AA/D607. Heparan sulfate levels were reduced in all groups. This was most pronounced in patients undergoing liver resection surgery. POD 1 plasma levels of biomarkers according to surgery type are presented in Supplemental Digital Content, Table 3, http://links.lww.com/AA/D607.

There were no differences observed in biomarker levels between patients with and without complications (Supplemental Digital Content, Figure 6, http://links.lww.com/AA/D607). Biomarker levels in patients with and without complications stratified by intervention are presented in Supplemental Digital Content, Figure 7, http://links.lww.com/AA/D607. The 25th and 75th percentile results are reported in Supplemental Digital Content, Table 2, http://links.lww.com/AA/D607. No differences were observed for other inflammatory markers (WCC and neutrophil/lymphocyte ratio). Data on 24-hour postoperative fluid intervention are presented in Supplemental Digital Content, Table 6, http://links.lww.com/AA/D607.

DISCUSSION

We performed a multicenter, randomized, open-label, blinded end point trial to investigate the effect of IV dexamethasone and albumin on EGL biomarker levels in patients undergoing major abdominal surgery. The combined treatment was feasible, supporting a future phase 2b/3 randomized trial. We showed that administering dexamethasone and albumin did not significantly reduce syndecan-1 levels at any time point. However, heparan sulfate values on the evening of surgery and POD 1, and CRP values on POD 1 were significantly lower in the Dex-alb group. We also found that biomarker levels reached their peak on the evening of surgery. Moreover, the presence of one or more postoperative complications on POD 3 was significantly lower in the Dex-alb group.

The discordant changes in syndecan-1 and heparan sulfate levels may be explained by the structure of the endothelial glycocalyx (Supplemental Digital Content, Figure 1, http://links.lww.com/AA/D607). Syndecan-1 is a single-pass transmembrane proteoglycan composed of a core protein with up to 5 GAGs side chains attached.[5] The side chains include heparan sulfate and chondroitin sulfate. The core protein may be more stable, and the GAGs side chains may be more sensitive to injury, which may explain why we observed no changes in syndecan-1 levels for either group but significantly less elevation in heparan sulfate levels in the Dex-Alb group.

There are few observational studies assessing syndecan-1 and heparan sulfate levels in patients undergoing surgery and only 1 randomized control trial assessing the impact of an anti-inflammatory intervention on EGL biomarkers postoperatively. Rehm et al[10] measured peak syndecan-1 levels of up to 200-fold

LWW	4 Color Fig(s):0	07/10/21	15:33	Art: AA-D-21-00011

Table 3. Efficacy and Safety Outcomes

Outcome	Dex-Alb group n = 36	Control group n = 36	Difference in medians or effect size (95% CIs)	*P*
Primary efficacy: syndecan-1 levels				
Syndecan-1: POD 1 (ng·mL^{-1})	39 (20–97)	41 (19–84)	−2.1 (−13 to 8.6)[a]	.69[a]
Secondary efficacy: endothelial glycocalyx and inflammatory markers at other time points				
Syndecan-I: postoperation (ng·mL^{-1})	41 (18–91)	33 (15–99)	0.4 (−7.2 to 8.0)[a]	Time-by-treatment interaction *P* = .99
Syndecan-I: POD 0 night (ng·mL^{-1})	52 (37–97)	80 (24–120)	−7.8 (−38 to 22)[a]	
Syndecan-I: POD 2 (ng·mL^{-1})	27 (20–99)	31 (16–54)	2.5 (−8.2 to 13)[a]	
Heparan sulfate: postoperation (ng·mL^{-1})	307 (214–552)	1137 (562–2602)	−709 (−1433 to 15)[a]	Time-by-treatment interaction *P* < .001
Heparan sulfate: POD 0 night (ng·mL^{-1})	583 (140–935)	1758 (938–3687)	−1455 (−2737 to −172)[a]	
Heparan sulfate: POD 1 (ng·mL^{-1})	319 (161–717)	1422 (670–2430)	−1085 (−1779 to −391)[a]	
Heparan sulfate: POD 2 (ng·mL^{-1})	608 (228–1765)	638 (385–1573)	−151 (−627 to 326)[a]	
Exploratory efficacy				
C-reactive protein: POD 1 (mg·L^{-1})	48 (29–77)	85 (49–133)	−48 (−75 to −21)[a]	NA
White cell count: POD 1 (10^{9} L^{-1})	12 (10–14)	11 (9.6–13)	1.1 (−0.7 to 2.8)[a]	
Neutrophil/lymphocyte ratio: POD 1	10 (6.2–13)	8.9 (7.4–15)	1.3 (−2.0 to 4.6)[a]	
Safety				
Peak blood glucose within first 24 postoperative hours (mmol·L^{-1})	10 (8.2–12)	9.0 (7.3–10)	0.9 (−0.6 to 2.4)[b]	NA
ICU admission (%)	6 (17)	5 (14)	1.2 (0.3–4.3)[b]	
Presence of one or more postoperative complications on POD 3 (%)	6 (17)	18 (50)	0.2 (0.06–0.6)[b]	
In-hospital mortality (%)	1 (2.8)	0 (0)	NA	
Days alive at home at 30 d	20 (10–23)	19 (14–21)	1.0 (−3.7 to 5.7)[b]	

Abbreviations: CI, confidence interval; Dex-Alb, dexamethasone and albumin; ICU, intensive care unit; NA, not applicable; POD, postoperative day.
[a]Adjusted by baseline value and surgery strata.
[b]Adjusted by surgery strata.

from baseline in the setting of cardiac bypass surgery and up to 40-fold for heparan sulfate, and thus these markers were considered sensitive for endothelial glycocalyx injury. Steppan et al[24] showed that both syndecan-1 and heparan sulfate were elevated in the setting of abdominal surgery and sepsis. Interestingly, they found syndecan-1 levels to be higher in septic patients and heparan sulfate levels to be higher in surgical patients. This corresponds to the pattern of biomarker elevation observed in our study, with a relatively larger increase in heparan sulfate levels than syndecan-1 levels. We postulate that this reflects a different range of inflammatory mediators with varied effects on the endothelial glycocalyx. We identified only 1 study investigating the effect of corticosteroids (hydrocortisone 100 mg IV preoperatively) on the endothelial glycocalyx.[29] Patients undergoing cardiac surgery had lower levels of heparan sulfate and CRP in the hydrocortisone group and no significant difference in syndecan-1 levels.

Albumin could maintain EGL integrity and vascular fluid homeostasis more effectively than crystalloids. Albumin has been shown to reduce the release of syndecan-1 and heparan sulfate in a guinea pig cardiac model of ischemia-reperfusion injury.[15,30] In addition to albumin binding to the EGL by electrostatic forces, albumin facilitates the release of S1P from red blood cell. S1P preserves the barrier function of the vascular endothelium and is shown to reduce EGL degradation.[31,32]

The anti-inflammatory effect of glucocorticoids is also established.[33] Exposure to proinflammatory mediators such as interleukins is known to increase EGL breakdown.[34] The release of these mediators is reduced with the administration of glucocorticoids.[35] We found a significant reduction in respiratory complications that may be related to the endothelial-alveolar richness of the lung. In a preclinical lipopolysaccharide model of sepsis, there was reported thinning of the EGL in lung microvessels due to heparan sulfate degradation.[36]

The clinical significance of reduced perioperative heparan sulfate levels in the Dex-Alb group is unclear. Similar to our trial, prior research found that, in patients undergoing abdominal surgery, heparan sulfate levels rose more in the perioperative period than did syndecan-1, but the article did not report on any clinical outcomes.[24] Conversely, another study reported that heparan sulfate levels during the bypass period were associated with unfavorable microcirculatory perfusion, and that microcirculatory perfusion remained impaired for several days postoperatively.[37] Thus, further investigation is required to determine whether our results translate into improved microcirculatory perfusion.

Results of this study suggest that IV albumin and dexamethasone may reduce the postoperative inflammatory response and EGL disruption secondary to surgery as reflected by reduced postoperative heparan sulfate and CRP values. Moreover, the present

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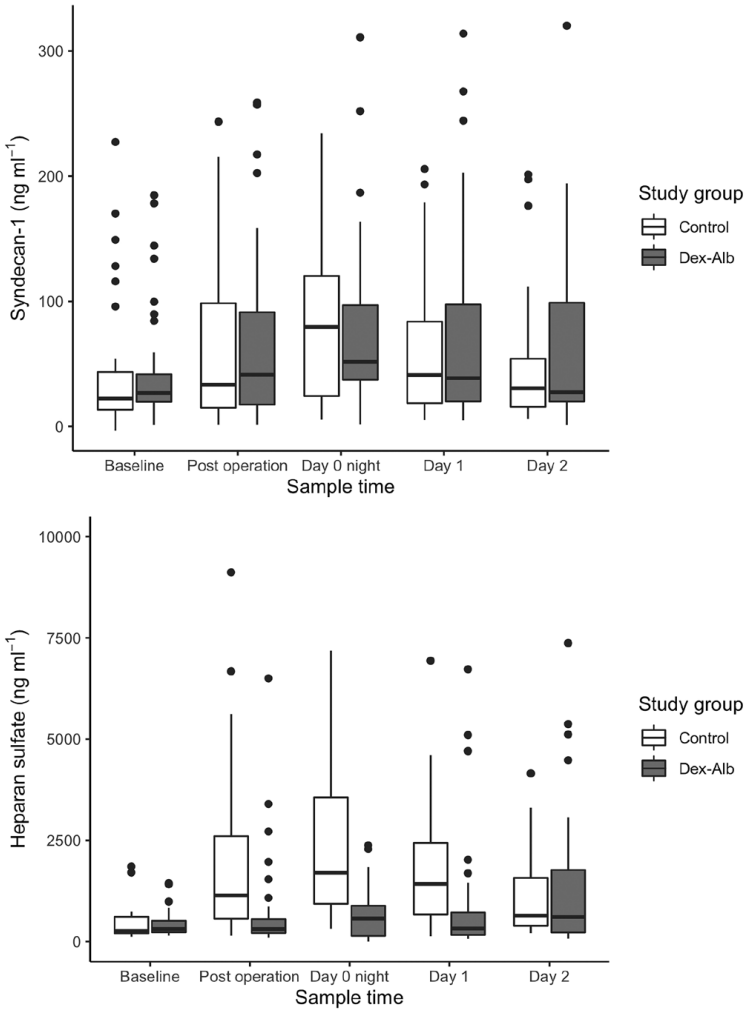

Figure 2. Perioperative time course of changes in plasma syndecan-1 and heparan sulfate levels. One patient in the control group reported a heparan sulfate value of 18,880 ng·mL^{-1} on the evening of the day of surgery, 1 patient in the Dex-Alb group reported a heparan sulfate value of 18,001 ng·mL^{-1} on the evening of the day of surgery. These were included for analysis. Dex-Alb indicates dexamethasone and albumin group.

results also suggest that dexamethasone and albumin can be administered safely. Finally, the results indicate that heparan sulfate levels may be a more sensitive marker of endothelial glycocalyx disruption.

This study has several limitations. First, the interventions were not blinded because of the physical appearance of albumin. Thus, a pragmatic decision was made to conduct the trial as an open-label blinded end point study. We chose a study design that involved coadministration of 2 interventional drugs. We considered that such an approach would have the disadvantage of precluding assessment of their individual effects. However, based on the evidence available at the time, we reasoned that these 2 interventions would likely be synergistic by acting on complementary protective pathways and would increase the likelihood of a meaningful biochemical/physiological/biologic result to better inform a future phase 2b or phase 3 study. Selecting syndecan-1 levels on POD1 as the primary outcome resulted in a negative study.

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Protecting the Glycocalyx During Abdominal Surgery

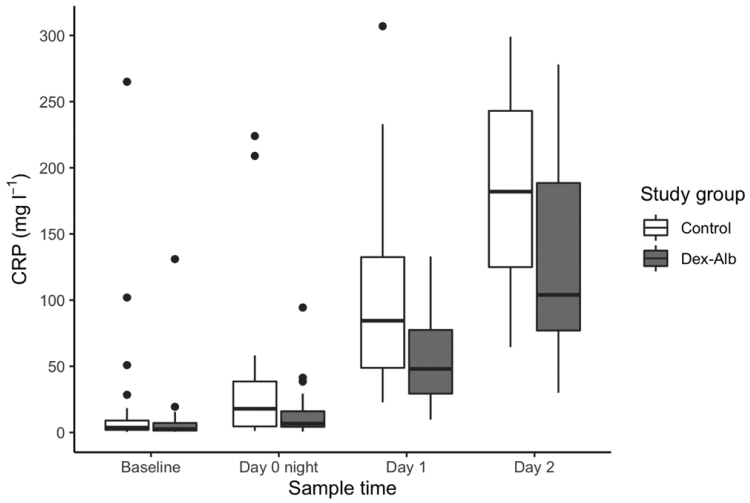

Figure 3. Perioperative time course of plasma CRP levels. CRP indicates C-reactive protein; Dex-Alb, dexamethasone and albumin group.

However, in our review, syndecan-1 was the biomarker most commonly used to assess EGL injury.[23] Eight patients received nonstudy fluids due to clinical necessity. Seven of these patients also received blood transfusion due to intraoperative bleeding. Five patients were excluded, as their planned procedure was canceled due to inoperable disseminated cancer diagnosed intraoperatively; however, inoperability could not have been detected before surgery.

This study has some strengths and provides important information to inform the design of a future phase 2b/3 trial. First, although the literature has reported marked variations in inflammatory biomarkers,[23] we observed significant differences in heparan sulfate levels between the 2 groups on the first postoperative night and on POD 1 (Table 3). We performed post hoc receiver operator curve (ROC) analysis to help determine which postoperative time point would have the best discriminatory threshold for measuring heparan sulfate levels in future studies. ROC analysis showed the most discriminatory time points to be on POD 0 night (ROC value = 0.83; 95% CI, 0.71–0.95), followed by the end of surgery (ROC value = 0.80; 95% CI, 0.66–0.93), and finally on POD 1 (ROC value = 0.80; 95% CI, 0.65–0.93). Moreover, at these time points, we observed a difference in median heparan sulfate values between the groups on the order of approximately 1000 ng·mL^{-1}. Considering the small IQRs in the intervention group (Figure 2), this is consistent with a medium-to-large effect size and provides valuable information to inform sample size calculations for a future phase 2b/3 trial. We are considering the next trial to be a seamless phase 2b/3 design, in which the phase 2b component will use the suitably chosen biomarker outcomes based on the evidence generated by our current study. Should the phase 2b trial demonstrate sufficient promise on the chosen biomarker outcome (heparan sulfate), we will seamlessly convert it into a phase 3 study with complications as the key clinical outcomes. This can be implemented as a multiarm, multistage trial, with 2 stages that correspond to the phase 2b and phase 3 components.

In conclusion, the administration of dexamethasone and albumin was feasible but did not reduce syndecan-1 levels in patients undergoing abdominal surgery. A reduction in postoperative pulmonary complications was observed; however, further investigation is required to determine whether this clinical outcome is causally related to biological interventions. Given the clinically important CIs observed between the groups for heparan sulfate, CRP, and postoperative complications, a larger trial assessing the associations between dexamethasone and albumin administration and these outcomes is warranted.

DISCLOSURES

Name: Fumitaka Yanase, MD.
Contribution: This author helped with study design, patient recruitment, data collection, data analysis, data interpretation, manuscript preparation, critical revision, and final approval.
Conflicts of Interest: F. Yanase received scholarships for his PhD course from the Japan Student Services Organization and Endeavour scholarship. The funding agencies had no role in the design and conduct of the study; collection, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit.
Name: Shervin H. Tosif, MBBS, MPH.

Contribution: This author helped study design, patient recruitment, data collection, data interpretation, manuscript preparation, critical revision, and final approval.
Conflicts of Interest: None.
Name: Leonid Churilov, BSc, PhD.
Contribution: This author helped with study design, data collection, data analysis, data interpretation, manuscript preparation, critical revision, and final approval.
Conflicts of Interest: None.
Name: Ken Yee, MD.
Contribution: This author helped with patient recruitment, data collection, data interpretation, critical revision, and final approval.
Conflicts of Interest: None.
Name: Rinaldo Bellomo, MD, PhD.
Contribution: This author helped with study design, data collection, data interpretation, manuscript preparation, critical revision, and final approval.
Conflicts of Interest: None.
Name: Kerry Gunn, MD.
Contribution: This author helped with patient recruitment, data collection, data interpretation, critical revision, and final approval.
Conflicts of Interest: None.
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Contribution: This author helped with study design, patient recruitment, data collection, data interpretation, manuscript preparation, critical revision, and final approval.
Conflicts of Interest: None.
Name: Laurence Weinberg, MD.
Contribution: This author helped with study design, patient recruitment, data collection, data interpretation, manuscript preparation, critical revision, and final approval.
Conflicts of Interest: None.
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Supplemental Table 1: Physiochemical differences of fluids administered compared to human plasma.

	Plasma	**Compound Sodium Lactate**	**Plasma-Lyte 148**	**Albumex 20% (200g l^{-1} human albumin)**	**Albumex 4% (40g l^{-1} human albumin)**
Sodium (mmol l^{-1})	136 - 145	129	140	48-100	140
Potassium (mmol l^{-1})	3.5 - 5.0	5	5	0	0
Magnesium (mmol l^{-1})	0.8 - 1.0	0	1.5	0	0
Calcium (mmol l^{-1})	2.2 - 2.6	2.5	0	0	0
Chloride (mmol l^{-1})	98 - 106	109	98	0	128
Acetate (mmol l^{-1})	0	0	27	0	0
Gluconate (mmol l^{-1})	0	0	23	0	0
Lactate (mmol l^{-1})	0.5-2.0	29	0	0	0
Octanoate (mmol l^{-1})	0	0	0	32	6.4
Theoretical osmolarity (mosmol l^{-1})	291	278	295	Not stated	Not stated
Actual or measured *osmolality (mosmol kg H_2O^{-1})	287	256	271	130	260
pH	7.35 - 7.45	5 - 7	4 - 8	6.7 - 7.3	6.7 - 7.3

* Freezing point depression
Plasma-Lyte 148 manufactured by Baxter Healthcare, Toongabie, NSW, Australia
Hartmann's solution manufactured by Baxter Healthcare, Toongabie, NSW, Australia
Albumex manufactured by CSL Behring Pty Ltd, Broadmeadows VIC, Australia

Supplemental Table 2. Results of quantile regression analysis for primary and secondary outcomes.

		25th percentile	75th percentile
		Adjusted	Adjusted
Postoperative Day 1: Syndecan-1 levels (ng ml^{-1})*	Difference	-0.3	6.2
	95% CI	-7.8 to 7.3	-21 to 33
	p-value	0.95	0.65
Postoperative Day 1: Heparan Sulfate levels (ng ml^{-1})*	Difference	-379	-1744
	95% CI	-907 to 150	-2852 to -637
	p-value	0.16	0.003
Postoperative Day 1: Peak blood glucose (mmol l^{-1})†	Difference	1.3	0.2
	95% CI	-0.1 to 2.7	-2.0 to 2.3
	p-value	0.075	0.89
Postoperative Day 1: White cell count (10^9 l^{-1})*	Difference	1.5	0.8
	95% CI	-0.3 to 3.4	-1.4 to 3.0
	p-value	0.10	0.46
Postoperative Day 1 Neutrophil/Lymphocyte ratio*	Difference	-0.9	-1.5
	95% CI	-4.2 to 2.3	-7.6 to 4.7
	p-value	0.58	0.63
Postoperative Day 1 C-reactive protein (mg l^{-1})	Difference	-18	-50
	95% CI	-45 to 8.6	-94 to -5.7
	p-value	0.18	0.028
Days Alive at Home within 30 days†	Difference	-5	2
	95% CI	-13 to 3.2	-1.4 to 5.4
	p-value	0.23	0.24

*: Adjusted by baseline value and study group.
†: Adjusted by surgery strata.

50th percentile data (median difference) are presented in Table 3.

Supplemental Table 3. Postoperative Day 1 plasma levels of biomarkers according to the surgery type.

	Dex-Alb group	Control group	Adjusted difference in medians and 95% CI	Adjusted P value
Syndecan-1 (ng ml^{-1})				
Colorectal surgery	24 (16 to 33)	22 (18 to 26)	0.8 (-9.8 to 11)	0.88
Pancreas surgery	39 (17 to 53)	38 (13 to 62)	8.7 (-17 to 34)	0.49
Liver surgery	121 (43 to 190)	84 (49 to 115)	-36 (-145 to 74)	0.51
Heparan sulfate (ng ml^{-1})				
Colorectal surgery	231 (173 to 1735)	1021 (434 to 1889)	-359 (-2490 to 1773)	0.73
Pancreas surgery	389 (155 to 976)	1558 (579 to 2499)	-1178 (-2539 to 182)	0.086
Liver surgery	300 (172 to 360)	1902 (1370 to 2320)	-1604 (-2463 to -745)	0.001

All results were adjusted by baseline biomarker values.

Supplemental Table 4. Postoperative Morbidity Survey (POMS) conducted on postoperative on day 3.

POMS domain	Dex-Alb group	Control group	P value
Patients with at least one postoperative complication (%)	6 (17)	18 (50)	0.005
Pulmonary complication (%)†	1 (2.8)	8 (22)	0.028
Infection (%)‡	3 (8.3)	3 (8.3)	>0.99
Renal complication (%)[#]	2 (5.6)	4 (11)	0.67
Gastrointestinal complication (%)^	2 (5.6)	5 (14)	0.43
Cardiovascular complication (%)*	1 (2.8)	4 (11)	0.36
Neurological complication (%)[§]	1 (2.8)	0 (0.0)	>0.99
Haematological complication (%)‡‡	1 (2.8)	2 (5.6)	>0.99
Wound complication (%)[##]	0 (0.0)	0 (0.0)	NA
Pain complication (%)^^	0 (0.0)	0 (0.0)	NA

† new requirement for oxygen or respiratory support; ‡ on antibiotics and/or has had a temperature of >38˚C in the last 24 h; [#] presence of oliguria <500 ml 24 h^{-1}, increased serum creatinine (30% from preoperative level); urinary catheter in situ for nonsurgical reason; ^ unable to tolerate an enteral diet for any reason including nausea, vomiting, and abdominal distension; * diagnostic tests or therapy within the last 24 h for any of the following, new MI or ischaemia, hypotension (requiring fluid therapy 200 ml hr^{-1} or pharmacological therapy, atrial or ventricular arrhythmias, cardiogenic pulmonary oedema and thrombotic event (requiring anticoagulation); [§] new focal neurological deficit, confusion, delirium, or coma; ‡‡ requirement for any of the following within the last 24 h: packed erythrocytes, platelets, fresh-frozen plasma, or cryoprecipitate; [##] wound dehiscence requiring surgical exploration or drainage of pus from the operation wound with or without isolation of organisms; ^^ new postoperative pain significant enough to require parenteral opioids or regional analgesia

Supplemental Table 5. Severity of complications on day 3 as defined by Clavien-Dindo classification. Data presented as counts in each allocation group.

Type of complication	Severity of complication	Dex-Alb group	Control group
Pulmonary			
	I	0	6
	II	1	2
Infection			
	I	1	1
	II	2	2
Renal			
	I	1	4
	IV-b	1†	0
Gastrointestinal			
	I	0	4
	II	1	1
	IV-b	1†	0
Cardiovascular			
	I	0	1
	II	0	3
	IV-b	1†	0
Neurological			
	IV-b	1†	0
Haematological			
	II	0	2
	IV-b	1†	0
Total			
	I	2	16
	II	4	10
	III	0	0
	IV	5†	0
	All Grades	11	26

† Note. All grade IV-b complications occurred in a single patient with multiple organ dysfunction.

Supplemental Table 6. 24-hour postoperative fluid requirements

	Dex-Alb group	Control group	P value
Crystalloids			
Use of crystalloid (%)	36 (100)	36 (100)	>0.99
Total volume of crystalloid (ml)	2000 (1600 to 2450)	1855 (1230 to 2500)	0.44
Colloid			
Use of 4% albumin (patients, %)	4 (11)	7 (19)	0.51
4% albumin volume* (ml)	500 (438 to 500)	500 (500 to 750)	0.32
Use of 20% albumin (patients, %)	2 (5.7)	5 (14)	0.43
20% albumin volume† (ml)	150 (125 to 175)	200 (200 to 200)	0.46
Transfusion			
Use of blood product (%)	2 (5.6)	2 (5.6)	>0.99
Red blood cell‡ (units)	1.5 (1.3 to 1.8)	1.5 (1.3 to 1.8)	>0.99
Fresh frozen plasma‡ (units)	0.0 (0.0 to 0.0)	0.0 (0.0 to 0.0)	N/A
Platelet‡ (units)	0.0 (0.0 to 0.0)	0.0 (0.0 to 0.0)	N/A
Total volume			
Total fluid volume§ (ml kg^{-1} $hour^{-1}$)	1.1 (0.8 to 1.5)	1.1 (0.7 to 1.7)	0.97

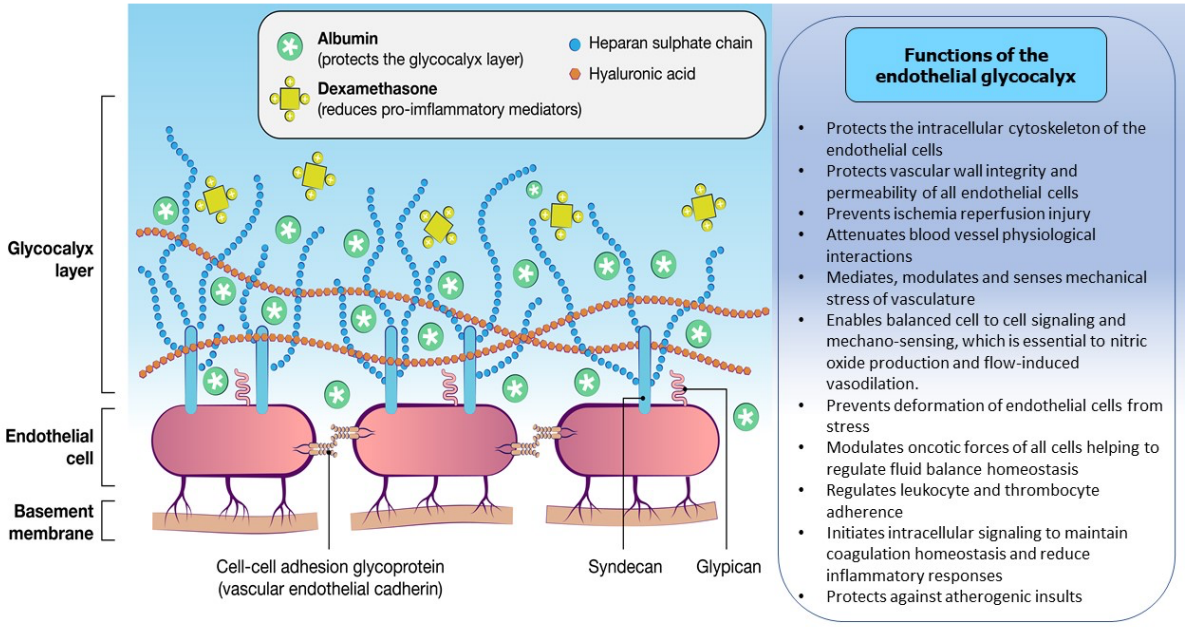
* Median (interquartile range) dose for patients receiving 4% albumin.

† Median (interquartile range) dose for patients receiving 20% albumin.

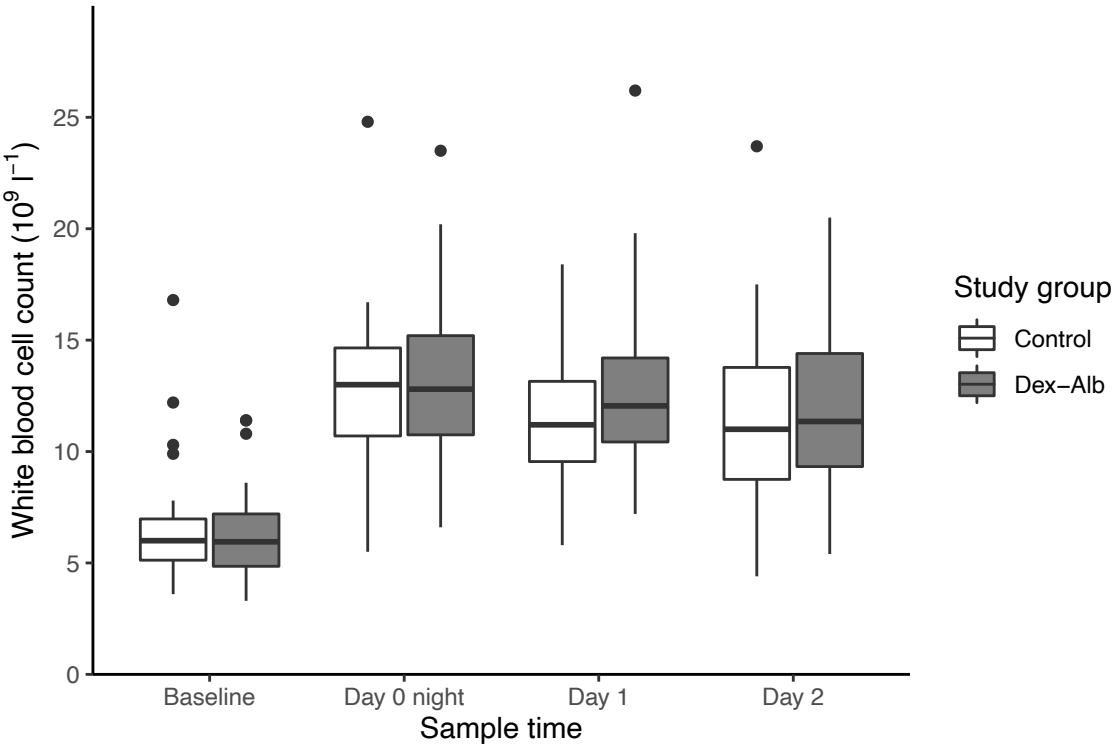
‡ Median (interquartile range) dose for patients receiving transfusion.

§ Sum of crystalloid, colloid and transfusion volume.

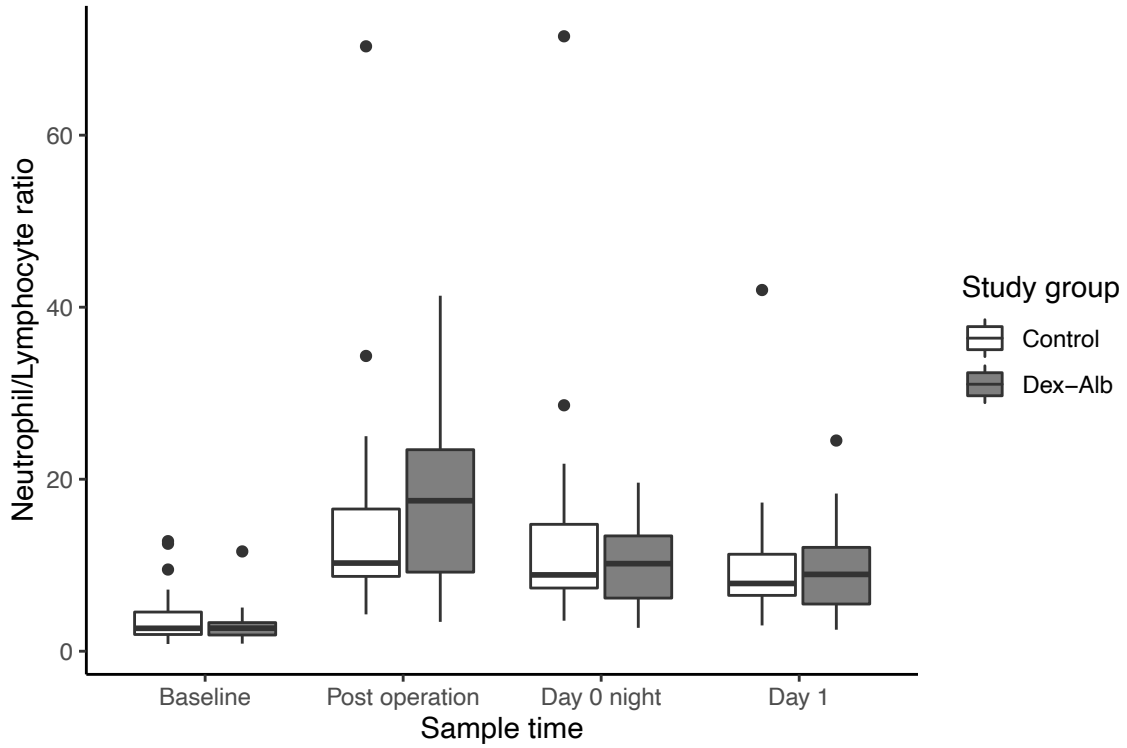
Supplemental Figure 1. Structure and functions of the endothelial glycocalyx layer.



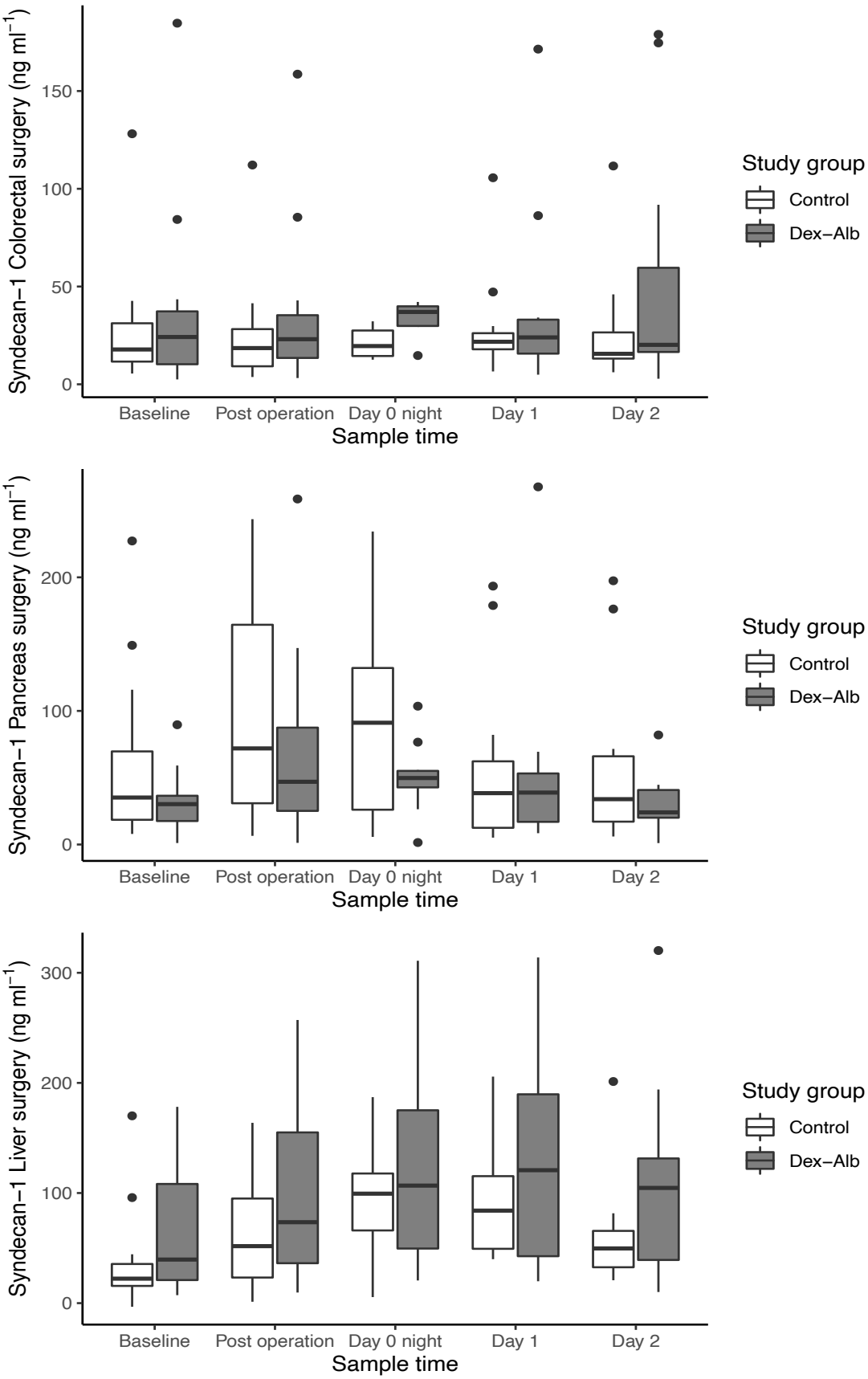
Supplemental Figure 2. Perioperative time course for white blood cell count



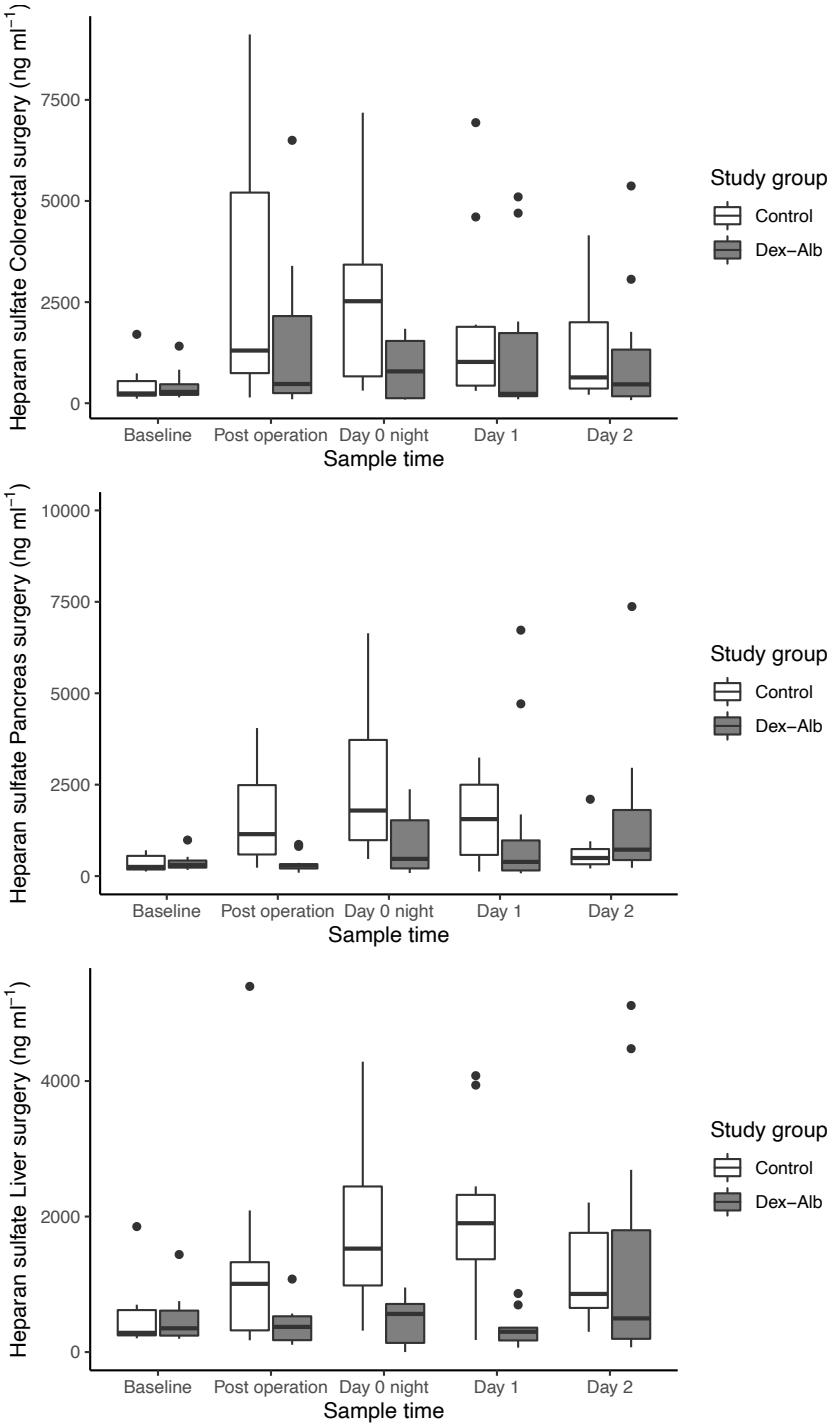
Supplemental Figure 3. Perioperative time course for neutrophil/lymphocyte ratio



Supplemental Figure 4. Perioperative time course for plasma syndecan-1 values in the three subgroups of abdominal surgery

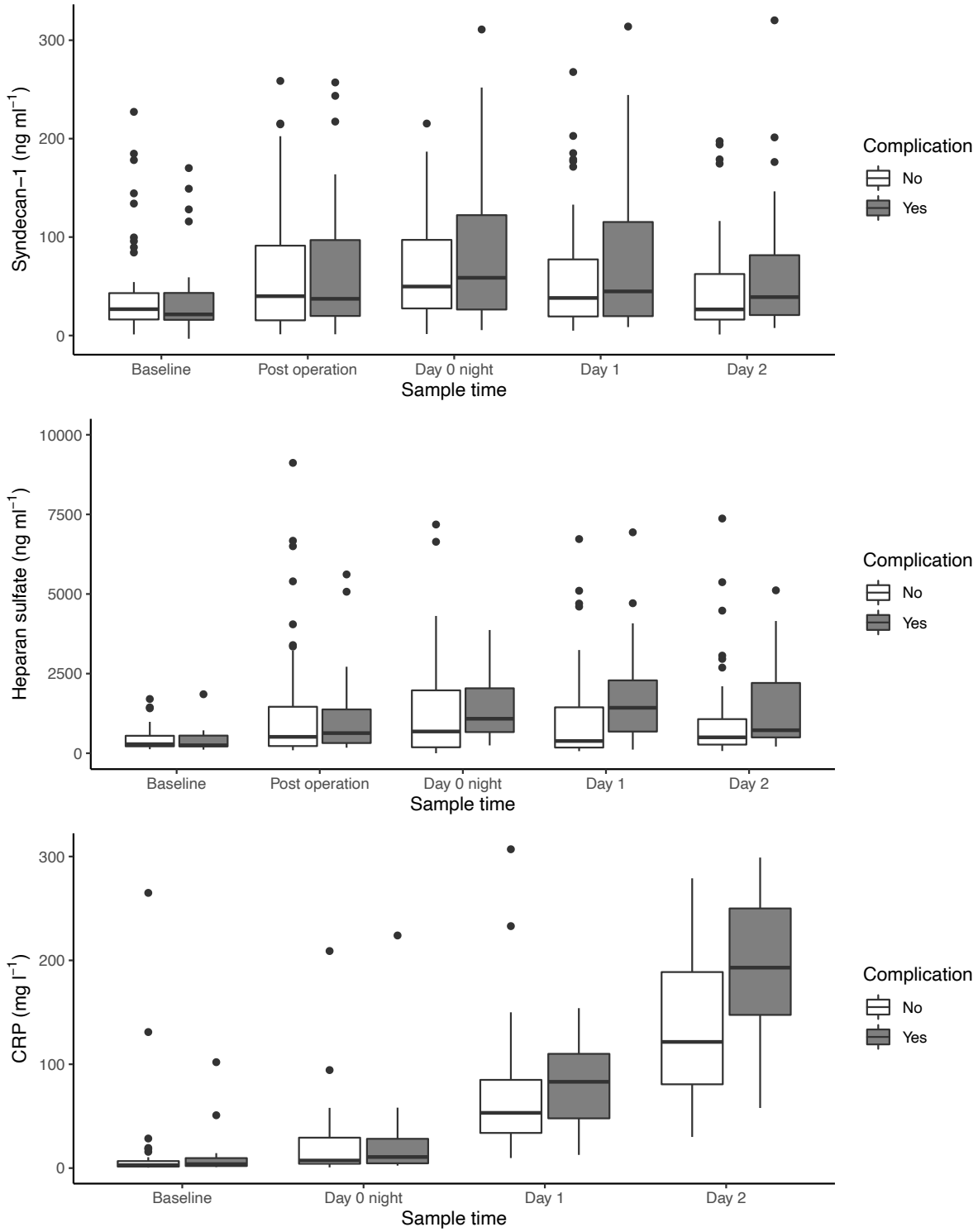


Supplemental Figure 5. Perioperative time course for plasma heparan sulfate levels in the three subgroups of abdominal surgery



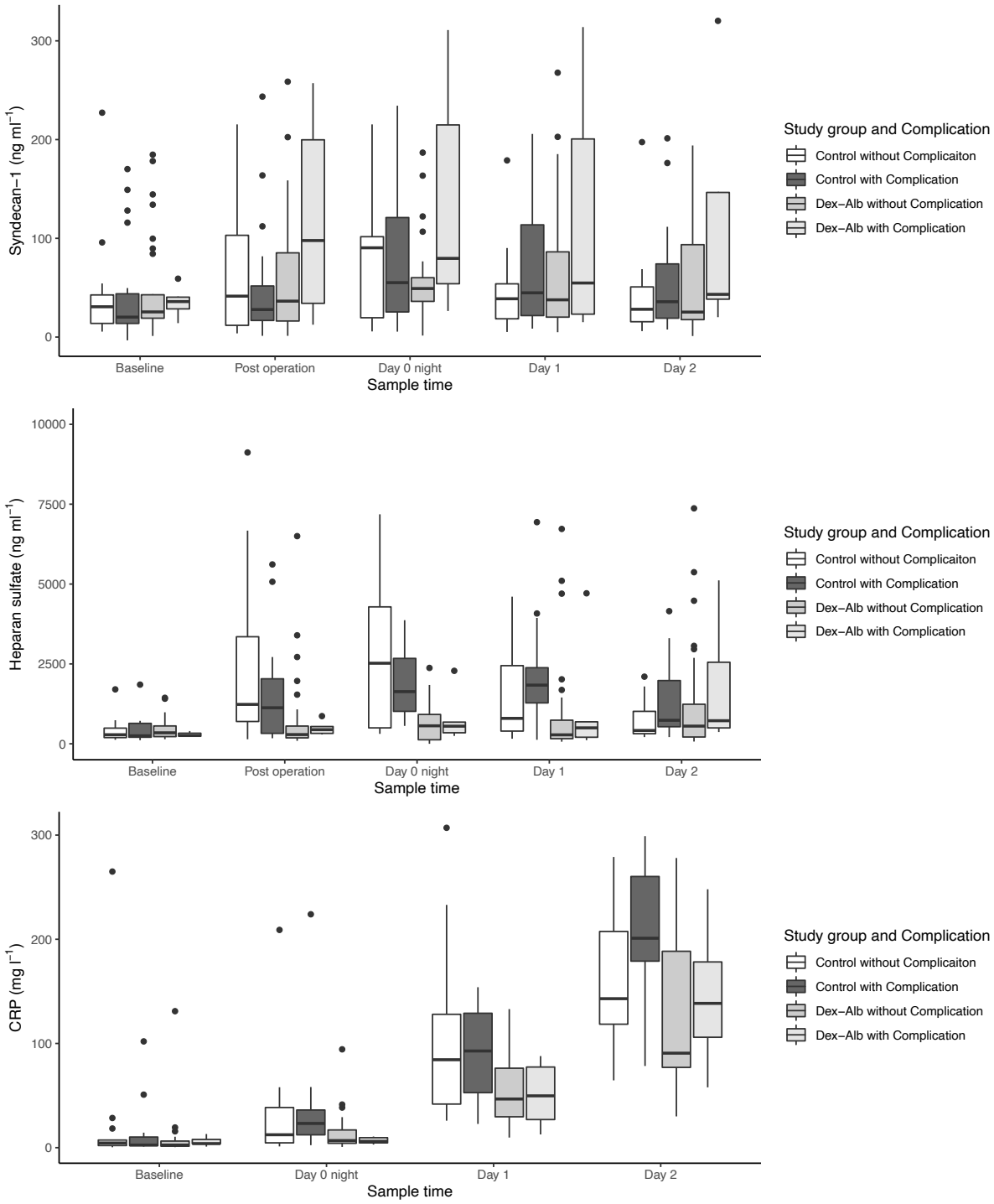
Note: one patient underwent pancreas surgery in the Control group reported a value of 18879.9 ng ml^{-1} on the evening of the day of surgery, one patient underwent pancreas surgery in the Dex-Alb group reported a value of 18000.8 ng ml^{-1} on the evening of the day of surgery

Supplemental Figure 6. Biomarker levels in patients with and without complications



Note: one patient underwent pancreas surgery without complication reported a heparan sulfate value of 18000.8 ng ml^{-1} on the evening of the day of surgery, one patient underwent pancreas surgery with complication reported a heparan sulfate value of 18879.9 ng ml^{-1} on the evening of the day of surgery.

Supplemental Figure 7. Biomarker levels in patients with and without complications stratified by intervention



Note: one patient underwent pancreas surgery in the Control group with complication reported a heparan sulfate value of 18879.9 ng ml^{-1} on the evening of the day of surgery, one patient underwent pancreas surgery in the Dex-Alb group without complication reported a heparan sulfate value of 18000.8 ng ml^{-1} on the evening of the day of surgery.

Chapter 10: Summary of this thesis

This chapter provides a summary of key findings of this thesis and discusses future directions to optimise heamodynamics in surgical patients.

Fluid bolus therapy

Usually, intravenous fluid bolus is given at room temperature within short duration. However, if fluid is given at body temperature or given slowly, it may cause different haemodynamic changes compared to usual fluid bolus therapy. In a before-and-after trial, I led a controlled study that compared different fluids and fluid temperatures (Warm vs Cold 4% albumin and Warm vs Cold 20% albumin) in patients after cardiac surgery patents (Chapter 2 and Chapter 3). The study found that, although warm fluids could prevent blood temperature drops, their differential cardiac index or mean arterial pressure effect was limited.

Also, I led a further controlled before-and-after trial to compare two different approaches to the speed of infusion of 4% albumin and concluded that the rapid infusion group showed more sustained mean arterial pressure increase compared to the slow infusion, which was contrary to previous physiological opinion (Chapter 4).

In addition, I led a study that compared the haemodynamic effects of different fluid types (500ml crystalloid, 500ml 4% albumin and 100ml 20% albumin) at rapid infusion rate in patients after cardiac surgery. This comparative study concluded that, compared to crystalloid fluid bolus, 4% and 20% albumin bolus achieved higher mean arterial pressure effects (Chapter 5).

However, we only assessed physiological outcomes (cardiac index or blood pressures) and did not measure clinical outcomes because these studies were observational in nature and aimed to define the specific effects of these interventions in isolation on haemodynamics. In

addition, 20% albumin has theoretical advantages because it achieves higher mean arterial pressure effect with much less volume compared to crystalloid fluid bolus (Chapter 5). This could result in less fluid balance and some clinical benefits (20). Thus, the above physiological studies have now opened the door to clinical outcome studies. Therefore, to assess the clinical effect of 20% albumin fluid bolus on clinically relevant outcomes, such as mechanical ventilation free time or ICU free time, we have now started an open-label multicentre randomised controlled trial. This trial aims compare crystalloid bolus with 20% albumin bolus for patients after cardiac surgery with haemodynamic instability (HAS FLAIR-II trial, https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?id=378587). This study will include 470 patients after cardiac surgery who are receiving mechanical ventilation and who, in the opinion of the treating clinician, require fluid bolus therapy. Such patients are assigned to a crystalloid fluid bolus group or a 20% albumin fluid bolus group. The primary outcome will be vasopressor infusion duration and secondary outcomes will include fluid balance and time to ICU discharge. This ongoing study, has already recruited 200 patients, and will provide evidence on whether 20% albumin therapy can affect clinical outcomes.

Effect of high dose vitamin C

As discussed in my thesis, high-dose vitamin C has theoretical potential and benefits as an agent to improve haemodynamic instability. In addition, very high dose vitamin C (the maximum dose assessed was 224 g/day) appears safe in adult and paediatric patients (Chapter 7). Therefore, it appeared rational to consider giving high-dose vitamin C to patients with hypotension. However, as presented in my thesis, the pilot randomised controlled trial to compare the haemodynamic effect of intravenous high dose vitamin C (6 g/day) with placebo in vasoplegic patients after cardiac surgery (Chapter 6) found no difference in vasoplegia resolution time or other clinical outcomes.

It is possible that lack of effect may have simply reflected that high-dose vitamin C is no better than placebo at modifying haemodynamics in vasoplegic states. However, it is also

possible that the high dose (6 g/day) given was not a high enough dose to achieved the desired haemodynamic impact. In keeping with this notion, among patients with sepsis, intravenous vitamin C 1.5 g 6 hourly infusion achieved supra-physiological vitamin C level (88), however, the intervention did not achieve a faster septic shock resolution which was also observed our study (89). Thus, we have now begun a series of animal experiments (sepsis in sheep model) to study whether much bigger doses of intravenous vitamin C (mega-dose vitamin C) can achieve more striking and persistent haemodynamic effects. In such animals, during septic shock induced by the administration of mega-doses of intravenous vitamin C (0.5 g/kg over 0.5 hr + 0.5 g/kg/hr for 6.5 hr, total 3.75 g/kg over 7 hours) achieved several clinical improvements, such as a massive increase in urine output, a marked increase in arterial blood pressure, a complete resolution of vasopressor dependence, decreased serum creatinine level and a complete recovery of the animal from a state of near death (90). Thus, our initial dose (6 g/day) might have been an inadequate dose and mega-dose (instead of high dose) vitamin C might be needed to achieve clinical effectiveness.

Therefore, we have now started a pilot feasibility randomised controlled trial to examine the physiological effects of giving mega dose vitamin C (60 g/day) to patients with sepsis (Mega vitamin C trial, https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?id=379778). In this study, we are now giving, 30 grams of intravenous vitamin C over 1 hour and an additional intravenous vitamin C 30g dose over 5 hours (total 60g over 6 hours) or placebo (5% dextrose) to patients with sepsis. We are measure urine output, vasopressor dose, vasopressor free time or other chemical and physiological outcomes. This is a small pilot trial that will include 30 patients (15 patients in each group), however, this will give first-in-the world information on the possible effects of mega dose vitamin C in sepsis.

Glyoccalyx protection strategy

As reported in this thesis, I led a pilot phase 2 trial to assess the efficacy of a glycocalyx protection strategy (dexamethasone and albumin) in patients undergoing major abdominal surgery (Chapter 9). We found that, in the intervention group, although syndecan 1 value was the same to that of the control group, heparan sulphate, C-reactive protein and the number of patients with postoperative complications were lower in the intervention group. However, as syndecan 1 was the most frequently measured biomarkers in clinical setting and as we assume that observing a difference of postoperative complication in a phase 2 trial would not be pragmatic, syndecan 1 was our primary outcome. Now the outcomes observed in this pilot study justify the conduct of further studies with different outcomes informed by the pilot itself. Therefore, we are now designing a phase 3 trial to assess clinical outcomes, such as postoperative complication, as a primary outcome.

In conclusion, the research conducted as part of my PhD program and presented in my PhD thesis has produced a body of novel and clinically important findings. These findings have led to nine academic publications in peer reviewed journals. More importantly, they have opened the door to the conduct of a multicentre phase II randomized trial (now almost half way in its planned recruitment), the development of a further research program studying the novel concept of mega-dose vitamin C in sepsis, and the inception of a phase III trial of glycocalyx protection therapy in patients having major abdominal surgery.

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