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Benefits and harms of red blood cell transfusions in patients with septic shock in the Intensive Care Unit

PhD thesis

Lars Broksø Holst

Department of Intensive Care 4131
Rigshospitalet, Copenhagen
Denmark

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Author

Lars Broksø Holst, MD
Department of Intensive Care
Rigshospitalet, Copenhagen University Hospital
Denmark

Supervisors

Anders Perner, MD, Professor, PhD
Department of Intensive Care
Rigshospitalet, Copenhagen University Hospital
Denmark

Jørn Wetterslev, MD, PhD
Copenhagen Trial Unit, Centre for Clinical Intervention Research
Rigshospitalet, Copenhagen University Hospital
Denmark

Pär Ingemar Johansson, MD, Professor, DMSc
Section for Transfusion Medicine
Rigshospitalet, Copenhagen University Hospital
Denmark

Nicolai Rosenkrantz Segelcke Haase, MD, PhD
Department of Intensive Care
Rigshospitalet, Copenhagen University Hospital
Denmark

Assessment Committee

Hanne Ravn (Chair), MD, Professor, DMSc
Department of Thoracic Anaesthesiology
Rigshospitalet, Copenhagen University Hospital
Denmark

Thomas Strøm, MD, Associate Professor, PhD
Department of Intensive Care
Odense University Hospital
Denmark

Kathryn Maitland, MD, Professor, PhD
Department of Paediatric Tropical Infectious Diseases
Imperial College, London
United Kingdom

Preface

The work forming the basis for this PhD thesis was carried out during my employment as a Research Fellow at the Department of Intensive Care, Copenhagen University Hospital, Rigshospitalet, from March 2011 to August 2014. I would like to thank all the people who participated and supported me during the process.

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Lars Broksø Holst
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Abbreviations

ALI	Acute lung injury
ARDS	Acute respiratory distress syndrome
ARR	Absolute risk reduction
CI	Confidence interval
CTU	Copenhagen Trial Unit
DMSC	Data monitoring and safety committee
DO_2	Oxygen delivery
EGDT	Early goal-directed therapy
FiO_2	Fraction of inspired oxygen in a gas mixture
Hb	Haemoglobin
HCT	Haematocrit level
ICU	Intensive care unit
mM	mmol/l
NISHOT	Non-infectious serious hazards of transfusion
RBCs	Red blood cells
RCT	Randomised clinical trial
RRR	Relative risk reduction
SAGM	Saline-Adenine-Glucose-Mannitol
SAR	Severe adverse reaction
SC	Steering Committee
$ScvO_2$	Central venous oxygen saturation
SIRS	Systemic inflammatory response syndrome
TACO	Transfusion associated circulatory overload
TBI	Traumatic brain injury
TRALI	Transfusion associated acute lung injury
TRICC	Transfusion Requirements in Critical Care trial
TRISS	Transfusion Requirements in Septic Shock trial
VO_2	Oxygen consumption
6S	Scandinavian Starch for Severe Sepsis/Septic Shock trial

Original papers

This thesis is based on the following papers:

I. **Holst LB**, Haase N, Wetterslev J, Wernerman J, Aneman A, Guttormsen AB, Johansson PI, Karlsson S, Klemenzson G, Winding R, Nebrich L, Albeck C, Vang ML, Bülow HH, Elkjær JM, Nielsen JS, Kirkegaard P, Nibro H, Lindhardt A, Strange D, Thormar K, Poulsen LM, Berezowicz P, Bådstøløkken PM, Strand K, Cronhjort M, Haunstrup E, Rian O, Oldner A, Bendtsen A, Iversen S, Langva JÅ, Johansen RB, Nielsen N, Pettilä V, Reinikainen M, Keld D, Leivdal S, Breider JM, Tjäder I, Reiter N, Gøttrup U, White J, Wiis J, Andersen LH, Steensen M, Perner A. Transfusion requirements in septic shock (TRISS) trial - comparing the effects and safety of liberal versus restrictive red blood cell transfusion in septic shock patients in the ICU: protocol for a randomised controlled trial. Trials. 2013 May 23;14:150

II. **Holst LB**, Haase N, Wetterslev J, Wernerman J, Guttormsen AB, Karlsson S, Johansson PI, Aneman A, Vang ML, Winding R, Nebrich L, Nibro HL, Rasmussen BS, Lauridsen JR, Nielsen JS, Oldner A, Pettilä V, Cronhjort MB, Andersen LH, Pedersen UG, Reiter N, Wiis J, White JO, Russell L, Thornberg KJ, Hjortrup PB, Müller RG, Møller MH, Steensen M, Tjäder I, Kilsand K, Odeberg-Wernerman S, Sjøbø B, Bundgaard H, Thyø MA, Lodahl D, Mærkedahl R, Albeck C, Illum D, Kruse M, Winkel P, Perner A. Lower versus Higher Hemoglobin Threshold for Transfusion in Septic Shock. N Engl J Med. 2014 Oct 9;371(15):1381-91.

III. **Holst LB**, Petersen MW, Haase N, Perner A, Wetterslev J. Restrictive versus liberal transfusion strategy for guiding red blood cell transfusion: systematic review of randomised trials with meta-analysis and trial sequential analysis. Submitted to BMJ October 7th 2014.

Summary

Background

Transfusion of red blood cells (RBCs) is widely used for non-bleeding patients with septic shock in the intensive care unit (ICU). The evidence for effect and safety are limited showing conflicting results and transfused RBCs have the potential to harm subgroups of critically ill patients.

Our aim was to assess the benefits and harms of RBC transfusion in patients with septic shock in a randomised clinical trial and to conduct an up-to-date systematic review with meta-analysis of all randomised clinical trials comparing different transfusion strategies.

Methods

We planned and conducted a randomised, partly blinded, clinical trial assigning patients with septic shock in the ICU and a haemoglobin level of 9 g/dl (5.6 mM) or below to receive single units of pre-storage leukoreduced RBCs at a lower haemoglobin threshold level of 7 g/dl (4.3 mM) or below or a higher haemoglobin threshold level of 9 g/dl (5.6 mM) or below. The primary outcome was death by day 90 after randomisation. Secondary outcomes were need for life support, severe adverse reactions, ischaemic events in the ICU and days alive and out of hospital.

Secondly, we conducted a systematic review of randomised controlled trials comparing benefits and harms of using restrictive (range of lower haemoglobin thresholds) versus liberal (range of higher haemoglobin threshold) transfusion trigger strategies to guide RBC transfusion and pooled results in meta-analyses and trial sequential analyses.

Results

Of the 1005 patients that underwent randomisation 998 were included in analysis of the primary outcome of mortality. 90 days after randomisation, 216 of 502 patients (43%) in the lower threshold group had died compared to 223 of 496 (45%) patients in the higher threshold group (relative risk 0.94, 95% confidence interval (CI) 0.78 to 1.09, P=0.44). The number of patients who required life support, who had ischemic events, severe adverse reactions and number of days alive and out of hospital were similar in the two groups. Patients in the lower threshold group received 1588 units of RBCs compared to 3088 units in the higher group. A total of 176 (36%) patients in the lower threshold group never received RBCs in the ICU compared with 6 patients (1%) in the higher threshold group.

The systematic review identified 31 trials with a total of 9813 patients in different clinical settings. In meta-analyses restrictive versus liberal transfusion strategies were not associated with the relative risk (RR) of death (0.89, 95% CI 0.76 to 1.05, 5607 patients in eight trials with lower risk of bias), overall morbidity (RR 0.98, 95% CI 0.85 to 1.12, 4517 patients in six trials with lower risk of bias), fatal or non-fatal myocardial infarction (RR 1.32, 95% CI 0.61 to 2.83, 4630 patients in six trials with lower risk of bias). Trial sequential analysis on mortality and myocardial infarction showed that required information sizes have not been reached but use of restrictive transfusion strategies was associated with reduced numbers of RBC units transfused (mean difference -1.43, 95% CI -2.01 to -0.86) and reduced proportion of patients transfused (RR 0.54, 95% CI 0.47 to 0.63).

Conclusion

The TRISS trial provided evidence for the safe use of 7 g/dl as transfusion trigger in patients with septic shock and reduced the number of units transfused with about half. In line with this, the updated systematic review including data from several recent trials showed no associations with mortality or other adverse events when comparing restrictive to liberal RBC transfusion strategies, however, restrictive transfusion strategies reduce the exposure of patients to RBC transfusions and reduce number of transfused RBC units.

Given the fact that liberal transfusion strategies have not been proven beneficial, a more restrictive approach should be considered. Results from the TRISS trial together with other recent trials have the potential to alter the international guidelines for transfusing critically ill patients. Several guidelines have been updated the last years recommending the use of 7-8 g/dl as the ‘universal’ trigger level. Patients with acute myocardial ischemia and patients with acute brain injury may need special considerations.

Summary in Danish (Dansk resumé)

Baggrund

Blod transfusioner anvendes ofte til kritisk syge patienter indlagt på intensivafdelingen med blodforgiftning og heraf følgende kredsløbsskollaps (septisk shock). Retningslinjerne for præcis hvornår patienter med septisk shock bør modtage blodtransfusioner hviler på begrænset viden fra få kliniske forsøg, som viser modstridende resultater. Der er forsøgsresultater som viser at for meget blod potentielt kan skade visse grupper af kritisk syge patienter. Vores mål var at undersøge gavn og skade ved brugen af blodtransfusioner til patienter med septisk shock indlagt på intensivafdelinger i et klinisk randomiseret forsøg. Dernæst ønskede vi at lave et opdateret systematisk review (systematisk litteraturgennemgang) for at undersøge de samlede effekter af blodtransfusioner i alle tilgængelige forsøg.

Metode

Vi planlagde og gennemførte et delvist blindet, klinisk forsøg som randomiserede patienter på intensivafdelinger med septisk shock og et hæmoglobinniveau på 5.6 mmol/l (mM) eller herunder til at modtage leukocytreducerede blodtransfusioner ved en hæmoglobin grænse på 4.3 mM eller en hæmoglobin grænse på 5.6 mM. Det primære effektmål var død 90 dage efter randomisering til forsøget. Sekundære effektmål var behovet for organunderstøttende behandling, alvorlige bivirkninger, iskæmiske hændelser på intensiv afdeling samt andelen af dage, patienterne var i live efter udskrivelse fra hospital.

Vi lavede en systematisk litteratursøgning og fandt andre randomiserede forsøg, som undersøgte gavn og skade ved at brug af lavere sammenlignet med højere hæmoglobin- eller hæmatokritgrænser for transfusion. Vi udførte metaanalyser (samlede analyser af forsøgsresultaterne) og belyste herefter den statistiske usikkerhed via 'Trial Sequential Analysis' (TSA).

Resultater

Af de 1005 patienter, som undergik randomisering i vores kliniske forsøg blev 998 inkluderet i analysen af det primære effektmål. 90 dage efter randomisering var 216 af 502 patienter (43%) døde i gruppen, som blev tildelt den lavere hæmoglobin grænse sammenlignet med 223 af 496 (45%) patienter i gruppen tildelt den højere hæmoglobin grænse (relativ risiko 0,94, 95% konfidensinterval (CI) 0,78 til 1,09, P=0,44). Antallet af patienter, der krævede organunderstøttende behandling, havde iskæmiske hændelser, alvorlige bivirkninger og antallet af dage i live udenfor

hospitalet var ikke forskellige i de to grupper. Patienterne i gruppen, som blev tildelt den lavere hæmoglobin grænse (4.3 mM) modtog samlet 1588 blodtransfusioner og 176 (36%) af patienterne modtog aldrig blodtransfusioner på intensivafdelingen, sammenlignet med 3088 blodtransfusioner givet i gruppen, som fik tildelt den højere grænse (5.6 mM)og 6 patienter (1%) i denne gruppe fik aldrig transfusion.

Det systematiske review identificerede 31 forsøg som i alt havde undersøgt 9813 patienter under forskellige kliniske omstændigheder. Metaanalyse af restriktive (lavere) sammenlignet med liberale transfusions strategier (højere) viste ikke en association med øget relativ risiko (RR) for død (0.89, 95 % CI 0.76 til 1.05, 5607 patienter i otte forsøg med lavere risiko for bias), overordnet morbiditet (RR 0.98, 95% CI 0.85 til 1.12, 4517 patienter i seks forsøg med lavere risiko for bias) og fatalt eller ikke-fatalt myokardieinfarkt (RR 1.32, 95% CI 0,61 til 2.83, 4630 patienter i seks forsøg med lavere risiko for bias). TSA viste at det ikke var muligt at drage konklusioner omkring mortalitet og forekomsten af myokardieinfarkt idet den statistiske usikkerhed var for stor. Der er brug for yderligere forsøg som undersøger dette. Brugen af lavere hæmoglobin grænser for transfusion var associeret med en reduktion i antallet af blodtransfusioner (gennemsnitlig reduktion -1.43, 95% CI -2.01 til -0.86) samt en reduktion i andelen af patienter som transfunderes (RR 0.54, 95% CI 0.47 til 0.63).

Konklusion

Vores systematiske review inkluderede flere nye undersøgelser og viste at brugen af restriktive transfusionsstrategier ikke medførte øget risici for død eller forekomst af andre bivirkninger sammenlignet med liberale transfusionsstrategier og restriktive transfusionsstrategier medførte reduktion i antallet blodtransfusioner og reduktion i antallet af patienter som modtager blodtransfusioner.

Den statistiske usikkerhed er stor fordi der fortsat mangler forsøgsdata for at sikker viden er til tilvejebragt men aktuelt er der ikke forsøgsresultater, som viser at liberale transfusion strategier overordnet gavner patienter. Resultater fra vores forsøg har vist at det er sikkert at anvende 4.3 mM som transfusionsgrænse hos patienter med septisk shock indlagt på intensiv afdelingen. Resultaterne kan få indflydelse på guidelines for hvordan kritisk syge patienter transfunderes fremover og flere opdaterede guidelines foreslår generel anvendelse af transfusionsgrænser på mellem 4.3 og 5.0 mM. Patienter med tegn på myokardie iskæmi og akut hjerneskade har muligvis gavn af en højere transfusionsgrænse.

Introduction

The primary treatment of patients with septic shock is to optimise circulation and support organ perfusion by prompt administration of antibiotics and infection source control and resuscitation with intravenous fluids and vasopressor/inotropic drugs. These interventions may be supplemented with transfusion of blood (red blood cells (RBCs)) in case of anaemia (low numbers of RBCs) and persistent hypoperfusion.[1] Scandinavian Intensive Care Units (ICUs) were among the most frequent users of RBC transfusions[2] for patients with septic shock, transfusing half of these patients with a median of between three and five units of RBCs during the ICU stay.[3,4] Clinical trials and observational studies trying to uncover the effects and safety of blood transfusions first started to emerge 15-20 years ago and showed that blood transfusions were associated with harmful effects in critically ill patients.[5–8]

Blood transfusion has been perceived as a safe and effective treatment for patients with anaemia for almost 100 years. Transfusion practice has slowly moved towards a more restrictive approach due to emerging trial data supporting still lower 'triggers' for transfusion of RBCs, subsequent revised clinical guidelines[9] and increased focus on the concept of blood management.[10] RBC transfusion is highly controversial because data from randomised clinical trials in different clinical settings are still lacking including patients with septic shock and practices are highly based on tradition and theory because of that.[9]

This thesis is based on a randomised clinical trial that assesses the benefits and harms of two different haemoglobin thresholds for guiding RBC transfusion in patients with septic shock in the ICU and a systematic review including other trials assessing trigger guided RBC transfusion in a variety of clinical settings. The thesis contains description of the undertaken trial and meta-analysis and a discussion of their methods. Finally, the evidence for trigger guided RBC transfusion will be discussed.

In the following text the terms lower and higher haemoglobin thresholds (used in paper II) will be used synonymously with the terms restrictive and liberal transfusion strategies (used in paper I and paper III). Blood haemoglobin levels will preferentially be presented with the unit g/dl as this is the international standard.

Background

Red blood cell transfusion

Transfusion medicine has become a specialist discipline since the first human to human transfusion (allogeneous transfusion) was performed and the discovery of the

AB0 blood group a century ago. The first transfusions were given as whole blood donations and later fractionated blood products were developed from whole blood into separated blood products as RBCs, plasma and platelets.[11] RBCs are currently suspended in mediums consisting of citrate-dextrose-phosphate or saline-adenine-glucose-mannitol (SAGM) and stored in plastic bags for up to 42 days under low temperature.[12]

Transfusion of RBCs can be lifesaving in bleeding patients but most RBCs are transfused in non-bleeding patients with low haemoglobin levels (anaemia) as this is still the only means of achieving a momentary increase in haemoglobin level.[9,13,14]

Sepsis, severe sepsis and septic shock

Sepsis is a medical condition characterised by a deleterious whole-body inflammatory host response (systemic inflammatory response syndrome (SIRS)) to infection often taking place in the lungs, abdomen or urinary tract, inducing endothelial dysfunction leading to vascular leakage and vasodilatation.[15] Ultimately sepsis may result in relative and absolute hypovolaemia leading to organ hypoperfusion (severe sepsis) and manifest cardiovascular compromise with diminished oxygen delivery and impaired tissue oxygenation (shock) not reversed by initial fluid therapy (septic shock).[1,16,17] If shock persists, the result is progressive multiple organ failure and mortality rates close to 50% and in some subgroup of patients up to 75%.[18]

The course of sepsis evolving from sepsis to severe sepsis to septic shock dependent upon the causative organism(s), genetic constitution and underlying health status of the host patient but also on the timeliness of identification and therapeutic intervention.[19]

Treatment of patients with septic shock is a complicated task and is primarily undertaken in the ICU. Initial management is about controlling the infection and reversing the detrimental effects of shock by sustaining tissue oxygenation using fluids to restore intravascular volume, use of vasopressors to restore vascular tone and RBC transfusion to augment oxygen delivery. Sepsis is one of the leading causes of death worldwide and may in developed countries account for 8-9% of all deaths, thus representing a major global health problem.[20]

Oxygen delivery

The main function of RBCs is to transport oxygen (O_2) from the pulmonary to the peripheral capillaries and return carbon dioxide (CO_2) from the microcirculation to the

lungs. Haemoglobin is the oxygen binding molecule encapsulated in the red blood cells and most oxygen is carried to the organ tissues this way. Delivery of oxygen (DO_2) to the body tissues is defined by[21]:

$$DO_2 = CO \times CaO_2 = SV \times \text{pulse rate} \times ((SaO_2 \times 1.34 \times (Hb) + (0.0031 \times PaO_2))$$

Cellular hypoxia develops when oxygen consumption (VO_2) in the tissues exceeds DO_2 and below this threshold (DO_{2crit}) an oxygen uptake-to-supply dependency is present. Acute onset of anaemia to levels as low as 5 g/dl are well tolerated in resting healthy humans because of compensatory mechanisms to sustain tissue oxygenation.[22] The DO_2 is between three and four times greater than global VO_2 and with increasing cardiac output (CO), redistribution of blood flow to vital organs, a right shift in oxygen dissociation curve (a decrease in haemoglobin affinity for oxygen), recruitment of capillaries (increased capillary density), lowered blood viscosity, and increased oxygen extraction (O_{2ER}) the body will preserve DO_2 above the critical level in otherwise healthy anaemic patients.[21,23]

Tolerance to anaemia

Tolerance to anaemia is highly dependent on patient volume status, physiological reserve and the dynamics of the anaemia (chronic versus acute onset). Critically ill patients with septic shock are relative and absolute hypovolemic, have heterogeneous microcirculation and many endure severe comorbidity - and together with abrogated circulatory mechanisms - make these patients less capable of counteracting the deleterious effects of anaemia without resuscitation including RBC transfusion.[23,24]

Other groups of patients probably less susceptible to anaemia are patients with coronary artery disease and acute myocardial infarction but also patients with acute brain injury. Oxygen delivery to the myocardium is highly flow-dependent since the heart O_{2ER} is high in its resting state, and myocardial ischemia might occur or worsen with lower haemoglobin levels.[25,26] Due to the injured brains inability to compensate for decreased oxygen delivery, patients with traumatic brain injury might also require higher levels of haemoglobin to prevent secondary cerebral ischaemic insults.[27] But the impact of increasing haemoglobin levels are complicated by the possibility that this also may increase the risk of ischemia in both groups of patients.[28,29]

Ideally, RBCs should be transfused before reaching DO_{2crit} thereby restoring the blood oxygen-carrying capacity in the transfused patients to prevent tissue hypoxia and shock and thereby multiple organ failure. However the relationship between DO_2 and VO_2 in different subgroups of critically ill patients, including patients with septic shock, have been difficult to predict as global DO_2 increases with RBC transfusion but without a corresponding increase in oxygen consumption VO_2.[30,31]

Red blood cell storage lesion and leukocyte depletion

One explanation for the lack of increase in VO_2 following increase in DO_2 may be that tissue hypoxia in the early phase of septic shock are caused by heterogeneous microcirculation and perfusion (stagnant hypoxia)[32] which may not be resolved by RBC transfusion because stored RBCs do not deliver oxygen as well as genuine cells. The reduced ability may be caused by a combination of storage related biochemical and biomechanical alterations, modifying RBCs and the storage medium, the so-called storage lesion. Intracellular changes include depletion of 2.3-diphosphorglycerate (2.3 DPG) and depletion of adenosine triphosphate (ATP). Structural changes in the RBCs during storage include loss of cellular membrane integrity with phospholipid vesiculation and protein oxidation. Because of this RBCs undergo a shape change with loss of deformability and increased osmotic fragility leading to increased red cell-endothelial interaction (Figure 1).[33]

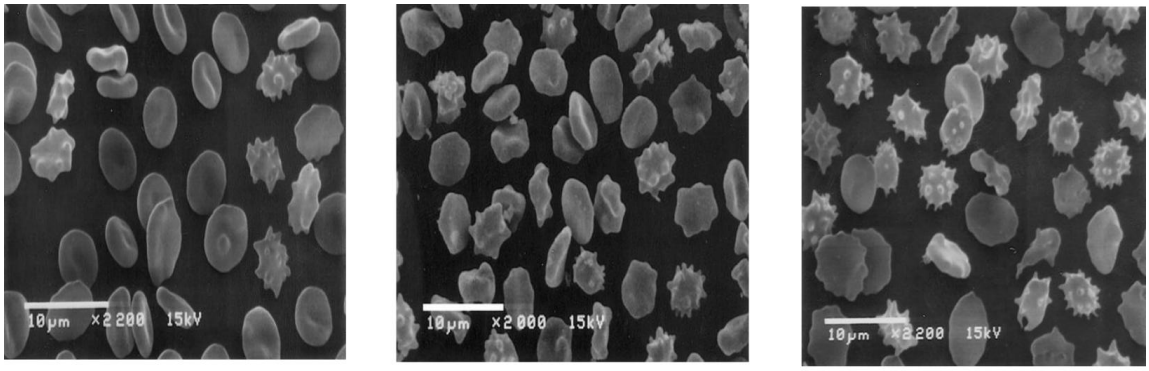

Figure 1. Electron microscope images showing corpuscular changes in red blood cells during storage.[34]

Furthermore, changes in the storage medium takes place as decrease in pH, increase in plasma potassium, release of free haemoglobin and iron, and accumulation of bio-reactive substances.[35] Together storage lesion mechanisms

decrease the RBCs ability to deliver oxygen to the tissues and increase the immunomodulatory potential within the storage medium. However the clinical implications of these alterations remain unknown and large RCTs are in progress.[33,36] Another explanation for the lack of increase in VO_2 with DO_2 increase may be that the organ cells are unable to exploit the increase in available oxygen due to mitochondrial changes and this type of hypoxia (cytopathic hypoxia)[37] will not be resolved by increased DO_2.[17,38]

Transfusion related complications and adverse events

The decision to transfuse a patient with RBCs should ideally balance the potential risks with transfusion against the risks of not transfusing (e.g. anaemia) (figure 2).

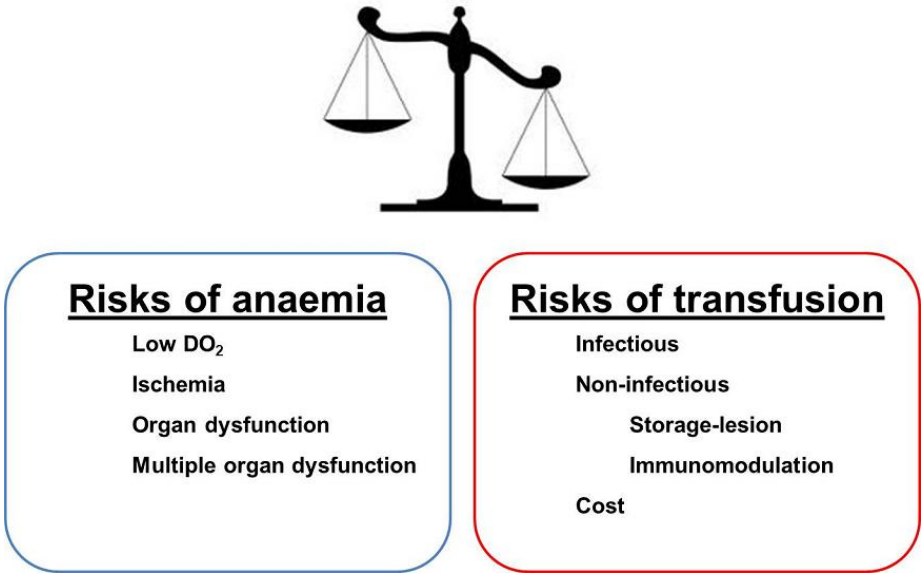

Figure 2. Showing risks to be outweighted before decision to transfuse.

Transfusion related risks can be defined as infectious or non-infectious serious hazards of transfusions (NISHOT) and the NISHOTs may be mediated by immune response or not. The risk of transfusion transmitted viral infections such as humane immune deficiency virus (HIV) and Hepatitis (HBV, HCV) have almost been eliminated in high income countries and bacterial infections and prion infections are few.[24,39,40] Procedural errors in relation to transfusion together with the leading cause of transfusion related mortality - transfusion-related circulatory overload (TACO) - are the greatest hazards related to transfusion of red blood cells (Table 1).[41,42]

RBC transfusion may also be associated with a transfusion-related modulation of the immune system (TRIM) potentially linked to storage lesion. Especially in critically ill patients TRIM may represent a significant “second-hit” when

added to pre-existing systemic inflammatory response syndrome (SIRS) underlying sepsis, causing increased number of infections and multiple organ failure,[35,43,44] and increased risk of transfusion-related acute lung injury (TRALI).[45,46] A number of observational studies have tried to uncover possible associations between RBC transfusions and clinical adverse outcomes (mortality, infections, acute respiratory distress syndromes, myocardial infarction) also in critically ill patients but a causal relationship is still questionable.[8,13,14,47]

The use of leukocyte reduction, a process reducing the number of white blood cells (WBCs), have showed to decrease the immunomodulating properties of stored RBCs but the clinical benefit is still unknown. However, leukoreduction is now routinely performed in most European countries.[48–50]

Table 1. Selected infectious and non-infectious hazards with transfusion of red blood cells.[40,51] The large range of incidences in numbers of hazards especially in transfusion related acute lung injury (TRALI)[46,52] and transfusion related circulatory overload (TACO)[42,53] are due to differences in clinical setting, definition of entities and type of surveillance systems being used.

Transfusion complications	Estimated frequency (event/no of transfusion)	Comment
INFECTIOUS		
Human immunodeficiency virus (HIV)	1:2.350.000	
Hepatitis B virus (HBV)	1:350.000	
Hepatitis C virus (HCV)	1:1.800.000 - 1:2.800.000	
Human T-cell lymphotrophic virus (HTLV)	1:2.000.000	
Clinical sepsis related to bacterial contamination	1:250.000	Often Yersenia and pseudomonas species
NONINFECTIOUS (NISHOT)		
Immune-mediated		
Haemolytic transfusion reaction	1:10.000 - 1:50.000	Due to IgM and IgG
Anaphylactic reaction	1:20.000 - 1:50.000	Associated with IgA deficiency
TRALI (Transfusion-related acute lung injury)	1:534 - 1:17.000	Within 6 hours of transfusion
Graft versus host disease	Very rare	Immunocompromised patients
Nonimmune-mediated		
"Wrong unit – wrong patient"	1:14.000 - 1:38.000	Mostly related to AB0 incompatibility
TACO (Transfusion-associated circulatory overload)	1:18 - 1:356	Major cause of transfusion related death

Anaemia in critical illness and in patients with septic shock

Anaemia is defined as a haemoglobin level of less than 13 g/dl (8.0 mM) in men and 12 g/dl (7.5 mM) in women and severe anaemia is defined as a haemoglobin level

below 8 g/dl (5.0 mM).[54] Anaemia is highly prevalent in critically ill patients and appears early in the ICU course with 65% of patients with a haemoglobin level below 12 g/dl (7.5 mM) at time of admission to the ICU, and 97% of patients becoming anaemic by day 8.[13,47,55] Anaemia is more prominent in patients with septic shock with a mean admitting haemoglobin level of 10.5g/dl (6.5mM) and more than half of patients with septic shock decreases to haemoglobin level below 9 g/dl (5.6 mM) during the first 3 days of shock.[4,13,55]

Anaemia in the critically ill patient is multifactorial and results from two fundamental processes; a shortened circulatory life span and/or diminished production of RBCs. Only 10-15% of patients have chronic anaemia before ICU admission and most critical ill patients show a phenotypical normochromic, normocytic anaemia with high ferritin concentrations, low serum ion and low transferrin saturation. Both haemodilution with administration of intravenous fluids, blood loss during procedural interventions and repeated blood sampling as well as rheologic changes inducing RBC removal via the reticuloendothelial system are among the most important aetiologies for anaemia in the critical care setting.[56,57] Reduced RBC production are seen as consequences of decreased endogenous erythropoietin levels, hyporeactive bone marrow and immune-associated functional iron deficiency all associated with critical ilness.[56,58]

Alternatives to RBC transfusion

Erythropoietin (EPO) has been tested in several RCTs and did not improve survival, but may increase the risk of thromboembolic events.[59] None of the artificial blood substitutes (Haemoglobin Binding Oxygen Carriers (HBOCS) or perflurochemicals (PFCs)) are currently approved for human use in Europe or the US and their use in the critical illness setting would probably be limited because of their short half-life of 12 to 48 hours and adverse effects.[9,60] Iron supplementation is a known intervention to treat iron deficiency anaemia but needs further investigation in critically ill patients before recommended because of the potential increased risk of infections.[56]

Transfusion triggers

The goal of transfusing non-bleeding patients is to avoid organ ischemia. When evidence of poor oxygenation exists, clinicians must decide whether to increase the cardiac output using fluids and/or inotropic drugs; or improve the oxygen carrying capacity by RBC transfusion. The single most important driver for RBC transfusions (the transfusion trigger) is the haemoglobin value (or in certain clinical settings haematocrit values).[3,4,13,14] Physiological measures and clinical signs such as central

venous oxygen saturation ($ScvO_2$), blood lactate concentration, ST-segment dynamics and fluid resistant tachycardia might be useful to help guide blood transfusion decisions, but all methods lack specificity as diagnostic tests and future trials must define trigger values for these measurements before they act as primary drivers for RBC transfusion.[21,61]

Transfusion practice

The standard transfusion triggers for RBC transfusion has been a haemoglobin level of 10 g/dl (6.2 mM) or a haematocrit level of 30%[62–64] and these triggers were not questioned for many years.[5,65–67] The mean pre-transfusion haemoglobin level in ICU patients are reported to be around 8.5 g/dl (5.3 mM).[13,14] Two prospective cohort studies[3,4] in adult patients with septic shock admitted to Danish ICUs showed results comparable to earlier findings with a median pre-transfusion trigger value of 8.3 g/dl (interquartile range (IQR) 7.7 to 9.0 g/dl (4.8 to 5.6 mM) and 8.1 g/dl ((IQR) 7.4 to 8.9 g/dl (4.6 to 5.5 mM)). Furthermore, these values were independent of shock day (figure 3)[3,4] and data from the 6S-trial[68] and the SAFE TRIPS[2] study (Simon Finfer, personal communication) confirmed that pre-transfusion haemoglobin levels in patients with septic shock were independent of shock day.

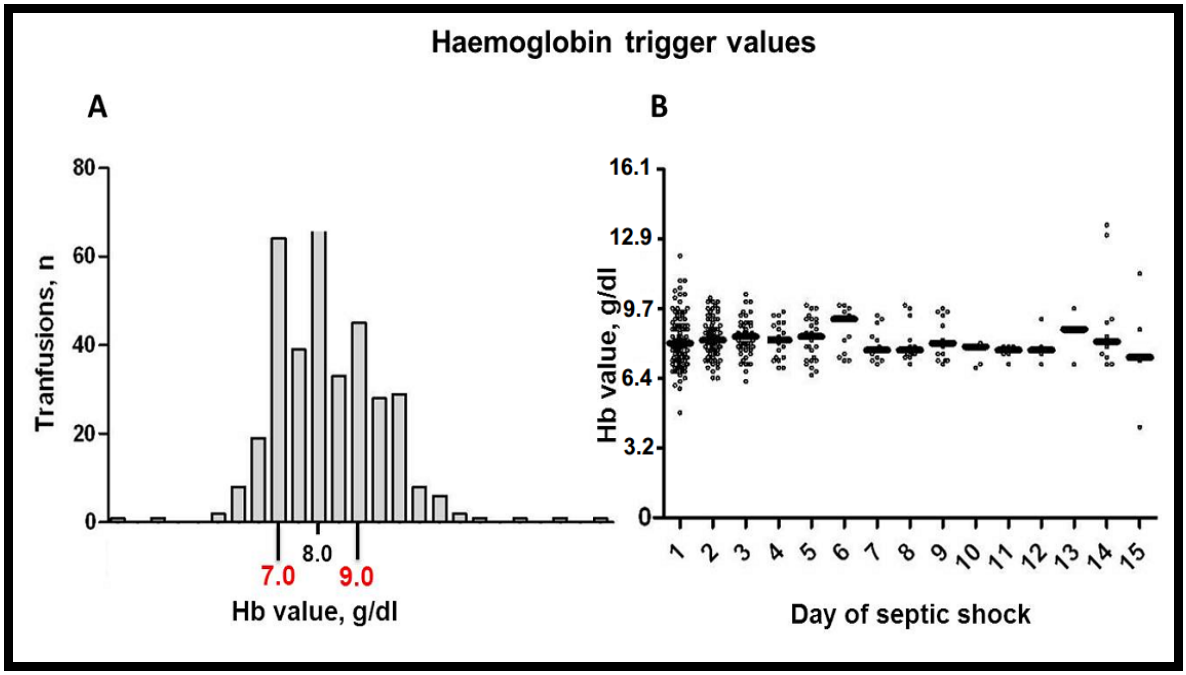

Figure 3. (Panel A) Showing pre-transfusion haemoglobin levels in 164 patients in 6 Danish ICUs, (Panel B) showing a scatter plot of pre-transfusion haemoglobin levels over time in the same patient group (thick bars representing the median).[3]

Evidence for RBC transfusion in patients with septic shock prior to the TRISS trial

Results from one randomised trial[69] assessing the effects of early goal-directed therapy (EGDT) were adopted by the Surviving Sepsis Campaign[70] as evidence for transfusion in the early resuscitation phase with signs of hypoperfusion. The trial by Rivers et al. was a single centre trial investigating the effect of target controlled and protocol based resuscitation in 263 patients with severe sepsis or septic shock in the first six hours after admittance to an emergency department. Patients were randomised to either control (usual care) or a protocol including a number of interventions such as resuscitation fluids, inotropic agents and blood transfusion to haematocrit above 30% (approximately 10.0 g/dl (6.2 mM)) if hypoperfusion persisted ($ScvO_2$<70%). Trial results showed a significant reduction in mortality (RR 0.58, 95% CI 0.38 to 0.87) with the use of EGDT protocol however; the clinical benefit of single interventions in the complex protocol is difficult to comprehend (e.g. RBC transfusion).

In the later phase of sepsis (when hypoperfusion has resolved) and without the presence of myocardial ischemia, severe hypoxemia, acute haemorrhage or ischemic coronary artery disease the haemoglobin level should be targeted at levels of 7-9 g/dl (4.3 to 5.6 mM) according to guidelines.[70] The evidence were based on data form the Transfusion Requirements in Critical Care trial (TRICC) trial conducted by Hebert et al. 15 years ago.[5] A broad range of critically ill normovolaemic ICU patients were randomised to a transfusion trigger of 7 g/dl (4.3 mM) or 10 g/dl (6.2 mM) in this trial. Results showed no statistically significant difference in 30-day mortality (primary outcome) between the two groups, but a trend towards increased hospital mortality and significantly increased risk of cardiopulmonary complications in the liberal group. Predefined subgroup analyses showed significantly lower mortality in the lower threshold group in younger (age < 55 years) and less severely ill patients (Acute Physiology and Chronic Health Evaluation (APACHE) II-score below 20).Trial results were subsequently repeated in the a paediatric population in the TRIPICU trial.[71] The trial randomly assigned 637 haemodynamically stable critically ill children to receive RBC at either 7 g/dl or 9.5 g/dl (5.9 mM) and no difference in the primary outcome of multiple organ-dysfunction syndrome or any of the primary outcomes (mortality, adverse events, nosocomial infections or length of ICU stay) were shown.

A systematic review of observational studies evaluating the effects of RBC transfusions on mortality and morbidity in different groups of critically ill patients was published by Marik & Corwin in 2008.[7] Authors reported that in 42 of 45 included studies, negative effects of RBCs outweighed any benefit. Later published

retrospective cohort studies investigated the association between anaemia, blood transfusion and mortality in patients with septic shock and showed that blood transfusion were associated with both decreased[47,72,73]and increased risk of mortality.[74] This cause-effect relationship is complicated in transfusion studies as severity of illness is associated with both transfusion and mortality (confounding by indication). Adjustment for volume of RBC transfusion and other known confounders can be done in observational studies but will most likely never be able to remove these effects and for sure not the possible effects of unknown confounders.[75]

When planning the Transfusion Requirements in Septic Shock (TRISS) trial in the early 2011 the use of RBC transfusions in patients with septic shock were controversial and guidelines were largely based on two trials showing somewhat conflicting results. No trial had assessed the effects and safety of RBC transfusion in patients with septic shock and the TRICC trial included critically ill patients already resuscitated, questioning the possibility of hypoperfusion in the subgroup with severe infections. Furthermore the patients were transfused with non-leukoreduced RBCs, making it difficult to adapt the results to clinical practice today.

Aims of studies

Our aim in study I (paper I and paper II) was to conduct a pragmatic trial to assess the effects and safety of haemoglobin based RBC transfusion trigger points, representing current RBC transfusion practice, on 90-day mortality (primary outcome measure), organ failure, severe adverse reactions (SARs) and ischaemic events in ICU patients with septic shock. Secondarily, in study II (paper III) we aimed at comparing our results with those of other randomised trials and subsequently perform an up-to-date systematic review with meta-analysis of evidence comparing benefits and harm of different RBC transfusion strategies.

Study outline

The present PhD thesis is based on two studies and three papers:

Study I is the Transfusion Requirements in Septic Shock (TRISS) Trial, a randomised multicentre trial assessing the effects and safety of a lower haemoglobin threshold versus a higher haemoglobin threshold in patients with septic shock in the ICU. Paper I is the design and rationale paper for the TRISS trial and paper II is the main

publication of the trial results, presenting data on mortality and other predefined outcomes.

Study II is a systematic review of randomised controlled trials comparing benefit and harm of using restrictive versus liberal transfusion trigger strategies to guide RBC transfusion.

Study I: Transfusion Requirements in Septic Shock (TRISS) TRIAL

Methods

Overview and design

The Transfusion Requirements in Septic Shock (TRISS) trial is a multicentre, parallel group clinical trial randomising patients in 32 ICUs in Denmark, Sweden, Norway and Finland from December 3rd 2011 to December 26th 2013. Allocation sequence was computer generated and centralised permuted block-randomisation with variable blocks size, stratified according to centre and the presence of hematological malignancy was used. The trial was partly blinded as it was not feasible to do so but assessors of mortality, our Data Safety and Monitoring Committee (DSMC) and the trial statistician were all blinded for the intervention.

Hypothesis

The evidence present in 2011 regarding the use of haemoglobin thresholds to guide RBC transfusion was not sufficient to support either a lower or a higher transfusion threshold in patients with septic shock. The interventional transfusion triggers used in the trial were chosen based on the transfusion practice observed during the pre-trial phase. However, data from the TRICC Trial[5] showed that a lower transfusion threshold (7 g/dl) had the potential to reduce the relative risk of death by 20% (9% ARR) compared with a higher threshold (10-12 g/dl) in the subgroup of patients with severe infection.

Ethics

The trial was approved by the ethics committee and data protection agency in the participating countries and registered at the ClinicalTrials.gov website prior to enrolment of the first patient (ClinicalTrials.gov Identifier: NCT01485315). The trial was conducted in adherence to the Helsinki Declaration and to the standards of good clinical practice.[76]

Consent procedure

Most patients included in the trial were temporarily incompetent due to the course of septic shock or as a consequence of sedation as part of the treatment in ICU. In Denmark and Finland deferred consent procedure was used meaning that patients were randomised and enrolled before obtaining informed consent. As soon as

possible after enrolment proxy consent was obtained from the patient's relatives and general practitioner/the regional medical officer of health. Patients who regained consciousness, were asked for informed consent as soon as possible.

Patients discontinued the trial protocol if consent was withdrawn by the proxy-consenter or by the patient, but we asked for permission to continue data registration. Only the patient could demand deletion of already registered data and if so, data were destroyed and a new patient randomised to obtain the full sample size.

Patients

Adult patients in the ICU with septic shock and haemoglobin level of 9 g/dl (5.6 mM) or below were eligible for randomisation. Exclusions included documented wish against transfusion**,** previous severe adverse reactions with blood products (not excluding TACOs), presence of acute myocardial ischaemia, life-threatening bleeding, transfusion of RBCs during current ICU admission but prior to randomisation, withdrawal from active therapy or brain death, lack of informed consent and acute burn injuries.

Intervention

Patients were randomised in a 1:1 ratio to receive single units of pre-storage leukoreduced RBCs suspended in SAGM when reaching a haemoglobin level of 7 g/dl (4.3 mM) or below in the lower threshold group versus a haemoglobin level of 9 g/dl (5.6 mM) or below in the higher threshold group. The intervention lasted during the entire ICU stay or to a maximum of 90 days after randomisation. Haemoglobin level assessments were done by point-of-care testing within 3 hours of termination of the RBC transfusion or before the initiation of a new transfusion. Clinicians were able to suspend the protocol during life-threatening bleeding events (haemorrhagic shock defined by the attending clinician), during ischemic events (defined as cerebral, myocardial, intestinal or peripheral limb) or during extracorporeal membrane oxygenation (ECMO) therapy. All other interventions were decided by the attending clinicians. After unblinding the 6S trial[68] we recommended against the use of Hydroxyethyl starch (HES) but use of HES was not regarded as a violation to protocol.

Outcomes

The primary outcome was death by day 90 after randomisation. Secondary outcomes were need for life support at day 5, 14 and 28 (as need for mechanical ventilation, renal replacement and vasopressor/inotropic therapy)[77], SARs in the ICU, ischaemic

events in the ICU (including myocardial, cerebral, intestinal and peripheral limb) and days alive and out of hospital (Table 2).

Statistical analysis

The primary analysis was a multiple regression analysis adjusted for stratification variables in the modified intention-to-treat population comparing death by day 90 in the two groups. Unadjusted analyses and analyses adjusted for design and baseline variables (stratification variables, age, previous cardiovascular disease, Hb level, SAPS II, SOFA score and RBC transfusion in the 24 h prior to randomisation). We pre-published the statistical analysis plan (SAP) in paper I prior to analysing data. P-values lower than 0.05 were considered statistically significant.

Table 2. Primary and secondary outcomes.

Outcome	Lower Hb-threshold	Higher Hb-threshold	Relative Risk* (95% CI)	P-value
Primary outcome measure	*no./total no. (%)*			
Dead by day 90	216 / 502 (43.0)	223 / 496 (45.0)	0.94 (0.78 - 1.09)	0.44
Secondary outcome measures	*no./total no. (%)*			
Use of life-support				
Day 5	278 / 432 (64.4)	267 / 429 (62.2)	1.04 (0.93 - 1.14)	0.47
Day 14	140 / 380 (36.8)	135 / 367 (36.8)	0.99 (0.81 - 1.19)	0.95
Day 28	53 / 330 (16.1)	64 / 322 (19.9)	0.77 (0.54 - 1.09)	0.14
Ischemic events	35 / 488 (7.2)	39 / 489 (8.0)	0.90 (0.58 - 1.39)	0.64
Severe adverse reaction	0 / 488 (0.0)	1 / 489 (0.2)	-	1.00
	mean percent of days			
Days alive without vasopressor /inotropic therapy	73	75	-	0.93
Days alive without mechanical ventilation	65	67	-	0.49
Days alive without renal replacement therapy	85	83	-	0.54
Days alive and out of hospital	30	31	-	0.89

Interim analysis

We conducted a pre-planned interim analysis 90 days after randomising patient number 500, in July 2013. The Data Monitoring and Safety Committee (DMSC) recommended finalising the trial and randomisation was closed December 26th 2013, 25 month after inclusion of the first patient.

Results

Of 1224 patients evaluated for eligibility, 1005 were randomised. Due to five post-randomisation exclusions during the trial and two exclusions after ending the trial, 998 patients were included in the analyses of mortality. Consent for the use of mortality only, were given in 21 patients, leaving 977 patients to be included in analyses of secondary outcomes (figure 4). Baseline characteristics were similar between the intervention groups.

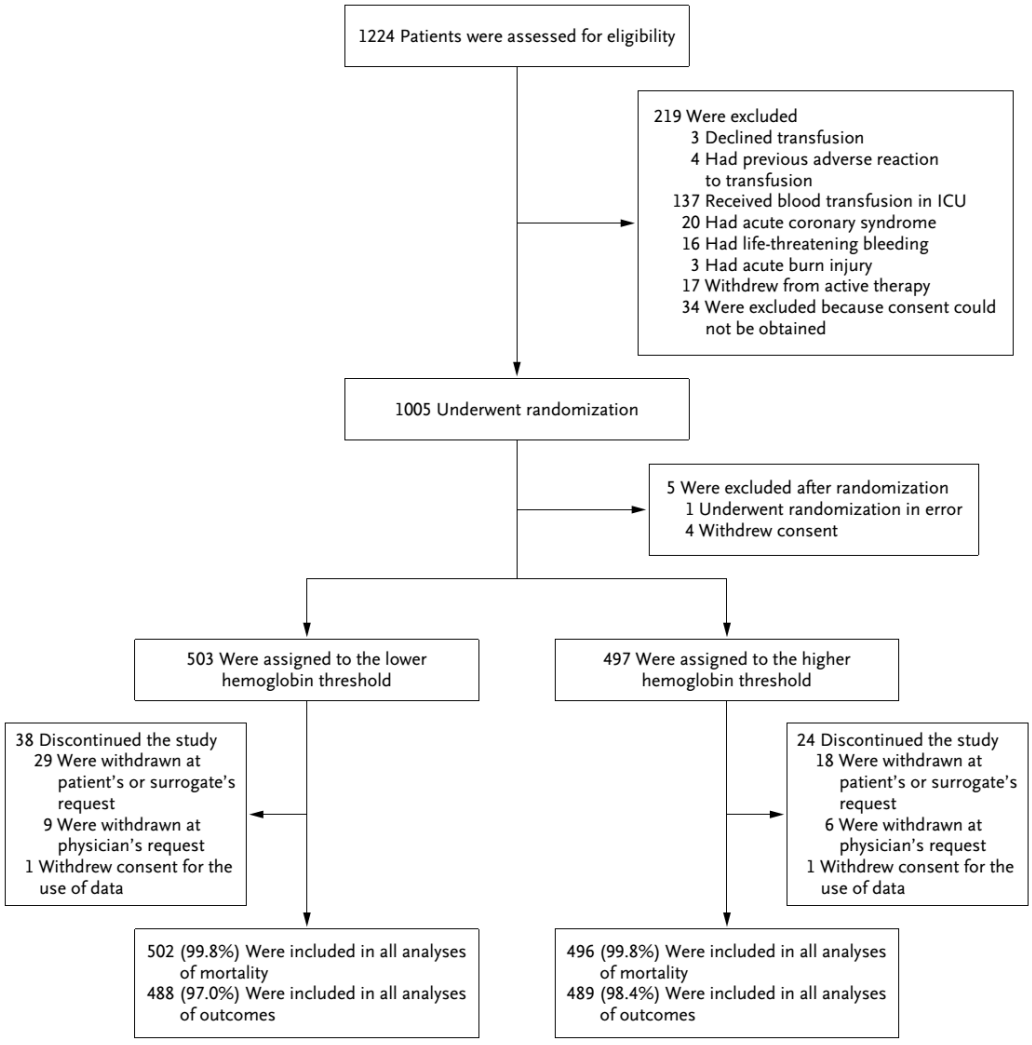

Figure 4. Flow of patients in the TRISS trial.

Red blood cell use and number of patients transfused

Daily lowest median haemoglobin level differed significantly ($p<0.001$) from a baseline level of 8.4 g/dl at randomisation (figure 5). A total of 4633 RBC units were transfused, 1545 units in the lower threshold group and 3088 units in the higher

threshold group. 176 (36%) patients in the lower threshold group did not receive transfusions as compared to 6 (1%) patients in the higher threshold group. The median number of RBC units transfused in the lower threshold group was 1 (IQR) 0-3) versus 4 (IQR 4-7) in the higher threshold group.

Predefined outcome measures

Death by day 90

216 patients in the lower threshold group and 223 patients in the higher threshold group fulfilled the primary outcome of death by day 90 after randomisation (relative risk 0.94, 95% confidence interval (CI) 0.78 to 1.09, P=0.44). We could not rule out the possibility of a 22% risk decrease or a 9% risk increase with the use of a lower haemoglobin threshold. Results of the primary analysis were supported by fully adjusted, unadjusted and per-protocol analyses. There was no significant heterogeneity between pre-defined subgroups in analysis of the primary outcome.

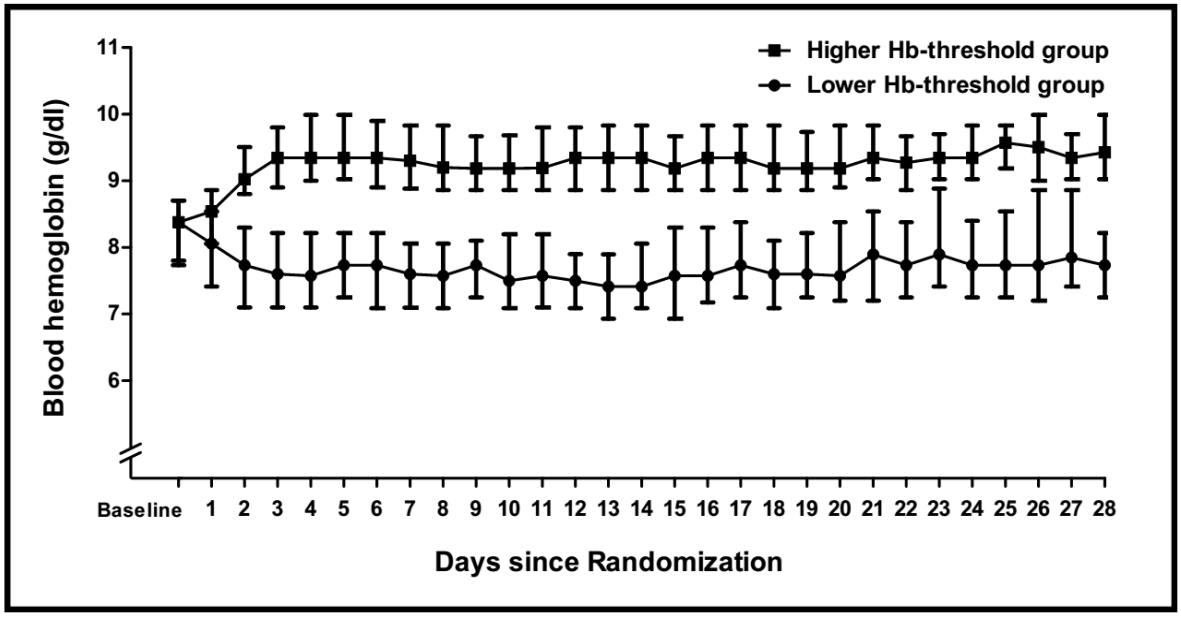

Figure 5. The daily lowest median haemoglobin level.

Kaplan-Meier analysis using a Cox-model including stratification variables showed that survival times did not differ significantly between groups (p=0.41) (figure 6).

Life support and days alive and out of hospital

Number of patients in need of life support on days 5, 14 and 28 were similar between the intervention groups and no differences in the mean percentage of days alive and

without mechanical ventilation, vasopressor or inotropic therapy, renal replacement therapy (RRT) or percentage of days alive and out of hospital.

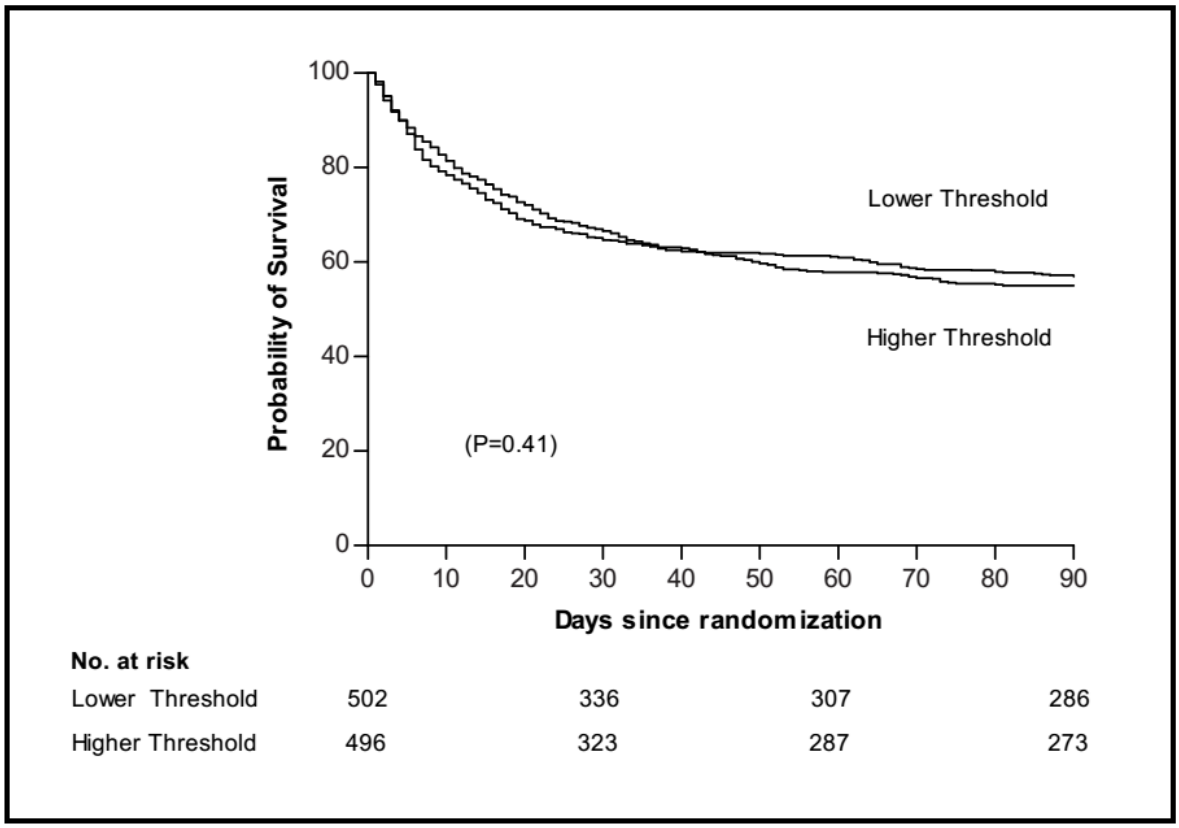

Figure 6. Survival curves censored at 90 days.

Ischemic events

No statistical significant differences were shown in the number of ischemic events in the ICU since 35 (7.2%) patients in the lower threshold group compared to 39 (8.0%) patients in the higher threshold group fulfilled this outcome.

Severe adverse reactions

One patient with acute haemolysis allocated to the higher threshold group was registered.

Other pre-defined outcomes

One year mortality and Health-Related Quality of Life (HRQoL) for Danish patients (78%) using the Physical and Mental Component Summary scores in the Short Form health survey questionnaire (SF-36)[78,79] will be reported in future publications.

Conclusion

No differences were shown in death by day 90, use of life support, ischemic events or in the mean per cent of patient days alive and out of hospital when comparing the use of a lower haemoglobin threshold (7 g/dl or below (4.3 mM)) and a higher haemoglobin threshold (9 g/dl or below (5.6 mM)) to guide transfusion of single units of pre-storage leukocytereduced RBCs in patients with septic shock in the ICU. Furthermore, the use of a lower haemoglobin threshold resulted in reduced numbers of RBC units transfused and reduced numbers of patients receiving transfusions.

Study II: Restrictive versus liberal transfusion strategy for guiding red blood cell transfusion: systematic review of randomised trials with meta-analysis and trial sequential analysis

Methods

Overview and design

The systematic review focused on updating the current Cochrane review and was conducted in accordance with recommendations from the Cochrane collaboration[80]. A review protocol was pre-published with the PROSPERO (registration no. CRD42013004272)[81] register before literature search was performed.

Eligibility criteria

Randomised trials were included if the comparison groups were assigned clearly defined transfusion "triggers" or "thresholds", described as haemoglobin or haematocrit level(s) that had to be reached before RBC transfusion were administered regardless of the clinical setting. Trials including preterm or very low birth weight neonates were excluded. Trials using factorial design without interaction effects between interventions were included and cluster randomised trials were included regarding assessment of harm.

Search strategy

Relevant RCTs were identified without language restrictions updating the Cochrane review search strategy. Records were sought in Cochrane Central Register of Controlled Trials, MEDLINE, EMBASE, Science Citation Index Expanded and clinical trial sites up until October 1st 2014. References of published literature were reviewed and expert in transfusion medicine were contacted to identify additional records.

Data extraction

Two authors independently identified trials and extracted data using a pre-planned data extraction form. Predefined primary outcomes were mortality and overall morbidity. Secondary outcomes were adverse events (transfusion reactions, cardiac events (e.g. myocardial infarction, cardiac arrest, acute arrhythmia, angina), renal failure, thromboembolic events, infections, haemorrhagic events, stroke or transitory cerebral ischemia, proportions of patients transfused and number of units of RBC transfused (Table 3).

Bias assessment and GRADING

All trials were reviewed for risk of bias in major domains recommended by the Cochrane Collaboration.[80] Trials with low risk of bias in all other domains than blinding were characterised as trials with lower risk of bias as blinding were not feasible in any of the included trials. The quality of evidence for mortality, overall morbidity and fatal- and non-fatal myocardial infarction were assessed using the GRADE methodology.[82]

Statistical analyses

Pooled estimates of intervention effects in primary and secondary outcomes were calculated using conventional meta-analysis with the software package Review Manager 3.1 (RevMan) version 5.3.3 (The Nordic Cochrane Centre, The Cochrane Collaboration, 2014). Trial sequential (TSA) analysis were performed as a sensitivity analysis correcting for repetitive significance testing and sparse data using TSA program version 0.9 beta (www.ctu.dk/tsa).

Results

Trial characteristics

31 trials[5,6,65–67,71,83–107] randomising 9813 patients were included. Trial population size ranged from 25 to 2016 patients and 8 trials included more than 500 patients. The included trials were heterogeneous regarding type of patients, clinical setting and intervention trigger values. Two trials used partly autologous transfusion (re-transfusion of own blood), 12 trials used only leukoreduced RBCs and 8 trials were judged as lower risk of bias trials. The intervention trigger value varied between trials with restrictive transfusion triggers ranging from haemoglobin levels of 7.0 to 9.7 g/dl (4.3 to 6.0 mM), haematocrit of 24 to 30% or symptoms of anaemia as defined by authors. The liberal transfusion trigger values ranged from haemoglobin levels of 9 to 13 g/dl (5.6 to 8 mM) and haematocrit of 30 to 40%. 10 trials used 7 g/dl as the restrictive intervention trigger.

Table 3. Results of conventional meta-analysis and Trial Sequential Analysis.

Outcomes	No. of trials	Restrictive events/total	Liberal events/total	Relative Risk (95% confidence interval)	TSA adjusted (95% confidence interval)
Mortality					
Lower risk of bias	8	426/2809	469/282	0.89 (0.76 - 1.05)	0.68 - 1.17
All trials	23	558/4167	586/4154	0.95 (0.81 - 1.11)	0.74 - 1.21
Leukocyte reduced	12	367/2993	404/2985	0.85 (0.70 - 1.03)	
Follow-up >90 days	5	82/337	88/341	0.93 (0.70 - 1.23)	
Patient age < 18 years	2	14/350	15/347	0.94 (0.46 - 1.90)	
Overall morbidity					
Lower risk of bias	6	858/2261	897/2256	0.98 (0.85 - 1.12)	0.81 - 1.19
All trials	12	1070/2982	1084/2993	1.06 (0.93 - 1.21)	
Fatal and non-fatal myocardial infarction					
Lower risk of bias	6	57/2318	41/2312	1.32 (0.61 - 2.83)	0.28 - 6.21
All trials	16	145/3259	137/3248	1.05 (0.82 - 1.36)	

Mortality

We did not show any overall difference between patients receiving a liberal versus a restrictive transfusion strategy when analysing the eight trials with lower risk of bias reporting data on mortality. Pooled analysis of all 23 trials reporting mortality data did not alter this result. TSA trials with lower risk of bias showed that no boundaries were crossed (figure 7). The quality of evidence was judged to be low. Differences in intervention effects were explored in pre-defined subgroups stratified by patient age, length of follow up, leukoreduction and found no differences. Post-hoc analysis of mortality stratified by clinical setting defined in accordance with the Cochrane review (trauma and acute blood loss, perioperative setting and critical care) did not show any differences.

Overall morbidity

No difference in overall morbidity were shown between restrictive and liberal transfusion strategies but trial sequential analysis showed that future trials will not be able to show an association with a 15% risk reduction with restrictive or liberal strategies given that the boundary for futility was crossed (figure 8). The quality of evidence was judged to be very low.

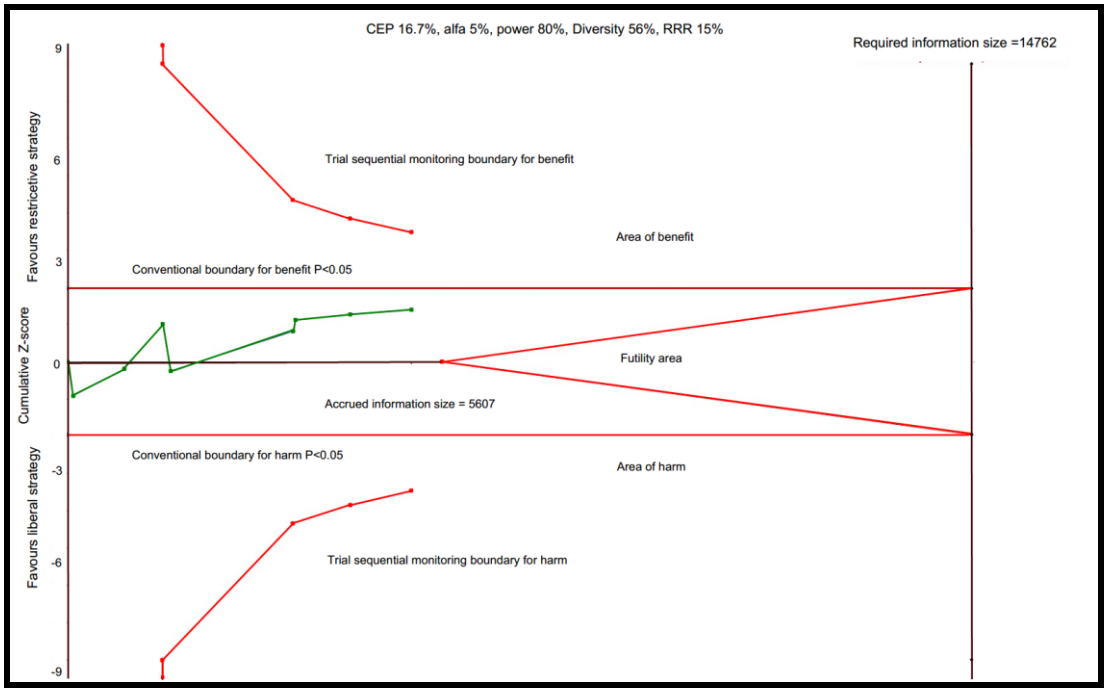

Figure 7. Trial sequential analysis of mortality. The required information size was not reached (right black line) and the green z-curve did not cross any of the boundaries for benefit, harm or futility leaving the meta-analysis inconclusive.

Fatal and non-fatal myocardial infarction

A restrictive transfusion strategy were not shown to be associated with a relative risk difference in fatal or non-fatal myocardial infarction regardless whether results were pooled in analyses from trials with lower risk of bias or all trials despite risk of bias. The quality of evidence was judged to be low.

Other adverse events, number of patients and units transfused

Analysis of eight trials reporting infectious complications in 5107 patients indicated a possible association in favour of using restrictive transfusion strategies. No associations with any other adverse events were shown. Restrictive transfusion strategies were shown to be associated with a reduction in number of patients transfused and number of RBC units transfused.

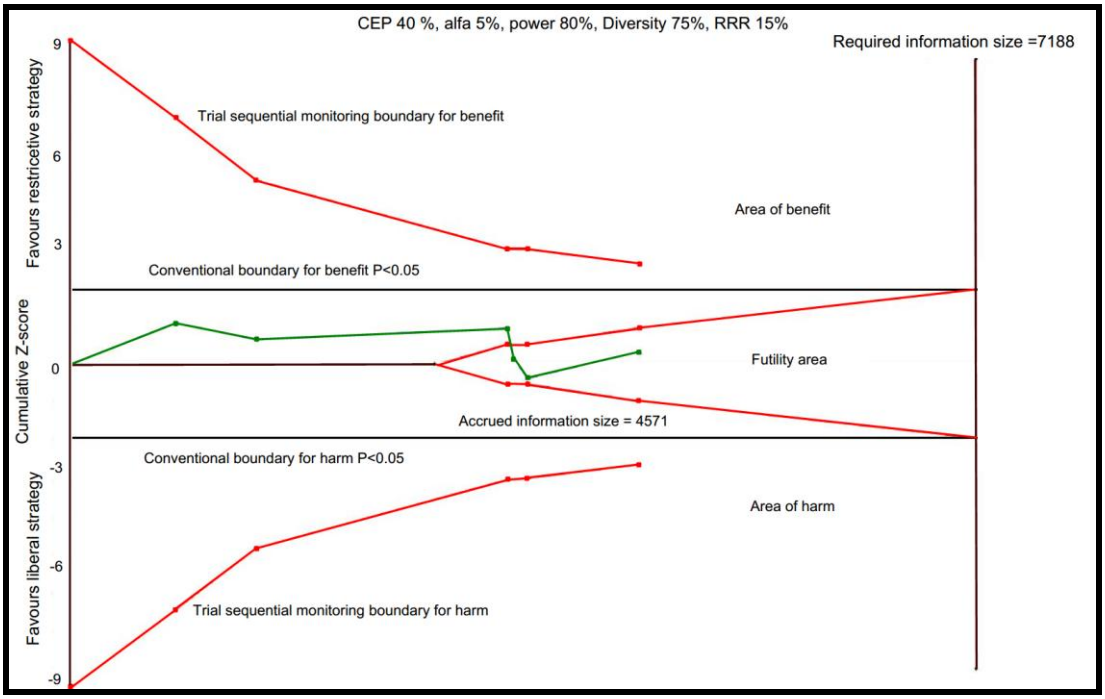

Figure 8. Trial sequential analysis of overall morbidity. The required information size was not reached (right black line) but the green z-curve entered the futility area meaning that future trials are unlikely to show a 15% risk difference in this outcome.

Conclusion

We added 12 RCTs and 3899 patients randomised in different clinical settings to the present Cochrane review including a total of 31 trials and 9813 patients. Conventional meta-analyses did not show associations with mortality, overall morbidity, or any of the secondary outcomes including fatal and non-fatal myocardial infarction with the use of restrictive as compared with liberal transfusion strategies. The overall quality of evidence was judged to be low and trial sequential analysis on mortality and myocardial infarction showed that the required information sizes have not been reached but a 15% risk difference in overall morbidity can be rejected. However, restrictive transfusion strategies reduced numbers of RBC units transfused and reduced proportions of patients receiving transfusions. We found a possible association between the use of restrictive transfusion strategies and reduced risk of infectious complications.

Discussion

Principal findings

The principal findings in TRISS were that using a lower haemoglobin threshold of 7 g/dl (4.3mM) reduced the number of transfusions with about half and reduced the number of patients transfused without harming patients. Death at 90 days, use of life support, rates of ischemic events, severe adverse reactions and number of days alive and out of hospital were similar between intervention groups.

The systematic review updated current Cochrane review and pooled analysis from data among the 31 included trials and 9813 patients showed that the use of restrictive transfusion strategies were not associated with harm but transfusion numbers and rates were reduced compared to liberal strategies. TSA analyses showed that further trials with lower risk of bias are needed to establish firm evidence but a 15% relative risk difference can be refuted regarding the overall morbidity outcome.

Limitations and strengths – Study I (the TRISS trial)

Design

It is generally not feasible to triple blind randomised transfusion trials. The TRISS trial was designed as what could be regarded as a transfusion trial with lower risk of bias using centralised computer based randomisation procedure, concealed allocation and blinding of assessors of mortality, DMSC members and trial statistician. The possibility of introduction of bias by the lack of blinding may still be present as clinicians were not unaware of the intervention. On the other hand the primary outcome of mortality is probably less likely to be influenced by this.

The trial was designed as a pragmatic trial with the aim of providing urgently needed safety data with high generalisability by assessing current transfusion practice in patients with septic shock. Thus, the pragmatic trial design supported the aim of TRISS but on the other hand did not allow us to describe or explain the biological mechanism and effects underlying the trial results.

Patient selection

Investigating the effects and safety of RBC transfusions in patients with septic shock in the ICU was an obvious choice. Transfusions were frequent and the evidence base behind guidelines for this patient group was limited. Moreover, patients with septic shock are among the most critically ill patients and RBC transfusion could

potentially worsen outcome[5], but higher transfusion threshold could also pose great benefit in these patients characterised by oxygen dept.[1,69]

A strength in our trial is that we used few and broad inclusion criteria to avoid selection bias and to ensure external validity. Finalising large RCTs have been shown to be difficult in this setting[5] and easy enrolment procedure was important. Our network managed to include an average of 1.3 patients per day during the enrolment period and we reached pre-planned inclusions in 25 month. We randomised 80% of the patients assessed for eligibility. Different reasons could explain this and the fraction of included patients varied between trial sites. National legislation allowed the use of deferred consent in Denmark and Finland accounting for more than 80% of the patients, allowing for immediate inclusion of patients. We asked investigators for mandatory data registration on patients fulfilling all four inclusion criteria despite also fulfilling one or more exclusions and we did not register all patients with septic shock in the participating ICUs in the totality of the trial period. The inclusion ratio in TRISS was high but a great variability were seen in other ICU trials.[18,108–110] Important is that our cohort is comparable to those of other large RCTs including patients with septic shock.[18,110,111]

A limitation regarding the patient population is clearly that some patients (11%) received RBC transfusion before ICU admission, which tends to minimise the treatment effect. But no group difference in number of patients transfused, units transfused or haemoglobin level was present at baseline. We considered that surgical and haematological patients in particular would have been excluded in larger numbers and we chose only to exclude patients receiving RBC transfusion in the ICU prior to randomisation to increase external validity. However, RBC transfusions given in the 24 hours prior to randomisation were a covariate included in the fully adjusted analysis and results supported that of the primary analysis. The majority of patients excluded due to RBC transfusions being given in the ICU prior to randomisation were excluded in the early phases of trial site initiation because clinicians were not aware of trial inclusion and procedure. Other patients received RBC transfusion in the ICU before randomisation during a non-septic ICU stay and then later became eligible. It is less likely that excluding these patients from the trial has influenced outcome.

We did not control for the RBC transfusion strategy after leaving the ICU. It was not feasible to control for transfusions after ICU discharge, but it is reasonable to state that the effects of RBCs in the critical ill patients with septic shock are most influential in the earlier phase of critically illness.

Intervention

One of the primary strengths of this trial is that the protocol managed a clear separation between intervention groups in terms of numbers of RBC units transfused and also in terms of the median lowest haemoglobin level. But there were differences in the number of protocol violations between groups. We chose to regard any transfusion as a transfusion decision because the protocol addressed single unit administration. Because of this absolute numbers may seem high. The lower threshold group had more 'giving blood too early violations' and the higher threshold group had more 'not giving blood violations' but per protocol analyses excluding patients with violations were not different from the primary analysis. The reporting of non-adherence to protocol in transfusion trials are highly variable and could relate to differences in defining violations.[5,6,71,87,104] The overall numbers of non-adherence in TRISS are comparable to those of other trials conducted in the critical care setting.[5,104] Any event of non-adherence could somehow reflect un-awareness of trial protocol or be a result of a deliberate action from the attending clinician. We did not register data on the reasons for non-adherence to protocol which is somehow a limitation.

Clinical equipoise (uncertainty) provides the ethical basis for medical research allocating patients to different treatment arms.[112] There should be no ethical imperative for investigators to support any of the chosen treatment arms in a randomised trial. When planning the TRISS trial we observed a variety of transfusion threshold in patients with septic shock in the ICU. Most frequently observed pre-transfusion haemoglobin levels in our cohort studies were 8.1-8.4 g/dl supported by data from large RCTs and observational data.[2,13,14,68] Haemoglobin trigger levels of 7 and 9 g/dl, were chosen as representatives for the current practice. We did not observe differences in the use of haemoglobin levels between the first and second day of septic shock which would have been expected according to the guidelines.[70] Thus we chose not to assess differences in the trigger levels between early and late phase of septic shock. Recent trials have questioned this use of different RBC transfusion thresholds in patients with septic shock at least in the early phase of resuscitation.[113,114]

Based on our cohort data collective equipoise appeared to be present prior to the trial. But the range of pre-transfusion haemoglobin levels show that some patients are transfused based on other pre-transfusion haemoglobin levels. This may question the principle of equipoise on the individual level but could also indicate that other parameters or information than haemoglobin was used to trigger RBC

transfusion. Data from our cohort study showed that haemoglobin concentration was the only measure that consistently differed between transfused and non-transfused patients with septic shock.[4] Thus it is reasonable to state that transfusion decisions at least in our setting were mainly based on haemoglobin levels and this is supported by data from large observational studies.[13,14]

We did not assess all co-interventions during the entire trial period but the relative large trial size and stratification for trial site during randomisation makes it less possible that results are influenced by confounding with differences in concomitant interventions.

Outcomes

The strength in our trial is that we reached the pre-planned inclusion, powered to inform on 90 day mortality. The validity of this indisputable outcome can be questioned in terms of the time of measurement, however, 90 day mortality has proven itself as the golden-standard in critical care as delayed survival differences related to interventions have been observed in previous critical care trials.[68,115–117]

Result of the primary analysis seems robust as this is supported by fully adjusted, unadjusted, per-protocol analyses and the fact that pre-defined subgroup analyses did not show any significant heterogeneity. We achieved 100% follow up in the primary outcome and 97% follow-up for the secondary outcomes eliminating bias due to drop-outs.

Our trial showed no statistically differences between groups in the primary outcome of death at 90 days. In terms of sample size, the assumption of a 9% or 20% relative risk reduction is a fairly large difference when regarded as a biologically plausible treatment effect. However, we based our trial size upon the only RCT-data to support our sample size calculation indicating a large increase in mortality with a lower compared to a higher transfusion strategy in ICU patients with severe infection (29.7% vs. 22.8%, RR increase 23%).[5] Obviously a mortality difference less than 9% would still be clinically relevant, but we found it realistic to fund and include 1000 patients with septic shock within our time frame thereby adding important high-quality data to this field of research.

Our results are consistent with but somewhat different from the TRICC trial results as we did not show any trends towards higher mortality or increased adverse events in the higher threshold group. Contrary to the TRICC trial all patients in TRISS received leukoreduced RBCs potentially minimising storage lesion and immunomodulatory effects and our results may represent increased product safety with the standard RBCs transfused nowadays. Differences between trial results could

also be due to the higher threshold group in TRISS (9 g/dl) being more restrictive compared to the higher threshold group in the TRICC trial (10 g/dl) imposing a protective effect of anaemia towards adverse effect of transfusion. Whether the result of TRISS is due to the lack of effect of anaemia (our defined levels) on outcomes or because physiologic benefits of RBC transfusions are outweighed by the storage lesion or the presence of heterogeneous microcirculation in patients with septic shock can only be speculative. A complex interplay exists between anaemia, RBC transfusion, critical illness and clinical outcomes.

The secondary outcomes should be interpreted with caution as power is low and they are all surrogate measures and may lead to overestimation of intervention effects.[118] However the secondary outcomes defined as the use of mechanical ventilation, vasopressor or inotropic therapy and renal replacement therapy (life-support) have been associated with mortality.[109,119–122]

Many physicians are concerned with the risks of myocardial ischemia as very few data on the association between this and lower haemoglobin thresholds have been published.[54,123] Data on myocardial infarction from the TRISS trial should be interpreted cautiously since we chose against using a continuous surveillance plan including ECGs and biomarkers.[124,125] Instead we registered episodes of myocardial ischemia defined by clinicians according to the clinical trial site in question and should furthermore result in reperfusion strategies or initiation/increased antithrombotic drug treatment. A clear limitation is that some cases could be missed and reporting could be influenced by detection bias.

The included patient population represents a heterogeneous cohort in terms of co-morbidity, onset of septic event, aetiology and focus of infection and our trial was not able to identify whether subgroups of patients with septic shock could benefit from either of the interventions but pre-defined subgroup analyses and fully adjusted analyses including baseline variables supported the primary result.

Statistics

The trial results are strengthened by the fact that the statistical analysis plan was pre-published in paper I. Furthermore, our primary analysis was a logistic regression analysis adjusted for stratification variables (site and haematological disease) accounting for correlation between patients within each stratum.[126,127] The primary analysis was done in the intention-to-treat population[128] but due to different reasons 7 patients were post randomly excluded, in fact making this a modified ITT population.[129] When doing trials in the acute setting, time is important and may lead to randomisation of patients wrongly assessed for eligibility. Handling these patients

is a balance between not excluding patients that may reduce group differences but on the other hand skewing the distribution of baseline variables when excluding these patients.[128] We only excluded one patient that did not fulfil inclusion criteria and this was realised immediately. Moreover the use of deferred consent increased the risk of discontinuing the trial protocol (stopped intervention) and ultimately post-randomisation exclusion (deletion of all trial data). This may lead to loss of power and introduction of bias if the reasons for dropouts are associated to outcome (e.g. patient fulfilling the primary outcome early in the trial period and because of that consent are not obtainable).[130–132] Intervention was stopped in 62 patients but data registration followed in 41 of these patients. Only 6 patients did not consent for the use of data and were excluded after randomisation making the influence on outcomes less likely. We were not able to obtain consent in 21 Danish patients because patients died before regaining consciousness and consent from relatives were not obtainable. The Danish Ministry of Health waived the consent and allowed the use of data after advice from the Danish Ethical Committee based on arguments from our trial Steering Committee.[133]

The statistical analysis plan included instructions for handling missing data. This was primarily a problem in the fully adjusted analysis of the primary outcome adjusted for design variables among these SAPS II[134] score and SOFA[135] score missing at baseline in 18% and 12 % of patients, respectively. The missing values were handled by worst-best case analysis predicting the limits for the true intervention effects. The results of these analyses showed that the result of the primary analysis was well within the limits of the worst-best case analyses and on the basis of that we did not perform multiple imputation procedure.[136]

Limitations and strengths – Study II (the systematic review)

Adherence to Cochrane methodology including a pre-published, peer-reviewed protocol in the PROSPERO register, structured and comprehensive record search in relevant databases with no language restriction and evaluation of all included trials strengthen our systematic review. We reported the results according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) guidelines, emphasised the results of trials with lower risk of bias in our conclusions and performed evidence quality evaluation by GRADE (Grading of Recommendation, Assessment, Development and Evaluation) recommendations[82] all of which strengthen our results.[80,137]

Although statistical heterogeneity was low to moderate among trials reporting primary outcomes, it is obvious that we have pooled data from heterogeneous trials

in regards to clinical setting, patient age and comorbidity and co-interventions. Moreover a variety of transfusion triggers were used in the intervention arms all increasing the risk of type-II error and making interpretation of analyses less intuitive. We have on the other hand conducted a broad meta-analysis resulting in increased power and precision of pooled analyses. Furthermore, we had the opportunity to assess the general effects of RBC transfusions across different clinical settings but also to explore the hypothesis that the effects of transfusion vary between different clinical settings. We found no significant differences between subgroups stratified by clinical setting inspired by the Cochrane review. However, this stratification may not reflect clinical relevant subgroups. We did a post-hoc analysis regrouping patients according to more strict clinical definitions (Figure 9) and found that the use of liberal strategies were associated with increased risk of mortality in patients with upper gastro intestinal bleeding. Results have to be interpreted very carefully as this is strictly post-hoc analysis but interestingly because these patients are being excluded in most transfusion trials. A broad review also reduces the risk of erroneous conclusion when undertaking narrowing scopes which can lead to the verification of desired hypotheses because trial inclusion are hampered by a priori knowledge of trials with desired outcomes.[80] We excluded preterm infants and neonates to increase clinical applicability.

Despite a thorough pre-planned search strategy in relevant databases supported by hand search we are not able to rule out the possibility of reporting and publication bias.

Trial Sequential Analysis

The overall strength of doing meta-analysis is the increased power and precision of pooled estimates however, the analyses may be influenced by systematic and random error.[138] We applied TSA as a sensitivity analysis to account for the increasing risk of type-I error when doing repetitive testing on accumulating data and to estimate whether information size was reached to draw firm conclusions.[139,140] TSA analyses can be regarded similar to the interim analyses in single trials and some argue that TSAs should be applied with the same methodological rigour.[138] The principle behind this analysis is that the p-value of the conventional meta-analysis is adjusted based on the number of patients needed and the required information size to show a pre-defined intervention effect. In TSA the required information size is adjusted for heterogeneity among trials and calculated based on the rates for type-I and type-II errors, control event proportion and size of the intervention effects. A limitation to this analysis is that these parameters can be based on different

assumptions formed by a priori knowledge or on the basis of results already performed in meta-analyses. We pre-planned and reported the procedure for TSA which is a major strength.

Current evidence for the use of RBC transfusion

Broad systematic reviews and overall use of RBC transfusion

The Cochrane review published in 2012 found 19 RCTs including 6264 patients comparing the effects of different transfusion thresholds on a variety of clinical outcome variables. Pooled analyses showed no association between increased risk of adverse events (mortality, cardiac events, stroke, pneumonia and thromboembolism) and the use of a restrictive transfusion strategy. Authors concluded that for most patients RBC transfusion is probably not essential until Hb levels drop below 7.0 g/dl (4.3 mM).

Our updated review supports the Cochrane review findings and we found no evidence to support an overall use of liberal transfusion strategies. Our review show that if restrictive transfusion strategies were widely implemented, exposure of patients to RBC transfusions would decrease by approximately 45% and reduce the mean number of transfused units by approximately 1.4 units for those patients transfused. This could have potentially impact on the risks for transfusion complications.

The critically ill patient

Walsh et al. conducted a feasibility trial including 100 critically ill patients with age above 55 years enduring prolonged mechanically ventilation (more than 4 days). Haemoglobin triggers of 7 or 9 g/dl were assessed. The trial was not powered to show differences in any of the patient-centred outcomes (mortality or quality of life) but a trend towards lower mortality with the use of the restrictive transfusion strategy and should be assessed in a larger trial.

All together five RCTs[5,67,71,97,104] have now been conducted in the critical care setting including a total of 2639 patients all using 7 g/dl as the lower transfusion threshold. None of the trials showed harm with the use of the lower threshold. A post-hoc meta-analysis of mortality stratified by clinical setting, different from the stratification done in our review, showed that the use of 7 g/dl were not associated with increased risk of death (RR 0.92, 95% CI 0.82 to 1.03) (Figure 9). Again this was not a pre-planned analysis and results have to be interpreted carefully.

Patients with septic shock

Two recent trials[113,114] randomising a total of 2941 patients have questioned the complex early goal- directed therapy (EGDT) protocol by Rivers.[69] There were no differences in the overall mortality at 90 days in both these trials despite the fact that twice the number of patients in the goal-directed groups as in the usual-care groups received RBCs. Currently no evidence exists to support differences in transfusion thresholds between early and late stages of septic shock. Based on the present evidence from the TRISS trial being the only trial conducted in patients with septic shock and in the scope of broad systematic reviews, the use of a transfusion threshold of 7 g/dl is safe and should be the future trigger for RBC transfusion in these patients.

Patients with cardiovascular disease

The largest transfusion trial published to date, the FOCUS trial included 2016 patients with age above 50 years and known atherosclerotic disease, undergoing primary hip fracture osteosynthesis.[87] No difference were shown between patients receiving RBCs at 8 g/dl (or symptoms of anaemia) or 10 g/dl in terms of mortality, postoperative complications or activities of daily living.

A subgroup analysis of 357 patients with cardiovascular disease randomised in the TRICC trial supported the FOCUS trial results and showed no differences in 30 day mortality.[141]

Acute myocardial ischemia

In a recent meta-analysis including both observational studies and randomised trials, Chartterjee et al. showed that the risk of secondary myocardial infarction was increased (RR 2.04 (95% CI 1.06 to 3.93)) with liberal transfusion strategy or transfusion as compared to restrictive transfusion strategy or no transfusion.[8]

To date only two small pilot RCTs comprising 155 patients have compared transfusion triggers in patients with acute myocardial infarction (Figure 9).[88,142] One trial showed higher incidence of adverse outcomes including exacerbation of congestive heart disease with liberal use of RBCs. The trial was not powered to show differences in mortality or recurrent myocardial infarction.[90] The other trial including 110 patients showed a trend towards lower incidences of cardiac events and mortality with the use of a restrictive strategy.[88] We found another four trials in different clinical settings reporting data on fatal and non-fatal myocardial infarction

but TSA were inconclusive. Newly updated guidelines state the inability to make recommendations regarding RBC transfusion in this group because of lacking evidence.[143]

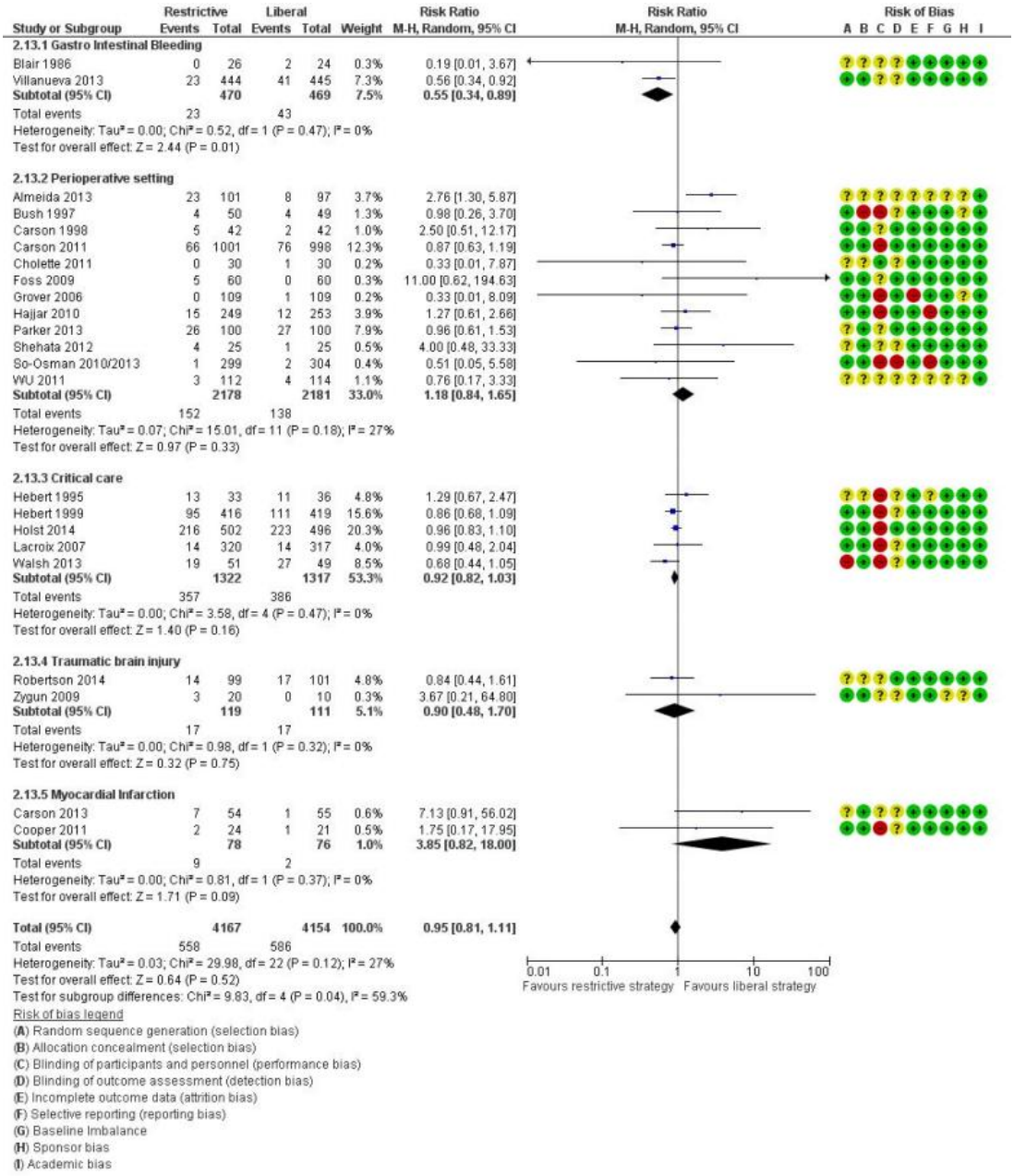

Figure 9. Forest plot of mortality stratified by clinical setting (post-hoc). Size of squares for risk ratio reflects weight of trial in pooled analysis. Horizontal bars represent 95% confidence intervals.

Traumatic brain injury

Two trials have randomised patients with traumatic brain injury.[101,107] One recent published RCT used a factorial design to randomise 200 patients with closed head injury and compared the effects of erythropoietin and two different Hb-thresholds for

RBC transfusion (7 g/dl versus 10 g/dl).[101] The trial showed no difference in neurological outcome six months after randomisation but the trial was not powered to show differences in mortality.

A small subgroup analysis of 67 patients from the TRICC trial with closed head injuries did not show any difference in mortality or organ dysfunction between groups.[144] Whether patients with traumatic brain injury (TBI) needs a higher transfusion level or not remain unknown and further data from high-quality RCTs are needed to guide transfusion practice in this group of patients.

Conclusion and future perspective

The TRISS trial provided evidence for the safe use of 7 g/dl as transfusion trigger in patients with septic shock and reduced the number of units transfused with about half. In line with this, the updated systematic review including data from several recent trials showed no associations with mortality or other adverse events when comparing restrictive to liberal RBC transfusion strategies, however, restrictive transfusion strategies reduce the exposure of patients to RBC transfusions and reduce number of transfused RBC units.

Given the fact that liberal transfusion strategies have not been proven beneficial, a more restrictive approach should be considered. Results from the TRISS trial together with other recent trials have the potential to alter the international guidelines for transfusing critically ill patients. Several guidelines have been updated the last years recommending the use of 7-8 g/dl as the 'universal' trigger level.[9,143,145] Patients with acute myocardial ischemia and patients with acute brain injury may need special considerations.

Time will show how clinicians will adapt to the evidence supporting restrictive transfusion strategies. In the meantime trials are warranted in subgroups of patients and other transfusion triggers than haemoglobin need investigation and could be important if trials like TRISS motivate for an even more restrictive use of RBC transfusions in the future.

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Conflicts of interests

None

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Papers I-II-III

Paper I

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TRIALS

STUDY PROTOCOL Open Access

Transfusion requirements in septic shock (TRISS) trial - comparing the effects and safety of liberal versus restrictive red blood cell transfusion in septic shock patients in the ICU: protocol for a randomised controlled trial

Lars B Holst[1*], Nicolai Haase[1], Jørn Wetterslev[2], Jan Wernerman[3], Anders Åneman[4], Anne B Guttormsen[5], Pär I Johansson[6], Sari Karlsson[7], Gudmundur Klemenzson[8], Robert Winding[9], Lars Nebrich[10], Carsten Albeck[10], Marianne L Vang[11], Hans-Henrik Bülow[12], Jeanie M Elkjær[12], Jane S Nielsen[13], Peter Kirkegaard[14], Helle Nibro[15], Anne Lindhardt[16], Ditte Strange[16], Katrin Thormar[16], Lone M Poulsen[17], Pawel Berezowicz[18], Per M Bådstøløkken[19], Kristian Strand[20], Maria Cronhjort[21], Elsebeth Haunstrup[22], Omar Rian[22], Anders Oldner[23], Asger Bendtsen[24], Susanne Iversen[25], Jørn-Åge Langva[26], Rasmus B Johansen[26], Niklas Nielsen[27], Ville Pettilä[28], Matti Reinikainen[29], Dorte Keld[30], Siv Leivdal[31], Jan-Michael Breider[32], Inga Tjäder[3], Nanna Reiter[1], Ulf Gøttrup[1], Jonathan White[1], Jørgen Wiis[1], Lasse Høgh Andersen[1], Morten Steensen[1] and Anders Perner[1]

Abstract

Background: Transfusion of red blood cells (RBC) is recommended in septic shock and the majority of these patients receive RBC transfusion in the intensive care unit (ICU). However, benefit and harm of RBCs have not been established in this group of high-risk patients.

Methods/Design: The Transfusion Requirements in Septic Shock (TRISS) trial is a multicenter trial with assessor-blinded outcome assessment, randomising 1,000 patients with septic shock in 30 Scandinavian ICUs to receive transfusion with pre-storage leuko-depleted RBC suspended in saline-adenine-glucose and mannitol (SAGM) at haemoglobin level (Hb) of 7 g/dl or 9 g/dl, stratified by the presence of haematological malignancy and centre. The primary outcome measure is 90-day mortality. Secondary outcome measures are organ failure, ischaemic events, severe adverse reactions (SARs: anaphylactic reaction, acute haemolytic reaction and transfusion-related circulatory overload, and acute lung injury) and mortality at 28 days, 6 months and 1 year.
The sample size will enable us to detect a 9% absolute difference in 90-day mortality assuming a 45% event rate with a type 1 error rate of 5% and power of 80%. An interim analysis will be performed after 500 patients, and the Data Monitoring and Safety Committee will recommend the trial be stopped if a group difference in 90-day mortality with $P \leq 0.001$ is present at this point.

Discussion: The TRISS trial may bridge the gap between clinical practice and the lack of efficacy and safety data on RBC transfusion in septic shock patients. The effect of restrictive versus liberal RBC transfusion strategy on mortality, organ failure, ischaemic events and SARs will be evaluated.

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* Correspondence: Lars.Broksoe.Holst@regionh.dk
[1]Department of Intensive Care, Copenhagen University Hospital, Rigshospitalet, Denmark
Full list of author information is available at the end of the article

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Trial registration: ClinicalTrials.gov: NCT01485315. Registration date 30 November 2011. First patient was randomised 3 December 2011.

Keywords: Sepsis, Septic shock, Intensive care medicine, Red blood cell transfusion, Fluid therapy

Background

The first line treatments for patients with septic shock are antibiotics, source control and resuscitation with intravenous fluids and vasopressor/inotropic drugs to optimise circulation and organ perfusion. These interventions may be supplemented with red blood cells (RBCs) in case of anaemia and persistent hypoperfusion [1].

It is known from large prospective studies in European and North American intensive care units (ICUs) that anaemia is very common in critically ill patients; 65% of critically ill patients have haemoglobin (Hb) level <12 g/dl (7.4 mM) at time of admission to the ICU and a mean admission Hb level of 11.3 g/dl (7 mM) [2,3]. As a result of this, 40 to 50% of patients admitted to ICUs are transfused with RBCs during their stay, and 90% of transfusions are administered to non-bleeding patients with a mean of 5 units of RBC per transfused patient. The mean pre-transfusion Hb level - the trigger - in ICU patients is reported to be around 8.5 g/dl (5.3 mM) [4,5].

RBC transfusion has traditionally been perceived as an effective treatment for patients with anaemia - especially for patients with clinical signs of reduced tissue oxygenation [6]. The main function of RBCs is to transport oxygen from the pulmonary to the peripheral capillaries. Thus, RBCs are administered to increase Hb levels and thus blood oxygen-carrying capacity in patients with sepsis to prevent tissue hypoxia and thereby multiple organ failure. However in patients with septic shock, oxygen delivery (DO_2) may increase after RBC transfusion without a corresponding increase in oxygen consumption (VO_2) [7]. Several mechanisms may lie behind this observation.

Firstly, tissue hypoxia in the early phase of sepsis might be due to heterogeneous perfusion (stagnant hypoxia) [8], which may not be amenable to RBC transfusion. Secondly, the stored RBCs may not deliver oxygen as efficient as native cells, perhaps due to biochemical and rheological changes of the RBC suspension *ex vivo*, so called storage lesion [9,10]. Thirdly, organ cells may be unable to exploit the increase in oxygen tension due to mitochondrial changes and such cytopathic hypoxia [11] will not be amenable to increased DO_2 in general [12,13].

In addition, RBC suspensions may have immunomodulatory properties, which can be harmful to patients with sepsis [14,15].

The two trials randomising adult patients with sepsis to different RBC transfusion strategies have shown divergent results. The trial by Rivers and colleagues indicated increased survival with a complex early goal-directed protocol (the goal being central venous oxygen saturation ($ScvO_2$) ≥70%) including RBC transfusion. Mortality was 31% with early goal-directed therapy versus 47% in controls, but the role of RBC transfusion was unclear since transfusion was given only if hypoperfusion persisted after initiation of mechanical ventilation, fluid and administration of inotropic drugs [16]. On the other hand, the Transfusion Requirements in Critical Care (TRICC) trial randomising 838 resuscitated and normovolaemic ICU patients to a transfusion trigger of either 7 g/dl (4.3 mM) (restrictive) or 10 g/dl (6.2 mM) (liberal) found no significant difference in the primary outcome measure - 30-day mortality - between the two groups [17]. Hospital mortality was higher in the liberally transfused patients, who also had significantly more cardiopulmonary complications in the ICU than those in the restrictive group. Predefined subgroup analyses indicated lower mortality in the restrictive transfusion group in younger (age <55 years) and less severely ill patients (Acute Physiology and Chronic Health Evaluation (APACHE) II-score <20).

The results of this trial should be interpreted with caution, since the planned inclusion of 1,600 patients was not achieved due to slow recruitment. The patient population may not be representative for ICU patients in general because cardiovascular disease was more common in the excluded patients than in the included. Thus, potential negative effects of restrictive transfusion practice in cardiac patients may not have been discovered. Furthermore the patients were transfused with non-leuko-depleted RBCs stored in citrate suspension, making it difficult to adapt the results to clinical practice today, where pre-storage leuko-depleted blood is widely used. Finally, all patients were resuscitated and deemed normovolaemic by the clinicians when randomised and therefore less likely to have tissue hypoperfusion.

A cochrane review published in 2012 [18] found 19 randomised clinical trials, involving 6264 patients, examining the effects of transfusion thresholds on different outcome variables. Three trials included intensive care patients and one of these was in paediatric patients. Most of the mortality data (52%) came from the TRICC trial [17]. In this review, restrictive transfusion strategy did not increase the rate of adverse events (that is, mortality, cardiac events, myocardial ischaemia, stroke, pneumonia and thromboembolism) compared to liberal transfusion strategies. Furthermore restrictive transfusion strategies were associated

with a significant reduction in the rate of infections and hospital mortality but not 30-day mortality. The authors of the Cochrane review concluded that current evidence supports the use of a restrictive transfusions strategy for most patients, including patients with pre-existing cardiovascular disease, but more research is needed to evaluate the effects of restrictive transfusion in high-risk patients.

Taken together, RBC transfusion for patients with septic shock remains controversial because important efficacy and safety questions have not been firmly addressed in previous trials. The optimal Hb-guided transfusion strategy that outbalances risk and benefit remains to be established in this subgroup of high-risk patients.

Aim

The aim of the TRISS trial is to assess the effects of two well-defined Hb-trigger guided transfusion strategies on mortality and morbidity in ICU patients with septic shock.

Methods/Design

This is a multicentre trial with computer generated allocation sequence, centralised stratified randomisation, concealed allocation, and blinded outcome assessment of patients with septic shock. The patients will be stratified by centre and by the presence or absence of haematological malignancy and randomised 1:1 to RBC transfusion at Hb ≤7 g/dl (4.3 mM) or Hb ≤9 g/dl (5.6 mM). The latter stratification variable was chosen because these patients have very high mortality rates (70% at 90 days in the 6S trial [19]) and will only be included at few trial sites. Therefore, centre stratification alone may not ensure equal distribution of these patients into the two trial arms.

Hypothesis

The present evidence is not sufficient to support either restrictive or liberal transfusion strategy in ICU patients with septic shock underlining the need for this trial. The transfusion triggers chosen for this trial are well within the range of the current transfusion practice. We do not have *a priori* expectations on superiority/inferiority of one of the transfusion strategies in this trial. However, a restrictive transfusion strategy in patients with septic shock has the potential to reduce the relative risk of death by 20% (9% absolute risk reduction) compared with a liberal strategy based on the subgroup of patients with severe infection in the TRICC trial [17].

Trial interventions

Enrolled patients are given a RBC transfusion when they reach their assigned trigger level (Hb ≤9 g/dl (5.6 mM) or 7 g/dl (4.3 mM)) during the entire ICU stay to a maximum of 90 days after randomisation. After ICU discharge or 90 days after randomisation transfusions are given at the discretion of the clinicians despite group allocation. If the patient is readmitted to the ICU within 90 days after randomisation, the Hb-trigger value assigned at randomisation will be reused regardless of the readmission diagnose or status.

RBCs will be transfused as single units followed by renewed Hb assessment by point-of-care testing within 3 hours of termination of the last transfused unit or before the initiation of a new transfusion. All other interventions will be at the discretion of clinicians.

The choice of the two transfusion triggers is based on data from observational studies representing current transfusion practice in septic shock patients in Scandinavia [5,20] [Figure 1].

All trial sites will use pre-storage leuko-depleted RBCs suspended in saline-adenine-glucose-mannitol (SAGM). The intervention is to be administered as an intravenous infusion after making sure that a match of recipient and donor blood has been carried out. The exact amount of blood (ml) in each unit and the exact amount of blood transfused will be recorded by the clinical staff on a transfusion registration sheet when SAGM transfusions are initiated and terminated.

Concomitant medication/treatment

All other interventions will be at the discretion of the clinicians.

Inclusion criteria

Adult (age 18 years or above) patients in the ICU who:

- Have anaemia (Hb ≤9 g/dl (5.6 mM))
  AND

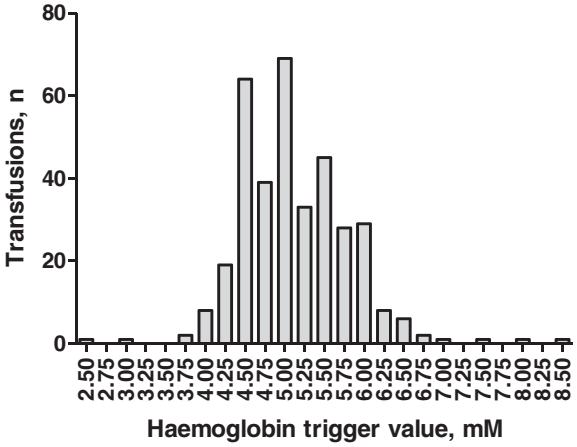

Figure 1 Transfusion trigger levels in Denmark. The figure shows the lowest haemoglobin level measured 0 to 2 hours before red blood cell (RBC) transfusion in 213 consecutive septic shock patients in 7 Danish ICUs. The data represent 358 transfused units of saline-adenine-glucose-mannitol (SAGM) [20]. To convert values in mM to g/l multiply with 1.6.

- Fulfil the criteria for septic shock [see full criteria in Additional file 1] [21]:
  a) Fulfil at least two systemic inflammatory response syndrome (SIRS) criteria within the last 24 hours [22]
  And
  b) Has a suspected or verified focus of infection
  And
  c) Has hypotension (systolic or mean arterial blood pressure ≤90 mmHg or ≤70 mmHg, respectively) despite fluid therapy OR requires infusion of vasopressor/inotropic agents to maintain blood pressure.

Exclusion criteria

Patients fulfilling one or more of the following criteria will not be included:

- Documented wish against transfusion
- Previous SAR with blood products (except transfusion-associated circulatory overload (TACO))
- Presence of ongoing myocardial ischaemia at time of randomisation ((defined as: 1) Patients diagnosed with : a) acute myocardial infarction (ST-elevation myocardial infarction or non-ST elevation myocardial infarction) or b) unstable angina pectoris during current hospital admission, according to the criteria in the clinical setting in question (for example, elevated biomarkers, ischaemic signs on ECG, clinical presence) AND 2) the patient has received treatment, initiated during current hospital admission, as a consequence of this (reperfusion strategies (PCI/thrombolysis) or initiated/increased antithrombotic drug treatment))
- Life-threatening bleeding at time of randomisation defined as: (1) Presence of haemorrhagic shock, as judged by research or clinical staff OR (2) the need for surgical procedure, including endoscopy to maintain Hb levels
- RBC transfusion during current ICU admission, administered before randomisation
- Withdrawal from active therapy or brain death
- Acute burn injuries regardless of severity or total burn surface area
- Lack of informed consent (in Sweden, Norway, Finland and Iceland consent is obtained from next of kin prior to randomisation; in Denmark delayed consent is obtained from next of kin and general practitioner after randomisation), [Figure 2].

Randomisation

Screening and randomisation are centralised, web-based, and accessible 24-hour around-the-clock according to the allocation list, the stratification variables and varying block size created by the Copenhagen Trial Unit (CTU) and kept secret from the investigators to allow immediate and concealed allocation to the intervention.

Primary outcome measure

- Mortality 90 days post-randomisation

Secondary outcome measures

- Mortality within the whole observation period reported at day 28, 6 months and 1 year after randomisation of the last patient
- Life support at day 5, 14 and 28 post randomisation as use of mechanical ventilation, renal replacement or vasopressor/inotropic therapy [23]
  - Severe adverse reactions in the ICU including anaphylactic/allergic reactions, acute haemolysis, transfusion-associated acute lung injury (TRALI), and transfusion associated circulatory overload (TACO)
  - Ischaemic events in the ICU including acute myocardial-, cerebral-, intestinal- and acute peripheral limb ischaemia
  - Days alive and out of hospital
  - Days alive without mechanical ventilation in the 90 days after randomisation
  - Days alive without dialysis/haemofiltration in the 90 days after randomisation
  - Days alive without vasopressor/inotropic therapy in the 90 days after randomisation
  - Health-related quality of life (HRQoL) for Danish patients assessed using the Physical and Mental Component Summary (PCS and MCS) scores of the country specific Short Form health survey questionnaire (SF-36 [24,25]) one-year after randomisation

Blinding

It will not be feasible to mask the assigned transfusion strategy from health care providers. Consequently, clinical staff caring for the patients will be aware of the allocation and correlated intervention bias as well as other bias mechanisms that may not be controlled. However, information on whether the primary outcome of death occurred will be acquired through the National Civil Registries immediately before the interim analysis and the final data analyses. Thus, steering committee members or investigators will have no knowledge to enable them to compare outcome variables with intervention group allocation for any patient. The independent trial statistician will also be blinded for the allocation during analysis. Information on the secondary outcomes, except long-term mortality, will be provided by the local investigators from

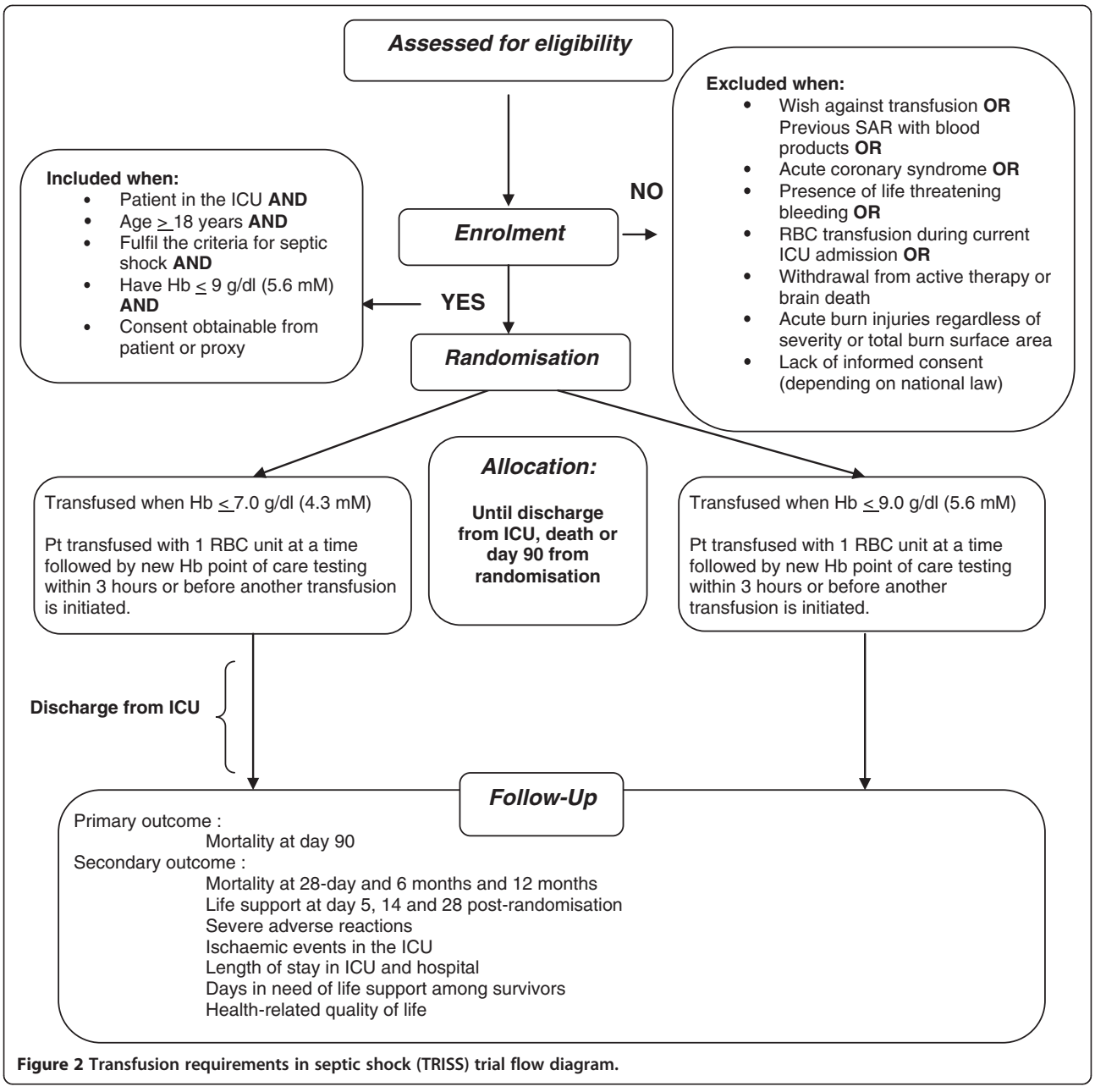

Figure 2 Transfusion requirements in septic shock (TRISS) trial flow diagram.

patient notes, but the statistician doing the final analyses will be blinded for the allocation. The members of the data and safety monitoring committee (DMSC) will remain blinded unless they request otherwise and after the interim analysis has provided strong indications of one intervention being beneficial or harmful.

Participant withdrawal

Patients may be withdrawn from the trial at any time if consent is withdrawn by the person(s), who has given proxy consent or by the patient.

The person(s) demanding withdrawal from trial intervention will be asked for permission to continue data registration. In the event the patient does not prohibit obtaining information on the primary outcome measure, it will be obtained centrally. Thus, there may be the following types of withdrawal:

- From intervention only (allowing for all data registration and follow-up)
- From intervention and further registration (but maintaining already registered data and centralised outcome assessment)
- From intervention, further registration, follow-up, and previously registered data demanding deletion of already registered data. Only the patient can

demand deletion of already registered data and only if the patient did not consent previously.

If patients deny use of data, we are obliged to delete all data. We expect few of these denials and that the trial will continue until full sample size has been reached to maintain statistical power without further violating the randomisation scheme [19].

Patients who are transferred to another ICU will be withdrawn from the transfusion protocol. However, if the new ICU is an active trial site, the allocated transfusion Hb-trigger level will be maintained in this new ICU. In any case, patients who are transferred to another ICU will be followed up for the primary outcome measure.

Suspension of protocol

The protocol may temporarily be suspended for the individual patient, at the discretion of the attending doctor, in case of [see Additional file 2 for details]:

- Life-threatening bleeding or
- Ischaemic events

After stabilisation in these instances, the patient will re-enter the protocol. For non-life-threatening bleeding, including surgical procedures, the protocol will be maintained.

Severe adverse reactions

Serious Adverse Reactions will be registered and are [see Additional file 3 for details]:

- Anaphylactic/allergic reactions
- Severe haemolytic complications
- Transfusion associated acute lung injury (TRALI)
- Transfusion associated circulatory overload (TACO)

Patients who experience a SAR will not be withdrawn from the trial protocol.

Use of hydroxyethyl starch

The recently completed Scandinavian Starch for Severe Sepsis/Septic Shock (6S) trial showed significantly increased mortality (51% versus 43%, $P = 0.03$) and use of renal replacement therapy (RRT) (22% versus 16%, $P = 0.04$) in patients with severe sepsis or septic shock who received HES 130/0.42 compared with those receiving Ringer's acetate [18]. These findings are supported by other recent trials [26,27]. Therefore, we prohibit the use of all starch preparations (that is, Voluven™, Tetraspan™ etcetera) in the TRISS trial.

Statistics

For this study, 2 × 500 patients will be needed to show a 9% absolute risk difference in 90-day mortality (relative risk reduction of 20% with restrictive transfusion among patients with severe infection in the TRICC trial) and mortality of 45% (obtained from 41% in the East Danish Septic Shock Cohort [28] and 51% in a later cohort of septic shock patients in Danish ICUs [29], alpha of 0.05 (two-sided) and power of 80% (1-beta). The Trial Sequential Analysis [30] showed that at least an information gap of 1,000 patients may be expected assuming a 19% relative risk reduction of mortality, and a diversity (D-square) of 0%, and a control event percentage of 11% as found in the traditional meta-analysis of the relevant trials. A type 1 and 2 error rate of 5% and 10%, respectively, were used for the trial sequential analysis [see Additional file 4].

The primary analyses will be by intention-to-treat comparing the two groups by logistic regression analysis for binary outcome measures adjusted for stratification variables (site and presence of haematological disease). An unadjusted Chi-square test for differences in the binary outcomes will be done as a co-primary analysis.

We will perform per protocol analyses of the primary outcome and the most important secondary outcomes excluding patients with one or more major protocol violations [see Additional file 5]. SAS software, version 9.3 (Cary, NC, US) will be used for data management and analysis.

Interim analysis

An interim analysis will be conducted when patient number 500 has been followed for 90 days [see Additional file 6 for details].

The independent DMSC will recommend pausing or stopping the trial if it finds:

- A group difference in the primary outcome measure $P < 0.001$ (Haybittle-Peto criterion) [31,32]. If an analysis of the interim data from 500 patients fulfils the Haybittle-Peto criterion the inclusion of further patients will be paused and an analysis including patients randomised during the analysis period will be performed. If this second analysis also fulfils the Haybittle-Peto criterion or if the group sequential monitoring boundaries are reached the DMSC will recommend stopping the trial.
- Results from other trials combined with the interim analysis from the TRISS trial show clear benefit or harm with RBC transfusion in meta-analysis using trial sequential analysis [30] with a diversity-adjusted required information size [33] based on an *a priori* relative risk reduction of 10%, an overall type 1 error rate of 5% and a type 2 error rate of 20% (power of

80%) and a control event proportion percentage of 45%.

Intervention accountability

Every patient will be allocated a transfusion registration sheet. This will be kept on site in the site master file. The transfusion registration sheet will include the allocated patient screening number, time for initiation of transfusion and unit volume.

Registration

Data will be registered into the electronic web-based case report form (eCRF) from patient notes (source data) by trial site personnel. The CTU in cooperation with the coordinating investigator will establish the trial database from an export of data from the eCRF. Paper CRF will be used in case of technical difficulties. Any deviation from the protocol will be captured either in the eCRF or in notes-to-file. Data registration is performed at each participating site by trained personnel.

The following data will be registered

Pre-randomisation and baseline characteristics: Basic patient characteristics (national identification number or date of birth and site of inclusion (dependent on national law), sex, estimated weight, suspected or confirmed site of infection, surgery during current admission (emergency, elective or not), date of admission to hospital and date and time of admission to ICU and from where the patient was admitted to ICU, co-morbidity (haematological malignancy or not (assessed at screening), chronic obstructive pulmonary disease, asthma or other chronic lung disease or not, cardiovascular disease or not (defined by history of acute myocardial infarction, stable/unstable angina pectoris, previous coronary intervention (CABG or PCI), chronic heart failure (NYHA class 3 to 4) [34], vascular disease (as previous central (aortic or iliac) or peripheral vascular intervention) or ischaemic stroke (including infarction and transitory cerebral ischaemia) and use of RRT.

24 hours prior to randomisation: Lowest/highest Hb level, volume of transfused blood components (specified as RBCs, plasma and platelets), lowest values of $ScvO_2$, highest value of p-lactate and data for Simplified Acute Physiology Score (SAPS) 2 [35] and Sepsis-related Organ Failure Assessment (SOFA) scoring [36].

Daily during the entire ICU stay: Hb-levels (daily minimum, maximum and number of assessments), volumes of transfused blood products (RBCs, plasma and platelets), time for initiation of RBC transfusion, unit ID, blood storage time, fluid in-/output, renal replacement therapy or not, vasopressor/inotropic infusion or not, mechanical ventilation or not, lowest PaO_2/FiO_2, lowest $ScvO_2$, highest p-lactate, surgery or not, any bleeding, ischaemic events, severe adverse reactions (SAR), and decision on not resuscitate in case of cardiac arrest.

90 days after randomisation: Survival status and hospital discharge status obtained from hospital or civil registries, and date of death if the patient has deceased.

Last day of any of the following interventions if the patient was discharged from the trial ICU receiving any of these: Renal replacement therapy, vasopressor/inotropic infusion and mechanical ventilation. We plan to perform a landmark mortality analysis for all randomised patients with a follow-up for each patients of 90 days, the primary analysis will be a logistic regression analysis adjusted for stratification variables. Further, we plan to perform survival analyses including Kaplan-Meier estimates within the total observation time. That is until the last randomised patient has been followed for 3 month. Within the total observation time we will also perform an adjusted proportional hazards analysis (Cox regression analysis), provided the criterion on proportional hazards is fulfilled, adjusting for all the pre-specified covariates listed in the protocol [37-39].

Twelve months after randomisation: Survival status obtained from hospital or civil registries and date of death if the patient is deceased. Days in need of life support (mechanical ventilation, renal replacement or vasopressor/inotropic therapy) in survivors: Status obtained from hospital or civil registries. Health-related quality of life in survivors obtained by posting of the SF-36 questionnaire followed by phone contact if the patient does not reply.

Data handling and record keeping

Data will be handled according to the data protection agencies of the different countries. All original records (including consent forms, eCRFs, and relevant correspondences) will be archived at trial sites or at CTU for 15 years. The clean electronic trial database file will be anonymised and delivered to the Danish Data Archive and maintained for 15 years.

Monitoring

Monitoring will adhere to good clinical practice (GCP [40]) principles and be performed according to a predefined monitoring plan including the following issues:

- Initiation visits at all sites
- For all patients: Documented informed consent
- For all patients: Primary outcome according to national or hospital registries
- For 100 patients being the first two patients at each trial site, and another two patients randomly chosen at each trial site: Documented delivery or non-delivery in the eCRF of the intervention according to the protocol compared with source data being patients' hospital records

- The coordinating centre will continuously monitor that all eCRFs are fulfilled according to the protocol
- Termination visit at all sites: Documenting informed consent for all participants.

A centralised day-to-day monitoring of the eCRF and adherence to the protocol (for example, the ability of individual centres to transfuse at assigned transfusion values only) will be done by the coordinating investigator or his delegates. Additional monitoring visit will be made to selected sites if the steering committee finds this necessary based on monitoring findings.

Ethical considerations

The trial will be conducted in adherence to the current version of the Helsinki Declaration [41] and to the standards of GCP. Screening of patients will only start after approval by the ethics committee and data protection agency in the countries of the trial sites.

There is no conclusive evidence from RCTs on the potential benefit or risk of RBC transfusion in adults with septic shock. RBC transfusion is part of the current treatment of septic shock, and the Hb-trigger values chosen for the present trial are well within those observed in clinical practice. Thus, the participants will not be exposed to known risks when included into the trial.

Furthermore, the research question is in the public's interest and the trial design will provide meaningful data with the potential to reach statistical significance and therefore lead to the acceptance or rejection of the null hypothesis.

Ethical approvals

By 8 January 2013 the study had been approved by: (Denmark) De Videnskabetiske Komiteer - Region Hovedstaden (H-3-2011-114); (Sweden) Regionala etikprövningsnämnden i Stockholm (2011/2:8) (2012/814-32); (Norway) Regionale Komiteer For Medicinsk og Helsefaglig Forskningsetikk (2011/2270/REK vest); (Finland) Tampereen Yliopistollisen Sairaalan Erityisvastuualueen Alueellinen Eettinen Toimikunta (R12269).

Informed consent

The majority of patients assessed for enrolment in the trial will be unable to give informed consent because of severe illness or as a consequence of the treatment (sedation). Some patients will thus be randomised and enrolled before obtaining informed consent if applicable by national law and after approval by the Ethics Committee for each of the participating ICUs.

There is no alternative to this approach as no clinically relevant model of septic shock exists and no conscious patients have the combination of severe infection, shock and multiple organ failure.

Furthermore, septic shock is an acute life-threatening condition and rapidly initiated resuscitation according to guidelines [1] is important to give the patient the best chance of survival. It would therefore be unacceptable to delay initiation of treatment while awaiting informed consent.

As soon as possible after enrolment proxy consent will be obtained from the patient's next of kin or general practitioner/regional medical officer of health according to national law. Patients who regain consciousness, will be asked for informed consent as soon as possible.

Duration

Patients from 31 Scandinavian ICUs are expected to be included during a 2-year inclusion period starting December 2011. Based on data from 6S trial it is realistic to include a mean of two patients per ICU per month [19] [Figure 3].

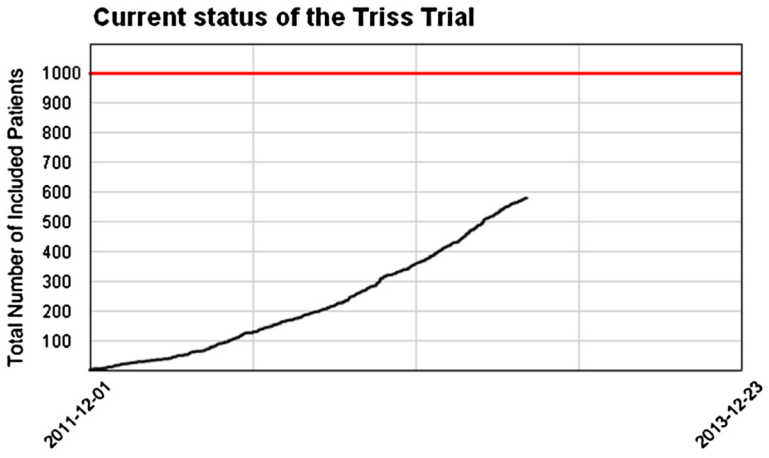

Figure 3 Trial status. Print from the ransfusion equirements n eptic hock (TRISS) electronic Case Record Form showing the trial status on 19 April 2013.

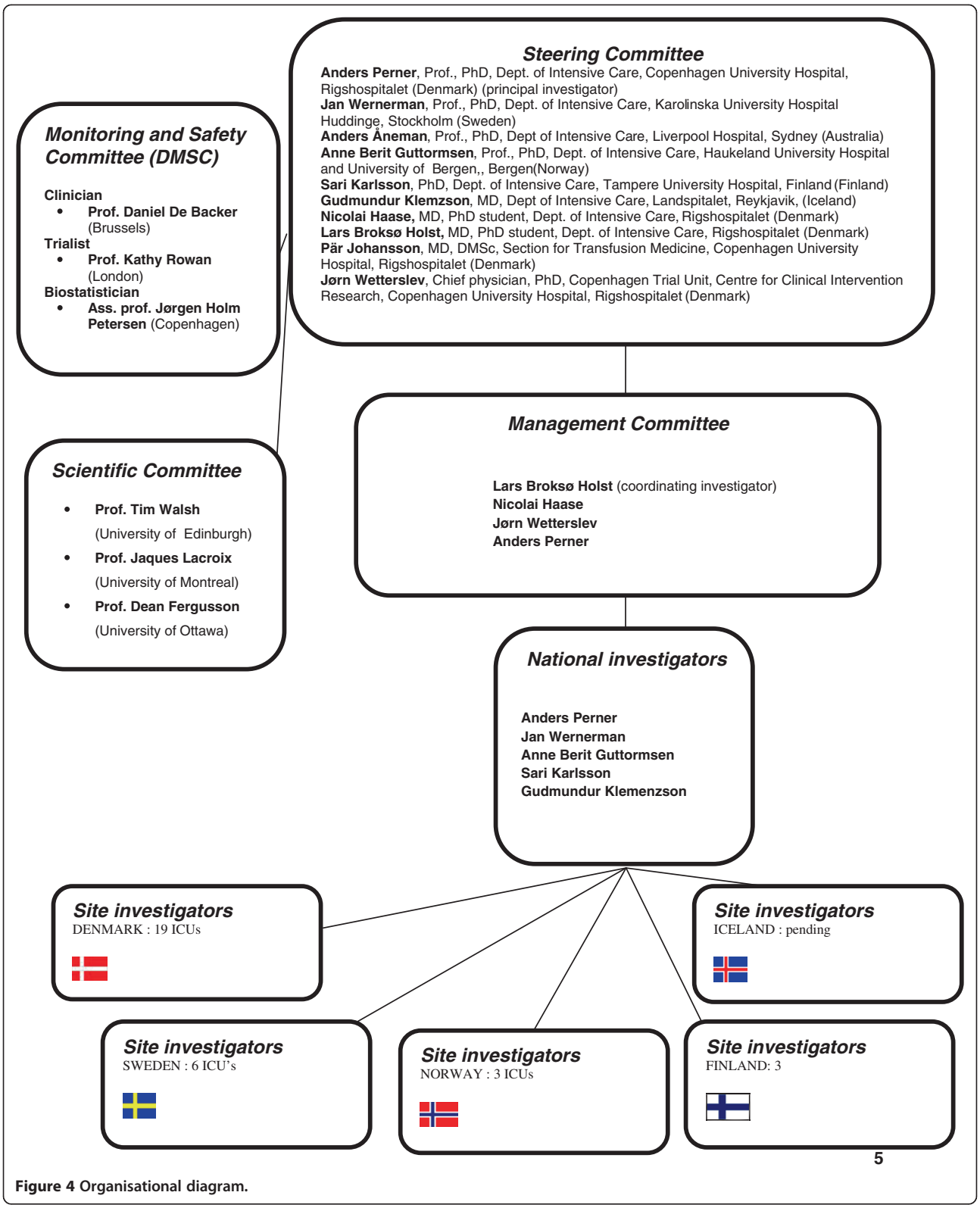

Figure 4 Organisational diagram.

Low recruitment contingency plan

In case of low recruitment we will involve new trial sites to reach the goal of including 1,000 patients within the 2-year time period.

Co-enrolment

We will assess the eligibility of patients included in the TRANSFUSE trial (ClinicalTrials.gov identifier: NCT01638416) but not of patients included in the ARISE trial (ClinicalTrials.gov identifier: NCT00975793).

Timeline

- 2011: Protocol, approvals from ethical committees, trial tool development (eCRF and randomisation system)
- 2012 to 2013: Inclusion of patients
- Mid-2013: Interim analyses
- 2014: The database is expected to be closed in March 90 days after the inclusion of the last patient. Data analyses and writing of the manuscript will be in April followed by submission for publication shortly thereafter

Trial organisation

This trial is investigator-initiated as a collaborative research programme between the Scandinavian Critical Care Trials Group, Rigshospitalet, Copenhagen Trial Unit and 31 ICUs in all the Nordic countries. [Figure 4]

Publication plan

The trial is registered on www.clinicaltrials.gov. Upon trial completion the main manuscript will be submitted to one of the major clinical journals regardless of the result, and the results will in any case be published at the SCCTG home page. The Steering Committee will grant authorship in adherence to the Vancouver guidelines [42] and number of patients enrolled by the individual investigator. If a trial site investigator is to gain authorship, the site has to include 25 patients or more. If the site includes 50 patients or more, two authorships will be granted per trial site, 75 patients will give three authorships per trial site and so on.

Finances

The TRISS trial is funded by the Danish Council for Strategic Research (09–066938) and Copenhagen University Hospital, Rigshospitalet. The funding sources will have no influence on trial design, trial conduct, data handling, data analysis, or publication.

Perspectives

Severe sepsis affects millions of patients worldwide with high rates of complications and mortality. Outcome differences between therapies for sepsis will therefore have a major impact on global health and healthcare costs. As far as the investigators are aware, no other RCTs are assessing the effects or safety of RBC transfusion in patients with septic shock.

Discussion

Performing the TRISS trial is in line with recommendations from the 2012 updated Cochrane review [18] and American Association of Blood Bankers [43] guidelines, both stating the need for trials assessing the effects of transfusion triggers in high risk populations.

The TRISS trial may bridge the gap between clinical practice and evidence providing urgently needed data on the efficacy and safety of RBC transfusion for patients with septic shock. The TRISS trial investigators have facilitated a network of Scandinavian ICUs enrolling a high number of patients with septic shock.

Trial Status

The first patient was randomised 3 December 2011. As of 19 March 2013 31 ICUs are participating, 779 patients have been screened, and 578 patients have been randomised. Ethical approvals in Iceland are pending, and we are expecting 2 to 3 new trial sites to be initiated in the following months.

Additional files

Additional file 1: Trial criteria for septic shock.

Additional file 2: Protocol suspension criteria.

Additional file 3: Severe adverse reactions (SARs).

Additional file 4: Trial sequential analysis.

Additional file 5: Statistical analysis plan.

Additional file 6: Charter for the independent Data Monitoring and Safety Committee (DMSC) of the TRISS trial.

Abbreviations

6S: Scandinavian tarch for evere Sepsis/eptic hock trial; ALI: Acute lung injury; ARDS: Acute respiratory distress syndrome; ARR: Absolute risk reduction; CTU: Copenhagen Trial Unit; DMSC: Data monitor and safety committee; DO_2: Oxygen delivery; eCRF: electronic (web based) case report form; FiO_2: Fraction of inspired oxygen in a gas mixture; Hb: Haemoglobin; ICH-GCP: Guidelines for Good Clinical Practice; ICU: Intensive care unit; PaO_2: Partial pressure of oxygen in arterial blood; POC: Point of care; Pt: Patient; RBC: Red blood cells; RCT: Randomised clinical trial; RRR: Relative risk reduction; RRT: Renal replacement therapy; SAE: Serious adverse event; SAGM: Saline-adenine-glucose-mannitol; SAR: Suspected adverse reaction; SC: Steering committee; SCCTG: Scandinavian Critical Care Trials Group; $ScvO_2$: central venous oxygen saturation; SIRS: Systemic inflammatory response syndrome; TACO: Transfusion associated circulatory overload; TRALI: Transfusion associated acute lung injury; TRICC: Transfusion Requirements in Critical Care; TRISS: Transfusion Requirements in Septic Shock trial; Tx: Transfusion; VO_2: Oxygen consumption.

Competing interests

The ICU at Copenhagen University Hospital receives research funds from Fresenius Kabi. Anders Perner, Nicolai Haase, Jørn Wetterslev, Anders Åneman, Anne Berit Guttormsen, and Gudmundur Klemenzson were members of the steering committee of the 6S trial, which was supported by B Braun Medical. The remaining authors declare that they have no competing interests.

Authors' contributions

All authors made substantive contributions to the TRISS trial as trial site investigators and revised and gave final approval of the manuscript. LBH drafted the manuscript together with NH, JW and AP. AP is the principal investigator and sponsor of TRISS. LBH designed the trial together with NH, JW, JWM, AÅ, ABG, SC, GK and AP. LBH is coordinating investigator and member of the steering and management committee. AP, JWM, ABG, SC and GK are national investigators.

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Author details

[1]Department of Intensive Care, Copenhagen University Hospital, Rigshospitalet, Denmark. [2]Copenhagen Trial Unit, Centre for Clinical Intervention Research, Copenhagen University Hospital, Rigshospitalet, Denmark. [3]Department of Intensive Care, Karolinska University Hospital, Huddinge, Sweden. [4]Department of Intensive Care, Liverpool Hospital, Sydney, Australia. [5]Department of Intensive Care, Haukeland University Hospital and University of Bergen, Bergen, Norway. [6]Section for Transfusion Medicine, Copenhagen University Hospital, Rigshospitalet, Denmark. [7]Department of Intensive Care, Tampere University Hospital, Tampere, Finland. [8]Department of Intensive Care, Landspitalet, Reykjavik, Iceland. [9]Department of Intensive Care, Herning Hospital, Herning, Denmark. [10]Department of Intensive Care, Hvidovre Hospital, Hvidovre, Denmark. [11]Department of Intensive Care, Randers Hospital, Randers, Denmark. [12]Department of Intensive Care, Holbæk Hospital, Holbæk, Denmark. [13]Department of Intensive Care, Kolding Hospital, Kolding, Denmark. [14]Department of Intensive Care, Næstved Hospital, Næstved, Denmark. [15]Department of Intensive Care, Århus University Hospital Nørreborgade, Århus, Denmark. [16]Department of Intensive Care, Bispebjerg Hospital, Bispebjerg, Denmark. [17]Department of Intensive Care, Slagelse Hospital, Slagelse, Denmark. [18]Department of Intensive Care, Vejle Hospital, Vejle, Denmark. [19]Department of Intensive Care, Gentofte Hospital, Gentofte, Denmark. [20]Department of Intensive Care, Stavanger Hospital, Stavanger, Norway. [21]Department of Intensive Care, Södersjukhuset, Stockholm, Sweden. [22]Department of Intensive Care, Horsens Hospital, Horsens, Denmark. [23]Department of Intensive Care, Karolinska University Hospital, Solna, Sweden. [24]Department of Intensive Care, Glostrup Hospital, Glostrup, Denmark. [25]Department of Intensive Care, Slagelse Hospital, Slagelse, Denmark. [26]Department of Intensive Care, Ålesund Hospital, Ålesund, Norway. [27]Department of Intensive Care, Helsingborg Hospital, Helsingborg, Sweden. [28]Department of Intensive Care, Helsinki University Hospital and University of Helsinki, Helsinki, Finland. [29]Department Of Intensive Care, North Karelia Central Hospital, Joensuu, Finland. [30]Department of Intensive Care, Århus University Hospital Skejby, Århus, Denmark. [31]Department of Intensive Care, Sønderborg Hospital, Sønderborg, Denmark. [32]Department of Intensive Care, Centrallasarettet, Växjö, Sweden.

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Additional file 1

Trial criteria for septic shock [21]

(1) ***AT LEAST TWO SYSTEMIC INFLAMMATORY RESPONSE SYNDROME (SIRS) CRITERIA****:*

1. ***CORE TEMPERATURE >38℃ or <36℃*** *. (Core temperature is rectal, urinary bladder, central line, or tympanic). If oral, inguinal or axillary temperatures are used, add 0.5℃ to the measured value. Hypothermia <36℃ must be confi rmed by core temperature. Use the most deranged value recorded* ***in the 24 hours before randomisation****.*
2. ***HEART RATE ≥90 beats/minute****. If patient has an atrial arrhythmia, record the ventricular rate. If patients have a known medical condition or are receiving treatment that would prevent tachycardia (for example, heart block or beta blockers), they must meet two of the remaining three SIRS criteria. Use the most deranged value recorded* ***in the 24 hours before randomisation****.*
3. ***MECHANICAL VENTILATION*** *for an acute process or respiratory rate ≥ 20 breaths per minute or a $PaCO_2$ < 4.3 kPa (32 mmHg). Use the most deranged respiratory rate or $PaCO_2$ recorded* ***in the 24 hours before randomisation****.*
4. ***WHITE BLOOD CELL COUNT of ≥12 x 10^9/l or ≤ 4 x 10^9/l****. Use the most deranged value recorded* ***in the 24 hours before randomisation****.*

AND

(2) ***SUSPECTED OR VERIFIED FOCUS OF INFECTION*** *as either:*

(i) An organism grown in blood or sterile site

OR

(ii) An abscess or infected tissue (e.g. pneumonia, peritonitis, urinary tract, vascular line infection, soft tissue, etc).

AND

(3) ***HYPOTENSION*** *(Systolic blood pressure ≤ 90 mmHg or MAP ≤ 70 mmHg) despite fluid therapy OR* ***VASOPRESSOR/INTROPE*** *infusion to maintain blood pressure.*

Additional file 2

Protocol suspension criteria

The protocol may temporarily be suspended for the individual patient, at the discretion of the attending doctor, if the patient is to be transfused with RBC during any of the following events:

- Presence of life-threatening bleeding :

  Defined as the presence of haemorrhagic shock, as judged by research or clinical staff.

- Ischaemic events defined as:

  1. Acute myocardial ischaemia:
     Defined as ***acute myocardial infarction*** *(ST-elevation myocardial infarction and non-ST elevation myocardial infarction) or* ***unstable angina pectoris*** diagnosed during current hospital admission, according to the criteria in the clinical setting in question (e.g. elevated biomarkers, ischaemic signs on ECG, clinical presence) **AND** the patient has received treatment as a consequence of this (reperfusion strategies (PCI/thromobolysis) or initiation/increased antithrombotic drug treatment), during current hospital admission.
  2. Cerebral ischaemia: Verified by CT- or MR scan
  3. Intestinal ischaemia: Verified by endoscopy or open surgery.
  4. Acute peripheral limb ischaemia: Clinical signs AND need of open/percutaneous vascular intervention, amputation or initiation/increased antithrombotic treatment.

The protocol will be resumed promptly once the patient no longer fulfils the suspension criterion. Suspension will not be considered a breach of protocol, and collection of data will continue during the suspension. These patients will be analysed according to their originally assigned groups on an intention-to-treat basis.

Additional file 3

Severe adverse reactions (SARs)

Patients will not be withdrawn from the trial protocol if Serious Adverse Reactions (SARs) occur but these will register as:

1. *Anaphylactic/allergic reactions after transfusion (occurrence within 6 hours of transfusion) of RBC*

2. *Severe haemolytic complications after transfusion (occurrence within 24 hours of transfusion) of RBC*

3. *Transfusion-associated acute lung injury (TRALI) after RBC transfusion*

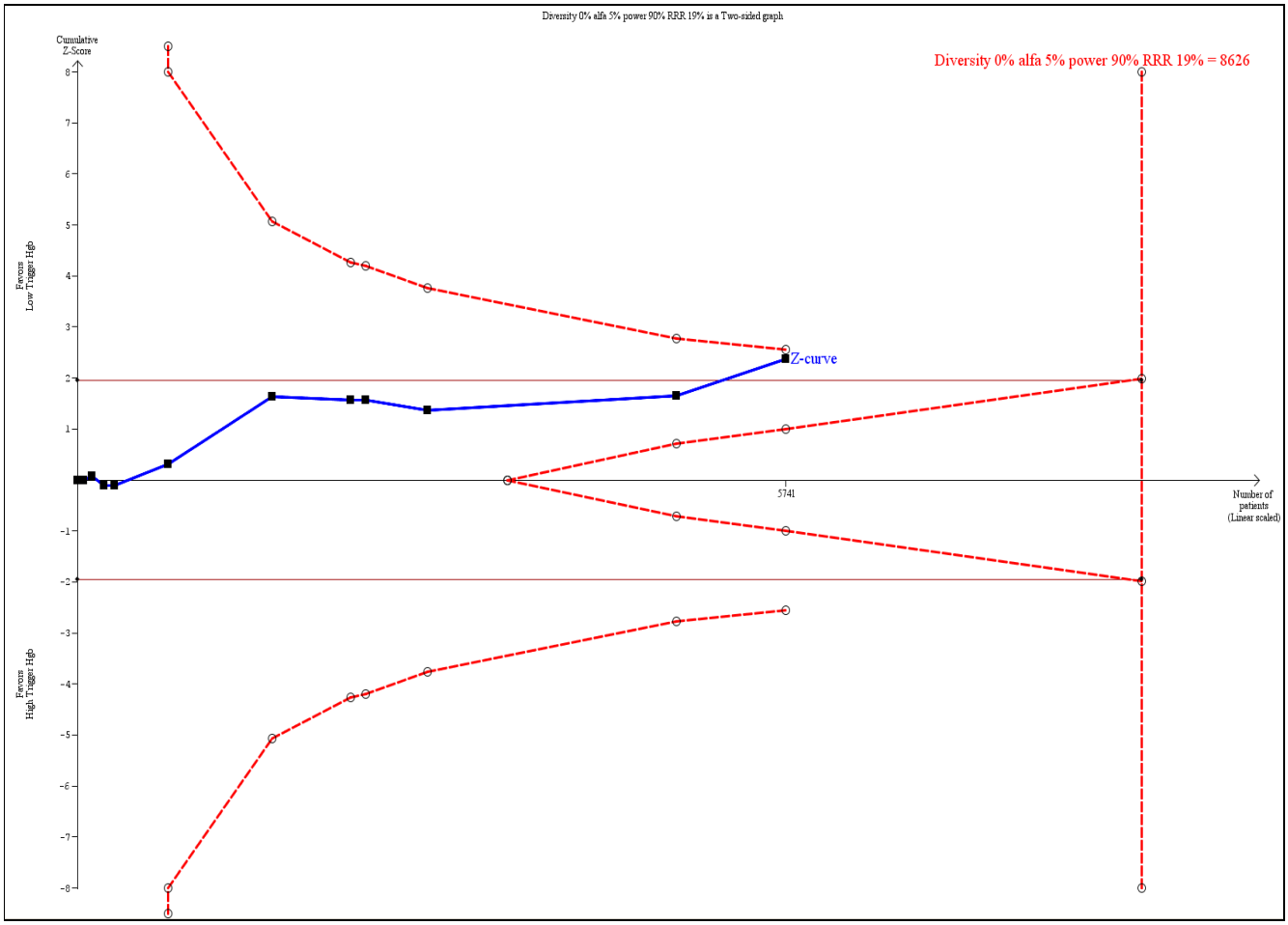
4. *Transfusion associated circulatory overload (TACO) after RBC transfusion*

The occurrence of SARs will be recorded in the eCRF during the ICU stay and compared for the two trial groups by the DMSC at the interim analysis. During the trial, sponsor will send yearly reports on the occurrence of SARs to the DMSC and the ethic committees.

SAEs will not be recorded as an entity, because the majority of septic ICU patients will experience several SAEs during their ICU stay. The most important SAEs will be captured as secondary outcome measures (life support).

Additional file 4

Trial sequential analysis (TSA)



The figure depicts Trial sequential analysis (TSA) of trials comparing the effect of low Hb trigger level with high Hb trigger level on all cause mortality, using a diversity of 0%, an anticipated intervention effect of 19% relative risk reduction, a control event proportion of 11% all of which was indicated in the meta-analysis by Carson et al. 2012 [18] up-dated with Villanueva et al. NEJM 2013 [44]. A type 1 and 2 error risk of 5% and 10% respectively was used. The required information size is estimated to be 8236 and neither boundaries for benefit or futility are crossed by the cumulative z-curve suggesting premature declaration of statistical significant benefit in the traditional random-effects meta-analysis (RR=0.81 95% CL 0.67-0.96) TSA adjusted 95% CL being 0.64-1.02.

Additional file 5

TRISS-Trial Statistical Analysis Plan

Populations

Intention-to-treat population: All randomised patients except those who withdrew their consent for the use of data.

Per-Protocol populations:

Per-protocol #1:

All randomised patients except patients having one or more major protocol violations defined as:

1. One or more RBC transfusions given despite Hb $\geq$ 7.0 g/dl in patients assigned to the restrictive strategy-group

   OR
2. One or more RBC transfusion not given within 24 hours after Hb $\leq$ 9.0 g/dl in patients assigned the liberal strategy-group

   OR
3. Monitoring revealed that one or more in- or exclusion criteria were violated

   OR
4. One or more transfusions of RBC unit(s) destined for another patient

   OR
5. One or more transfusions given despite lack of cross-match between donor and recipient. Administration of 0-neg blood without X-match between donor and recipient will not be regarded as a protocol violation

   OR
6. Any protocol suspension defined as transfusions administered when Hb > trigger level on days with the presence of ischaemic events and/or bleeding

   OR
7. Stopped/withdrawn patients

Per-protocol #2:

All randomised patients except patients having one or more protocol violations defined as

1. One or more transfusions *given* despite the patients Hb level being *above the trigger* level the patient was randomly assigned to

OR

2. *O*ne or more transfusions *not given* within a period of 24 hours after the patient is diagnosed with a Hb level *below the trigger* level that the patient was randomly assigned to

Per-protocol #3:

1. Patients who had one or more bleeding episodes

   OR

2. Patients who had one or more ischaemic episodes

Subgroups:

1. Patients with SAPS II > 53 at baseline
2. Patients age > 70 years
3. Patients with cardiovascular disease

Analyses

Primary analysis:
Will be a logistic regression analysis for binary outcome measures adjusted for stratification variables (site and presence of haematological disease). We will provide an unadjusted Chi-square test for differences in the binary outcomes as well. For rate data the generalized linear model (SAS proc genmod) will be used with distribution Poisson, link=log and offset.

Secondary analysis:
Multiple (logistic) regression and analysis of rate data with the following covariates:

Binary covariates

- Site (stratification variable)
- Hematological malignancy at time of randomisation (stratification variable) Y/N
- Previous cardiovascular disease Y/N
- Surgery during current hospital admission, but prior to randomisation Y/N

Continuous covariate

- Age
- SAPS II in the 24-hours prior to randomisation
- SOFA-score in the 24-hours prior to randomisation
- Hb-level at baseline
- Volume of transfused blood in the 24-hours prior to randomisation

Difference between groups in all-cause mortality within the whole observation period six-month and 1 year after randomisation of the last patient will be analysed using Cox proportional hazards method (Cox regression analysis) using unadjusted analysis and analysis adjusting for the design and patient variables listed above.

Outcomes

Primary outcome measure:

The primary outcome measure of 90-day mortality as retrieved from the National Civil Registries.

Secondary outcome measures:

- Mortality within the whole observation period reported at day 28, six-month and 1 year after randomisation of the last patient.
- Life support at day 5, 14 and 28 (i.e. need of mechanical ventilation, renal replacement therapy or vasopressor/inotropic therapy) post randomisation
- Severe adverse reactions (SARs) in the ICU
- Ischaemic events in the ICU (including myocardial, cerebral, intestinal and peripheral)
- Length of stay in ICU and hospital
- Days in need of life support among survivors
- Health-related quality of life (HRQoL) assessed using the SF-36

Level of statistical significance for all analyses: P = 0.05

Missing Data

Initially, we will perform a complete case-analysis. Then supplementary analyses using imputed data as described below will be performed:

Missing baseline data:

SAPS II
The score is based on values measured in the first 24 hours of ICU admission but we register SAPS II as a baseline score including values from the 24 hours prior to randomisation so patients randomised immediately after ICU admission may have missing values.

SOFA-score
This score does not depend on when the patient is admitted to the ICU but we register SOFA at baseline including values from the 24 hours prior to randomisation. Thus patients randomised immediately after ICU admission may also have missing values in this score.

For SAPS II and SOFA scores day 1 values may reflect patient's condition. However day 1 have variable length as it starts at time of randomisation and ends at the beginning of the next fluid-day. Thus variables may be missing at both baseline and day 1. In these situations data from day 2 may be representative of the patient's condition.

If the frequency of missing data after the above implemented logical imputation is > 5% we will perform *"best"/"worst" case scenarios* where 1) missing SAPS- or SOFA-components in group A will be given to the worst possible score AND missing SAPS- or SOFA-components in group B will be given the best score (zero) or 2) missing SAPS- or SOFA-components in group A will be given the best score (zero) AND missing SAPS- or SOFA-components in group B will be given the worst possible score. If there is no reasonable difference between the results of these two analyses, we will not do further imputation.

If the frequency of missing data after the above implemented "worst-best" scenarios is still > 5% and the complete case analysis is significant at the 10% value or less, we will perform an additional analysis using the multiple imputation method.

If the frequency of missing data after the above implemented "imputations" is > 5%, we will perform an additional analysis using the multiple imputation method.

Missing primary outcome data:
We do not expect missing data on the primary outcome as these will be obtained from hospital or civil registries. Only complete case analysis will be made.

Missing secondary outcome data
Only complete case analysis will be made.

To put significant results into perspective the following sensitivity analysis will be conducted: We define a worst case scenario as one where patients with missing data do not react on the treatment (whatever it may be). Missing data will be imputed according to this scenario. Let P be the estimate of the parameter reflecting the effect of the intervention calculated from the complete case analysis and P-imp be the corresponding estimate calculated from the analysis of the imputed data.
[(P-imp – P)/P-imp]*100% then a ball park figure of the bias is to be expected were the worst case scenario true.
P-imp/ (standard error of P-imp) is calculated and the corresponding p value found to assess the potential impact of this bias on the significance level.

Additional file 6

Charter for the independent Data Monitoring and Safety Committee (DMSC) of the TRISS-trial

Introduction

The Charter will define the primary responsibilities of the DMSC, its relationship with other trial components, its membership, and the purpose and timing of its meetings. The Charter will also provide the procedures for ensuring confidentiality and proper communication, the statistical monitoring guidelines to be implemented by the DMSC, and an outline of the content of the Open and Closed Reports that will be provided to the DMSC.

Primary responsibilities of the DMSC

The DMSC will be responsible for safeguarding the interests of trial participants, assessing the safety and efficacy of the interventions during the trial, and for monitoring the overall conduct of the clinical trial. The DMSC will provide recommendations about stopping or continuing the trial to the Steering Committee (SC) of the TRISS trial. To contribute to enhancing the integrity of the trial, the DMSC may also formulate recommendations relating to the selection/recruitment/retention of participants, their management, improving adherence to protocol-specified regimens and retention of participants, and the procedures for data management and quality control.

The DMSC will be advisory to the SC. The SC will be responsible for promptly reviewing the DMSC recommendations, to decide whether to continue or terminate the trial, and to determine whether amendments to the protocol or changes in trial conduct are required.

The DMSC is planned by protocol to meet physically in order to evaluate the planned interim analysis of the TRISS-trial. The interim analysis will be performed by an independent statistician selected by the members of the DMSC. The DMSC may additionally meet whenever they decide, contact each other by telephone or e-mail in order to discuss the safety for trial participants. Sponsor has the responsibility to report yearly to the DMSC the overall number of Serious Adverse Reactions (SAR).The DMSC can at any time during the trial request the distribution of events, including outcome measures and SARs according to intervention groups. The recommendations of the DMSC regarding stopping, continuing or changing the design of the trial should be communicated without delay to the SC of the TRISS-trial. The SC has the responsibility to inform as fast as possible, and no later than 48 hrs, all investigators of the trial and the sites including patients in the trial the recommendation of the DMSC and the SC decision hereof.

Members of the DMSC

The DMSC is an independent multidisciplinary group consisting of a clinician, a trialist and a biostatistician that, collectively, has experience in the management of ICU patients and in the conduct, monitoring and analysis of randomised clinical trials.

DMSC Clinician
Daniel De Backer (Brussels)

DMSC Trialist
Kathy Rowan (London)

DMSC Biostatistician
Jørgen Holm Petersen, Dept. of Biostatistics, University of Copenhagen

Conflicts of interest
DMSC membership has been restricted to individuals free of conflicts of interest. The source of these conflicts may be financial, scientific, or regulatory in nature. Thus, neither trial investigators nor individuals employed by the sponsor, nor individuals who might have regulatory responsibilities for the trial products, are members of the DMSC. The DMSC members do not own stock in the companies having products being evaluated by the TRISS-trial.
The DMSC members will disclose to fellow members any consulting agreements or financial interests they have with the sponsor of the trial, with the contract research organisation (CRO) for the trial (if any), or with other sponsors having products that are being evaluated or having products that are competitive with those being evaluated in the trial.
The DMSC will be responsible for deciding whether these consulting agreements or financial interests materially impact their objectivity.
The DMSC members will be responsible for advising fellow members of any changes in these consulting agreements and financial interests that occur during the course of the trial. Any DMSC members who develop significant conflicts of interest during the course of the trial should resign from the DMSC.
DMSC membership is to be for the duration of the clinical trial. If any members leave the DMSC during the course of the trial, the SC will appoint the replacement(s).

Formal interim analysis meeting
One 'Formal Interim Analysis' meeting will be held to review data relating to treatment efficacy, patient safety, and quality of trial conduct. The three members of the DMSC will meet when 90-day follow-up data of 500 patients have been obtained.

Proper communication
To enhance the integrity and credibility of the trial, procedures will be implemented to ensure the DMSC has sole access to evolving information from the clinical trial regarding comparative results of efficacy and safety data, aggregated by treatment group (0.1). An exception will be made to permit access to an independent statistician who will be responsible for serving as a liaison between the database and the DMSC.
At the same time, procedures will be implemented to ensure that proper communication is achieved between the DMSC and the trial investigators. To provide a forum for exchange of information among various parties who share responsibility for the successful conduct of the trial, a format for Open Sessions and Closed Sessions will be implemented. The intent of this format is to enable the DMSC to preserve confidentiality of the comparative efficacy results while at the same time providing opportunities for interaction between the DMSC and others who have valuable insights into trial-related issues.

Closed Sessions
Sessions involving only DMSC membership who generates the Closed Reports (called Closed Sessions) will be held to allow discussion of confidential data from the clinical trial, including information about the relative efficacy and safety of interventions. In order to ensure that the DMSC will be fully informed in its primary mission of safeguarding the interest of participating patients, the DMSC will be blinded in its assessment of safety and efficacy data. However, the DMSC can request unblinding from the SC.

Open Reports
For each DMSC meeting, Open Reports will be provided available to all who attend the DMSC meeting. The Reports will include data on recruitment and baseline characteristics, and pooled data on eligibility violations, completeness of follow-up, and compliance. The primary trial statistician will prepare these Open Reports.

Closed Reports will include analysis of the primary efficacy outcome measure. In addition, analyses of the secondary outcome measures and serious adverse events will also be reported. These Closed Reports will be prepared by an independent biostatistician, with assistance from the trial biostatisticians, in a manner that allow them to remain blinded.

The Closed Reports should provide information that is accurate, with follow-up on mortality that is complete to within two months of the date of the DMSC meeting.

The Reports should be provided to DMSC members approximately three days prior to the date of the meeting.

Minutes of the DMSC Meetings
The DMSC will prepare minutes of their meetings. The Closed Minutes will describe the proceedings from all sessions of the DMSC meeting, including the listing of recommendations by the Committee. Because it is likely that these minutes may contain unblinded information, it is important that they are not made available to anyone outside the DMSC.

Recommendations to the Steering Committee
After the interim analysis meeting, the DMSC will make a recommendation to the SC to continue, hold or terminate the trial.

If an analysis of the interim data from 500 patients fulfils the Haybittle-Peto criterion the inclusion of further patients will be paused and an analysis including patients randomised during the analysis and period of pausing the trial will be performed. If this second analysis also fulfils the Haybittle-Peto criterion or the group sequential monitoring boundaries the DMSC will recommend stopping the trial.

If the recommendation is to stop the trial the DSMC will discuss and recommend on whether the final decision to stop the trial will be made after the analysis of all patients included at the time (including patients randomised after patient number 500) and whether a moratorium shall take place (setting the trial at hold) in the further inclusion of patients during these extra analyses. If further analyses of the patients included after 500 patients is recommended rules for finally recommending stopping of the trial should obey the Lan DeMets stopping boundary.

This recommendation will be based primarily on safety and efficacy considerations and will be guided by statistical monitoring guidelines defined in this Charter and the trial protocol.

The SC is jointly responsible with the DMSC for safeguarding the interests of participating patients and for the conduct of the trial. Recommendations to amend the protocol or conduct of the trial made by the DMSC will be considered and accepted or rejected by the SC. The SC will be responsible for deciding whether to continue, hold or stop the trial based on the DMSC recommendations.

The DMSC will be notified of all changes to the trial protocol or conduct. The DMSC concurrence will be sought on all substantive recommendations or changes to the protocol or trial conduct prior to their implementation.

Statistical monitoring guidelines
The outcome parameters are defined in the TRISS-trial protocol. For the two intervention groups, the DMSC will evaluate data on:
The primary outcome measure
Mortality at 90 days after randomisation.

The secondary outcome measures
Need for life-support at days 5, 14 and 28
The occurrence of SARs in ICU
The occurrence of ischaemic events in ICU

The DMSC will be provided with these data from the Coordinating Centre as:
Number of patients randomised
Number of patients randomised per intervention group (0.1)
Number of patients stratified pr. stratification variable per intervention group (0.1)
Number of events, according to the outcomes, in the two groups

Based on evaluations of these outcomes, the DMSC will decide if they want further data from the Coordinating Centre and when next to perform analyses of the data.

For analyses, the data will be provided in one file as described below.

Based on the analyses of the primary outcome measure and SARs, the DMSC will use $P<0.001$ (Haybittle-Peto) as the statistical limit to guide its recommendations regarding early termination of the trial.

Based on 90-day mortality analyses, the DMSC will use $P<0.001$ (Haybittle-Peto) and group sequential monitoring boundaries as the statistical limit to guide its recommendations regarding early termination of the trial.

DMSC should also be informed about all SARs occurring in the two groups of the trial.

The DMSC may also be asked to ensure that procedures are properly implemented to adjust trial sample size or duration of follow-up to restore power, if protocol specified event rates are inaccurate. If so, the algorithm for doing this should be clearly specified.

Conditions for transfer of data from the Coordinating Centre to the DMSC
The DMSC shall be provided with the data described below in one file.

The DMSC will be provided with an Excel database containing the data defined as follows:

Row 1 contains the names of the variables (to be defined below).

Row 2 to N (where N-1 is the number of patients who have entered the trial) each contains the data of one patient.

Column 1 to p (where p is the number of variables to be defined below) each contains in row 1 the name of a variable and in the next N rows the values of this variable.

The values of the following variables should be included in the database:

1: PtID: a number that uniquely identifies the patient.

2: Rdcode: The randomisation code (group 0 or 1) – the DMSC is not to be informed on what intervention the groups received.

3: 90MInd: 90 day-mortality indicator (2 if patient is censored, 1 if patient was dead, and 0 if the patient was alive at day 90).

4: OF5ind: Life support at day 5. (1 if patient in need of life support and 0 if the patient did not).

5: OF14ind: Life support at day 14 (1 if patient in need of life support and 0 if the patient did not).

6: OF28ind: Life support at day 28 (1 if patient in need of life support and 0 if the patient did not).

7: SARInd: Severe Adverse Reaction indicator (1 if patient has had SAR in ICU and 0 if the patient did not).

8: ICHInd: Ischaemic event in ICU (1 if patient has had an ischaemic event in ICU and 0 if the patient did not).

Paper II

ORIGINAL ARTICLE

Lower versus Higher Hemoglobin Threshold for Transfusion in Septic Shock

Lars B. Holst, M.D., Nicolai Haase, M.D., Ph.D., Jørn Wetterslev, M.D., Ph.D., Jan Wernerman, M.D., Ph.D., Anne B. Guttormsen, M.D., Ph.D., Sari Karlsson, M.D., Ph.D., Pär I. Johansson, M.D., Ph.D., Anders Åneman, M.D., Ph.D., Marianne L. Vang, M.D., Robert Winding, M.D., Lars Nebrich, M.D., Helle L. Nibro, M.D., Ph.D., Bodil S. Rasmussen, M.D., Ph.D., Johnny R.M. Lauridsen, M.D., Jane S. Nielsen, M.D., Anders Oldner, M.D., Ph.D., Ville Pettilä, M.D., Ph.D., Maria B. Cronhjort, M.D., Lasse H. Andersen, M.D., Ulf G. Pedersen M.D., Nanna Reiter, M.D., Jørgen Wiis, M.D., Jonathan O. White, M.D., Lene Russell, M.D., Klaus J. Thornberg, M.D., Peter B. Hjortrup, M.D., Rasmus G. Müller, M.D., Morten H. Møller, M.D., Ph.D., Morten Steensen, M.D., Inga Tjäder, M.D., Ph.D., Kristina Kilsand, R.N., Suzanne Odeberg-Wernerman, M.D., Ph.D., Brit Sjøbø, R.N., Helle Bundgaard, M.D., Ph.D., Maria A. Thyø, M.D., David Lodahl, M.D., Rikke Mærkedahl, M.D., Carsten Albeck, M.D., Dorte Illum, M.D., Mary Kruse, M.D., Per Winkel, M.D., D.M.Sci., and Anders Perner, M.D., Ph.D., for the TRISS Trial Group* and the Scandinavian Critical Care Trials Group

ABSTRACT

BACKGROUND

Blood transfusions are frequently given to patients with septic shock. However, the benefits and harms of different hemoglobin thresholds for transfusion have not been established.

METHODS

In this multicenter, parallel-group trial, we randomly assigned patients in the intensive care unit (ICU) who had septic shock and a hemoglobin concentration of 9 g per deciliter or less to receive 1 unit of leukoreduced red cells when the hemoglobin level was 7 g per deciliter or less (lower threshold) or when the level was 9 g per deciliter or less (higher threshold) during the ICU stay. The primary outcome measure was death by 90 days after randomization.

RESULTS

We analyzed data from 998 of 1005 patients (99.3%) who underwent randomization. The two intervention groups had similar baseline characteristics. In the ICU, the lower-threshold group received a median of 1 unit of blood (interquartile range, 0 to 3) and the higher-threshold group received a median of 4 units (interquartile range, 2 to 7). At 90 days after randomization, 216 of 502 patients (43.0%) assigned to the lower-threshold group, as compared with 223 of 496 (45.0%) assigned to the higher-threshold group, had died (relative risk, 0.94; 95% confidence interval, 0.78 to 1.09; P=0.44). The results were similar in analyses adjusted for risk factors at baseline and in analyses of the per-protocol populations. The numbers of patients who had ischemic events, who had severe adverse reactions, and who required life support were similar in the two intervention groups.

CONCLUSIONS

Among patients with septic shock, mortality at 90 days and rates of ischemic events and use of life support were similar among those assigned to blood transfusion at a higher hemoglobin threshold and those assigned to blood transfusion at a lower threshold; the latter group received fewer transfusions. (Funded by the Danish Strategic Research Council and others; TRISS ClinicalTrials.gov number, NCT01485315.)

From the Department of Intensive Care (L.B.H., N.H., L.H.A., U.G.P., N.R., J. Wiis, J.O.W., L.R., K.J.T., P.B.H., R.G.M., M.H.M., M.S., A.P.), Copenhagen Trial Unit, Center for Clinical Intervention Research (J. Wetterslev, P.W.), and Section for Transfusion Medicine (P.I.J.), Rigshospitalet and University of Copenhagen, Copenhagen, Randers Hospital, Randers (M.L.V., H.B., M.A.T.), Herning Hospital, Herning (R.W., D.L., R.M.), Hvidovre Hospital, Hvidovre (L.N., C.A.), Aarhus University Hospital, Aarhus (H.L.N., D.I.), Aalborg University Hospital, Aalborg (B.S.R.), Holbæk Hospital, Holbæk (J.R.M.L.), Kolding Hospital, Kolding (J.S.N.), and Hjørring Hospital, Hjørring (M.K.) — all in Denmark; Karolinska University Hospital, Huddinge, Stockholm (J. Wernerman, I.T., K.K., S.O.-W.), Karolinska University Hospital, Solna (A.O.), and Södersjukhuset, Stockholm (M.B.C.) — all in Sweden; Haukeland University Hospital and University of Bergen, Bergen, Norway (A.B.G., B.S.); Tampere University Hospital, Tampere (S.K.), and Helsinki University Hospital and University of Helsinki, Helsinki (V.P.) — all in Finland; and Liverpool Hospital, Sydney (A.Å.). Address reprint requests to Dr. Perner at the Department of Intensive Care, Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen, Denmark, or at anders.perner@regionh.dk.

*Members of the Transfusion Requirements in Septic Shock (TRISS) Trial Group are listed in the Supplementary Appendix, available at NEJM.org.

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Blood transfusions are frequently given to patients with septic shock.[1-4] Some of these transfusions are given to patients who are bleeding, but many nonbleeding patients also undergo transfusion.[5]

The recommendations of the Surviving Sepsis Campaign regarding blood transfusion in patients with septic shock are complex and include a recommendation for transfusion to maintain a hematocrit of more than 30% in the presence of hypoperfusion in the first 6 hours.[6] After that, the transfusion threshold should be a hemoglobin level of less than 7 g per deciliter, aiming at levels between 7 g and 9 g per deciliter in patients who do not have myocardial ischemia, severe hypoxemia, acute hemorrhage, or ischemic coronary artery disease.[6] However, there are limited data supporting these recommendations,[6] and many clinicians may not follow them.[4,7] New trial data have been published recently,[8] and the use of a high hemoglobin threshold for transfusion may be at least questioned as part of an early resuscitation protocol for patients with septic shock.

Blood transfusion has been associated with increased mortality in subgroups of critically ill patients, both in cohort studies and in randomized trials,[9-12] but there have also been cohort studies in which transfusion was associated with improved survival,[13] including among patients with sepsis.[14] In some studies, nonleukoreduced blood was used, which may have influenced the results. Given the lack of efficacy data, in addition to concerns about safety, we conducted the Transfusion Requirements in Septic Shock (TRISS) trial to evaluate the effects on mortality of leukoreduced blood transfusion at a lower versus a higher hemoglobin threshold among patients with septic shock who are in the intensive care unit (ICU).

Methods

Trial Design and Oversight

After the approvals from ethics committees and data-protection agencies were obtained, patients in 32 general ICUs in Denmark, Sweden, Norway, and Finland underwent screening and randomization between December 3, 2011, and December 26, 2013. Written informed consent was obtained from all the patients or their legal surrogates before or after enrollment. In all cases, consent was obtained from the patient when possible. If consent was withdrawn or not granted, we asked the patient or surrogate for permission to continue registration of trial data and to use these data in the analyses. The protocol, including details regarding trial conduct and the statistical analysis plan, has been published previously[15] and is available with the full text of this article at NEJM.org. The management committee (see the Supplementary Appendix, available at NEJM.org) designed the trial and vouches for the adherence of the study to the protocol and for the accuracy of the data and the analyses. The members of the management committee wrote the drafts of the manuscript and made the decision to submit the manuscript for publication. The funders had no role in the design of the protocol, the trial conduct, or the analyses or reporting of the data.

This trial was a multicenter, stratified, parallel-group, clinical trial. Randomization was performed with the use of a centralized computer-generated assignment sequence, with stratification according to study site and the presence or absence of active hematologic cancer, because these characteristics may influence outcome.[16,17] Patients with septic shock were randomly assigned in a 1:1 ratio, with the use of permuted blocks of varying sizes of 6, 8, or 10, to blood transfusion at the higher hemoglobin threshold or the lower hemoglobin threshold. Treatment assignments were concealed from the investigators assessing mortality, the data and safety monitoring committee, and the trial statistician. The conduct of the trial and the safety of the participants were overseen by the data and safety monitoring committee, which performed an interim analysis after 500 patients had been followed for 90 days. The trial data were monitored by staff from the coordinating center.

Trial Patients

We screened patients 18 years of age or older who were in the ICU, fulfilled the criteria for septic shock,[18] and had a blood concentration of hemoglobin of 9 g per deciliter or less as measured by means of valid point-of-care testing (see the Supplementary Appendix). The reasons for the exclusion of some patients are shown in Figure 1 and listed in the Supplementary Appendix.

Intervention

Enrolled patients were given single units of cross-matched, prestorage leukoreduced red cells suspended in a saline–adenine–glucose–mannitol

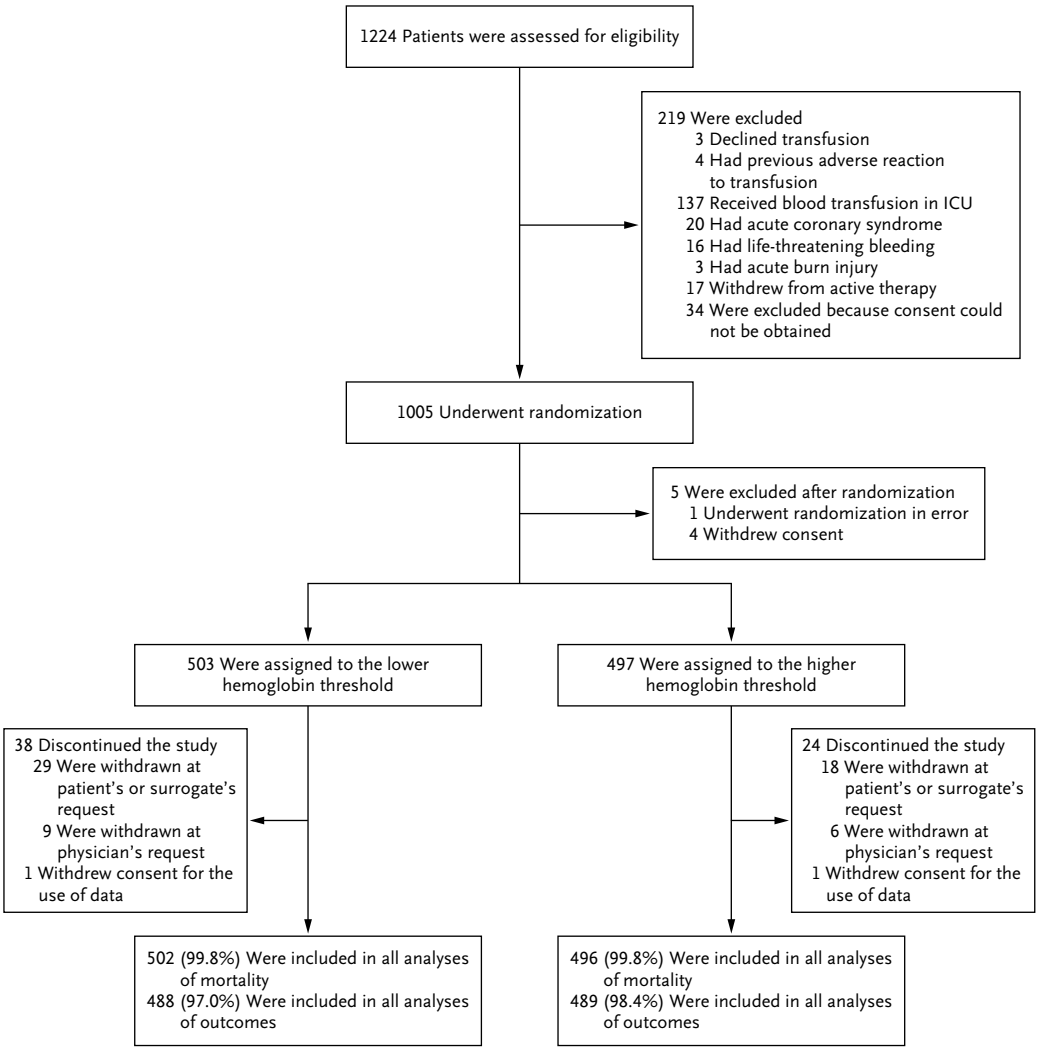


Figure 1. Assessment, Randomization, and Follow-up.

Patients were excluded if they had undergone randomization in this study previously, if there were medical reasons, if they had received a blood transfusion during the current intensive care unit (ICU) admission, if there was a documented wish not to receive a transfusion, or if informed consent could not be obtained. A total of 15 patients met two exclusion criteria. One patient was excluded immediately after randomization when it was determined that an inclusion criterion had not been met, and 4 were excluded because consent was withdrawn during the trial. Thereafter, 5 additional patients underwent randomization in order for the study to obtain the full sample. All the patients who withdrew from the trial at their own request or at a surrogate's request allowed the use of their data, but 14 patients or surrogates in the lower-threshold group (hemoglobin level, ≤7 g per deciliter) and 7 in the higher-threshold group (hemoglobin level, ≤9 g per deciliter) did not want further data registered except for mortality data, which were obtained from national registries. The process data (hemoglobin assessments and numbers of transfusions and temporary protocol suspensions and protocol violations) and some of the secondary-outcome data for these patients are missing.

solution when the blood concentration of hemoglobin had decreased to the assigned transfusion threshold (≤7 g per deciliter [lower threshold] or ≤9 g per deciliter [higher threshold]). These levels of hemoglobin have frequently been used as thresholds for transfusion in patients with septic shock.[15] Hemoglobin concentrations were reassessed within 3 hours after termination of the transfusion or before the initiation of another transfusion. The intervention period was the entire ICU stay, to a maximum of 90 days after randomization.

In the event that life-threatening bleeding or ischemia developed while a patient was in the ICU or a patient required the use of extracorporeal membrane oxygenation, the patient could receive a transfusion at a hemoglobin threshold decided by the attending doctor. The attending doctor decided when the patient again was to receive a transfusion at the assigned hemoglobin threshold. After the unmasking of trial data showing harm from hydroxyethyl starch,[3] we recommended against the use of all starch products in trial patients. All other interventions were at the discretion of the clinicians, including transfusion during surgery and after ICU discharge.

OUTCOME MEASURES

The primary outcome measure was death by 90 days after randomization. Secondary outcome measures were the use of life support (defined as the use of vasopressor or inotropic therapy, mechanical ventilation, or renal-replacement therapy) at days 5, 14, and 28 after randomization[19]; the number of patients with serious adverse reactions while in the ICU (allergic reaction, hemolysis, transfusion-associated acute lung injury, or transfusion-associated circulatory overload) (see the Supplementary Appendix); the number of patients with ischemic events while in the ICU, which included cerebral ischemia (identified from the results of imaging), acute myocardial ischemia (defined by symptoms, electrocardiographic signs, or elevated biomarker levels resulting in an intervention), intestinal ischemia (as observed during endoscopic examination or surgery), or limb ischemia (defined as clinical signs resulting in an intervention) (for full definitions, see the Supplementary Appendix); the percentage of days alive without vasopressor or inotropic therapy, mechanical ventilation, or renal-replacement therapy in the 90 days after randomization; and the percentage of days alive and out of the hospital in the 90 days after randomization. Data for the outcome measures were obtained by TRISS trial investigators or their delegates from patient files and national and regional registries for the entire 90-day follow-up period.

STATISTICAL ANALYSIS

We calculated that we would need to enroll 1000 patients for the trial to have 80% power to show mortality at 90 days that was 9 percentage points lower in the lower-threshold group than in the higher-threshold group, at a two-sided alpha level of 5%, assuming a mortality in the higher-threshold group of 45% (estimated from two previous cohorts).[20,21] The estimated difference of 9 percentage points was derived from the 20% reduction in relative risk observed with a restrictive versus liberal transfusion strategy in the subgroup of patients with severe infection in the Transfusion Requirements in Critical Care (TRICC) trial.[9] During our trial, 5 patients were excluded after randomization (4 patients did not allow the use of their data, and 1 did not have sepsis, which was realized immediately after randomization). A total of 5 additional patients underwent randomization in order for the study to obtain the full sample (Fig. 1).

An author who was the statistician for the study and who was unaware of the study-group assignments performed all the analyses according to International Conference on Harmonisation Good Clinical Practice guidelines[22] and the statistical analysis plan.[15] We performed the primary analyses in the intention-to-treat population, which included all the patients who underwent randomization, except for those whose data were deleted from the database during the trial (i.e., the 5 patients, noted above, who were excluded after randomization) and after the trial (2 patients who withdrew consent for the use of their data) (Fig. 1). In the per-protocol populations, we excluded patients who had one or more bleeding or ischemic episodes or one or more major protocol violations (see the Supplementary Appendix).[22]

In the primary analyses (including the analysis of the primary outcome measure), we compared data between the two groups by means of logistic-regression analysis for binary outcome measures with adjustment for the stratification variables (study site and presence or absence of active hematologic cancer),[23] and we converted odds ratios to relative risks.[24] We also performed unadjusted chi-square testing for binary outcome measures and Wilcoxon signed-rank testing for rate and ordinal data. We compared the primary outcome in the per-protocol populations and in prespecified subgroups defined according to the presence or absence of chronic cardiovascular disease (i.e., any history of myocardial infarction, any history of stable or unstable angina pectoris, previous treatment with nitrates, percutaneous coronary intervention, coronary-artery bypass grafting or noncoronary vascular interventions, any history of chronic heart failure [defined

as New York Heart Association class III or IV], or any history of cerebral infarction or transitory cerebral ischemia), an age of 70 years or younger versus an age older than 70 years, and a Simplified Acute Physiology Score (SAPS) II above 53 versus 53 or lower at baseline (with the score calculated from 17 variables and ranging from 0 to 163, with higher scores indicating higher severity of disease) and used multiple logistic-regression analyses in the intention-to-treat population to adjust for differences in prespecified risk factors at baseline. Details regarding the handling of missing data are provided in the Supplementary Appendix. We performed all analyses using SAS software, version 9.3 (SAS Software), and SPSS software, version 17.0 (SPSS). A two-sided P value of less than 0.05 was considered to indicate statistical significance.

RESULTS

TRIAL POPULATION

We obtained 90-day vital status for 998 patients (99.3%), including 502 in the lower-threshold group and 496 in the higher-threshold group (Fig. 1). The characteristics of the patients at baseline were similar in the two groups (Table 1, and Table S1 in the Supplementary Appendix). A total of 29 of 488 patients (5.9%) in the lower-threshold group and 11 of 489 (2.2%) in the higher-threshold group had the protocol temporarily suspended (P=0.004) (Table S2 in the Supplementary Appendix).

HEMOGLOBIN CONCENTRATIONS, BLOOD PRODUCTS, AND CIRCULATORY VARIABLES

The median value of the lowest concentration of hemoglobin in the 24 hours before randomization was 8.4 g per deciliter in both intervention groups. After randomization, the daily lowest concentrations of hemoglobin differed between the two groups (P<0.001) (Fig. 2). Additional details regarding hemoglobin assessments are provided in Table S3 in the Supplementary Appendix.

During the trial period, a total of 1545 blood transfusions were given in the lower-threshold group and 3088 transfusions in the higher-threshold group (P<0.001). The median cumulative number of blood transfusions after randomization was 1 unit (interquartile range, 0 to 3) in the lower-threshold group and 4 (interquartile range, 2 to 7) in the higher-threshold group (P<0.001). A total of 176 patients (36.1%) in the lower-threshold group did not undergo transfusion in the ICU, as compared with 6 (1.2%) in the higher-threshold group (P<0.001). Details regarding blood products, bleeding, cointerventions, fluid volumes and balances, and circulatory assessments are provided in Tables S4 through S9 in the Supplementary Appendix. The numbers of protocol violations differed significantly between the two groups (Table S10 in the Supplementary Appendix).

OUTCOMES

At 90 days after randomization, 216 patients (43.0%) in the lower-threshold group and 223 (45.0%) in the higher-threshold group had died (relative risk, 0.94; 95% confidence interval, 0.78 to 1.09; P=0.44) (Table 2 and Fig. 3, and Table S11 in the Supplementary Appendix). We obtained similar results in the analyses that were adjusted for prespecified baseline risk factors and in the per-protocol analyses (Table S12 in the Supplementary Appendix). The prespecified subgroup analyses showed no significant heterogeneity in the effect of the transfusion threshold on mortality at 90 days between patients with and those without chronic cardiovascular disease, patients 70 years of age or younger and those older than 70 years of age, and patients with a SAPS II of 53 or less and those with a SAPS II of more than 53 at baseline (Fig. 3).

A total of 7.2% of the patients in the lower-threshold group, as compared with 8.0% in the higher-threshold group, had one or more ischemic events in the ICU (Table 2, and Tables S13 and S14 in the Supplementary Appendix, which include the numbers of patients with myocardial ischemia and ischemia of other anatomical sites). One patient had a serious adverse reaction to transfusion (Table 2, and Table S13 in the Supplementary Appendix). The use of life support at days 5, 14, and 28 was similar in the two intervention groups (Table 2, and Tables S11 and S13 in the Supplementary Appendix), as were the percentages of days alive without vasopressor or inotropic therapy, without mechanical ventilation, and without renal-replacement therapy and the percentage of days alive and out of the hospital (Table 2).

DISCUSSION

In this international, multicenter, partially blinded, randomized trial involving patients with sep-

tic shock who were in the ICU, we observed no significant differences in mortality at 90 days, in the numbers of patients with ischemic events or with severe adverse reactions, in the use of life support, or in the numbers of days alive and out of the hospital between the group of patients who underwent transfusion at a lower hemoglobin threshold and the group of those who underwent transfusion at a higher hemoglobin threshold. Similar results were observed in subgroups of patients with chronic cardiovascular disease, with older age, or with greater disease severity. The patients in the lower-threshold group received 50% fewer units of blood than those in the higher-threshold group, and 36% of the patients in the lower-threshold group did not undergo transfusion in the ICU, as compared with 1% of the patients in the higher-threshold group.

Our results are consistent with those obtained in the TRICC trial, which assessed a lower versus higher hemoglobin threshold for blood transfusion in a broad population of adult patients in the ICU.[9] In that trial, there were no significant differences in mortality at 30 days in the full trial population (the primary outcome) or among patients 55 years of age or older or

Table 1. Characteristics of the Trial Patients at Baseline.*

Characteristic	Lower Hemoglobin Threshold (N=502)	Higher Hemoglobin Threshold (N=496)
Age — yr		
Median	67	67
Interquartile range	57–73	58–75
Male sex — no. (%)	272 (54.2)	259 (52.2)
Chronic cardiovascular disease — no. (%)†	75 (14.9)	66 (13.3)
Chronic lung disease — no. (%)‡	111 (22.1)	102 (20.6)
Hematologic cancer — no. (%)	39 (7.8)	36 (7.3)
Admission to a university hospital — no. (%)	323 (64.3)	324 (65.3)
Surgery during index hospitalization — no. (%)		
Emergency	191 (38.0)	217 (43.8)
Elective	59 (11.8)	53 (10.7)
Source of ICU admittance — no. (%)		
Emergency department	90 (17.9)	79 (15.9)
General ward	268 (53.4)	257 (51.8)
Operating or recovery room	113 (22.5)	121 (24.4)
Other ICU	31 (6.2)	39 (7.9)
Source of sepsis — no. (%)§		
Lungs	267 (53.2)	259 (52.2)
Abdomen	206 (41.0)	198 (39.9)
Urinary tract	58 (11.6)	61 (12.3)
Soft tissue	59 (11.8)	59 (11.9)
Other	50 (10.0)	47 (9.5)
Positive culture from blood or sterile site	188 (37.5)	160 (32.3)
Interval from ICU admission to randomization — hr		
Median	23	20
Interquartile range	7–50	7–43
SAPS II¶		
Median	51	52
Interquartile range	42–62	44–64

Table 1. (Continued.)

Characteristic	Lower Hemoglobin Threshold (N=502)	Higher Hemoglobin Threshold (N=496)
SOFA score‖		
Median	10	10
Interquartile range	8–12	8–12
Renal-replacement therapy — no. (%)**	68 (13.5)	53 (10.7)
Mechanical ventilation — no. (%)††	345 (68.7)	350 (70.6)

* None of the differences between the two groups were significant (P≥0.05). Additional details regarding baseline characteristics are provided in Table S1 in the Supplementary Appendix. The lower hemoglobin threshold was defined as a hemoglobin level of 7 g per deciliter or less, and the higher hemoglobin threshold as a hemoglobin level of 9 g per deciliter or less. ICU denotes intensive care unit.

† Patients were considered to have chronic cardiovascular disease if they had any history of myocardial infarction, stable or unstable angina pectoris, chronic heart failure (defined as New York Heart Association class III or IV), cerebral infarction or transitory cerebral ischemia, previous treatment with nitrates, percutaneous coronary intervention, coronary-artery bypass grafting, or noncoronary vascular interventions.

‡ Patients were considered to have chronic lung disease if they had any history of chronic obstructive pulmonary disease, asthma or other chronic lung disease, or any treatment with a drug indicated for chronic lung disease.

§ Some patients had more than one source of infection. Other sources of sepsis included a vascular catheter, meningitis, or endocarditis or were unclear.

¶ The Simplified Acute Physiology Score (SAPS) II[25] was assessed in the 24 hours before randomization. The SAPS II is calculated from 17 variables and ranges from 0 to 163, with higher scores indicating higher severity of disease. One or two of the 17 variables were missing for 77 patients in the higher-threshold group and for 99 in the lower-threshold group, so their values were not included here.

‖ The Sepsis-Related Organ Failure Assessment (SOFA)[26] score was assessed in the 24 hours before randomization. The SOFA grades organ failure, with subscores ranging from 0 to 4 for each of six organ systems (cerebral, circulation, pulmonary, hepatic, renal, and coagulation). The aggregated score ranges from 0 to 24, with higher scores indicating more severe organ failure. One variable was missing for 51 patients in the higher-threshold group and for 64 in the lower-threshold group, so their values were not included here.

** Renal-replacement therapy was defined as therapy for acute or chronic kidney failure at randomization.

†† Mechanical ventilation was defined as invasive or noninvasive ventilation in the 24 hours before randomization.

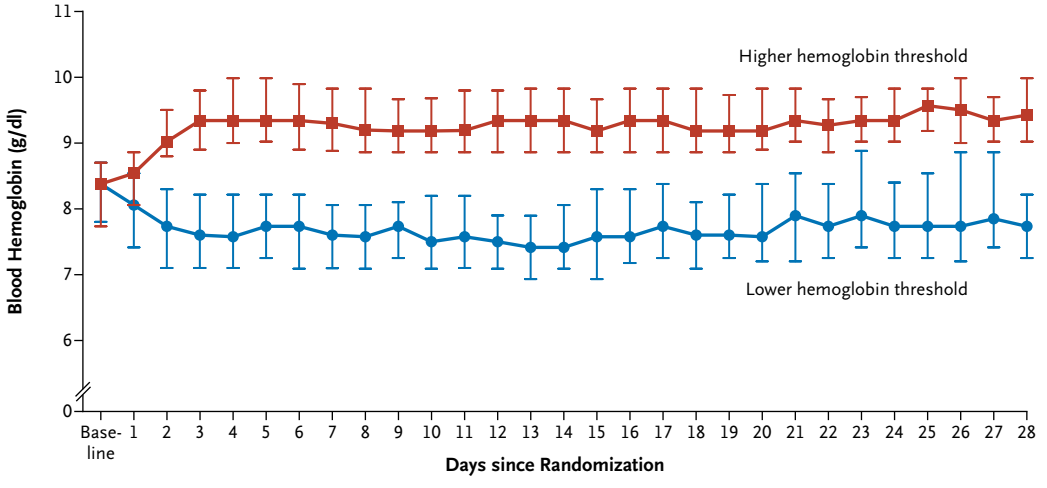


Figure 2. Blood Hemoglobin Levels in Patients in the ICU at Baseline and after Randomization.

The graphs show the median daily lowest levels of blood hemoglobin in the lower-threshold group and the higher-threshold group. Baseline values were the lowest blood hemoglobin level measured in the 24 hours before randomization. Day 1 was defined as the time of randomization to the end of that day and lasted a median of 15 hours in the lower-threshold group and 14 hours in the higher-threshold group. The I bars indicate the 25th and 75th percentiles.

Table 2. Primary and Secondary Outcome Measures.*

Outcome	Lower Hemoglobin Threshold	Higher Hemoglobin Threshold	Relative Risk (95% CI)	P Value
Primary outcome: death by day 90 — no./total no. (%)	216/502 (43.0)	223/496 (45.0)	0.94 (0.78–1.09)	0.44†
Secondary outcomes‡				
Use of life support — no./total no. (%)§				
At day 5	278/432 (64.4)	267/429 (62.2)	1.04 (0.93–1.14)	0.47†
At day 14	140/380 (36.8)	135/367 (36.8)	0.99 (0.81–1.19)	0.95†
At day 28	53/330 (16.1)	64/322 (19.9)	0.77 (0.54–1.09)	0.14†
Ischemic event in the ICU — no./total no. (%)¶	35/488 (7.2)	39/489 (8.0)	0.90 (0.58–1.39)	0.64‖
Severe adverse reaction — no./total no. (%)**	0/488	1/489 (0.2)	—	1.00
Alive without vasopressor or inotropic therapy — mean % of days††	73	75	—	0.93
Alive without mechanical ventilation — mean % of days††	65	67	—	0.49
Alive without renal-replacement therapy — mean % of days††	85	83	—	0.54
Alive and out of the hospital — mean % of days††	30	31	—	0.89

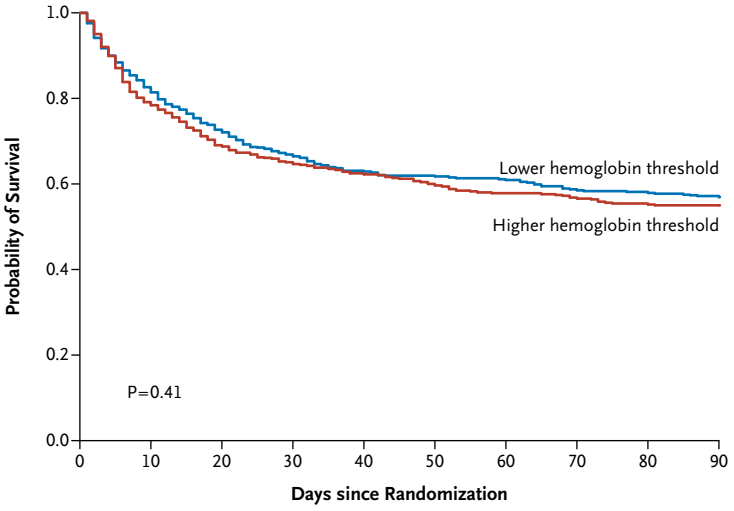
* CI denotes confidence interval.
† Logistic-regression analyses were adjusted for the stratification variables (study site and presence or absence of hematologic cancer). The results of the unadjusted outcome analyses are provided in Table S11 in the Supplementary Appendix.
‡ A total of 21 patients — 14 in the lower-threshold group and 7 in the higher-threshold group — did not wish to be included in the follow-up, so data regarding secondary outcome measures are missing for these patients.
§ Use of life support was defined as infusion of vasopressor or inotropic agents or the use of invasive or noninvasive mechanical ventilation or renal-replacement therapy on those days. The total number of patients decreased because patients died. See Table S13 in the Supplementary Appendix.
¶ An ischemic event in the ICU was defined as one or more events of acute myocardial, cerebral, intestinal, or limb ischemia. See Table S13 in the Supplementary Appendix.
‖ Logistic-regression analyses were adjusted for the presence of hematologic cancer. Adjustment according to study site was not possible, because there were zero events at four study sites.
** A severe adverse reaction was defined as allergic reaction, hemolysis, transfusion-associated acute lung injury, or transfusion-associated circulatory overload. See Table S13 in the Supplementary Appendix.
†† The mean percentage of days was calculated as the number of days without vasopressor, ventilator, or renal-replacement therapy, divided by the number of days alive during the 90-day follow-up period, or as the number of days out of the hospital, divided by the number of days alive during the 90-day follow-up period.

those with more severe disease; these two subgroups may best resemble our patients. Our results are also in line with those of a large trial involving high-risk patients after hip surgery, the Transfusion Trigger Trial for Functional Outcomes in Cardiovascular Patients Undergoing Surgical Hip Fracture Repair (FOCUS) trial,[27] and the Cochrane meta-analysis of trials of transfusion thresholds, both of which support restrictive transfusion to reduce the use of blood in patients with preexisting cardiovascular disease.[28] An important exception is patients with acute myocardial infarction, who were excluded both from our trial and from the FOCUS trial.[27] Research is needed to assess the safety of lower hemoglobin thresholds for transfusion in these patients.[12]

The effect of transfusion thresholds on rates of myocardial infarction may have differed among the three trials. In the TRICC trial, significantly increased rates of myocardial infarction were observed with a higher transfusion threshold,[9] whereas the opposite was observed in the FOCUS trial and in our trial, although the numerical differences were not significant in either of these two trials.[27] In our trial, myocardial infarction was not a prespecified outcome measure (the data are provided in the Supplementary Appendix); we did not specify surveillance testing for myocardial ischemia in the protocol and may have missed some events. This may also have resulted in detection bias because the clinicians and investigators were not unaware of the intervention assignments.

We observed no harm with an excess transfusion of a median of 3 units of blood, a finding that is contrary to most of the observational data

A Time to Death



No. at Risk

Lower hemoglobin threshold	502	334	306	286
Higher hemoglobin threshold	496	321	287	273

B Relative Risk of the Primary Outcome

Subgroup	Lower Hemoglobin Threshold	Higher Hemoglobin Threshold	Relative Risk (95% CI)	P Value for Heterogeneity
	no. of events/no. of patients in subgroup			
Age				0.85
>70 yr	93/173	98/185	0.98 (0.79–1.18)	
≤70 yr	123/329	125/311	0.94 (0.75–1.14)	
Chronic cardiovascular disease				0.25
Yes	42/75	33/66	1.08 (0.75–1.40)	
No	174/427	190/430	0.90 (0.75–1.06)	
SAPS II at baseline				0.06
>53	112/207	139/226	0.83 (0.64–1.04)	
≤53	104/295	84/270	1.10 (0.91–1.30)	
All patients	216/502	223/496	0.94 (0.78–1.09)	

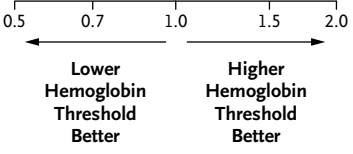


Figure 3. Time to Death and Relative Risk of Death at 90 Days.

Panel A shows the survival curves, with data censored at 90 days, in the two intervention groups in the intention-to-treat population. Kaplan–Meier analysis showed that the survival time did not differ significantly between the two groups (P=0.41 by Cox regression analysis, with adjustment for the stratification variables). Panel B shows the relative risks (black boxes) with 95% confidence intervals (horizontal lines) for the primary outcome measure of death by day 90 in the lower-threshold group, as compared with the higher-threshold group, among all the patients and in the three prespecified subgroups, as assessed by means of logistic-regression analysis, with adjustment for the stratification variables. The size of each black box is proportional to the size of the corresponding subgroup. Chronic cardiovascular disease was defined as any history of myocardial infarction, any history of stable or unstable angina pectoris, previous treatment with nitrates, percutaneous coronary intervention, coronary-artery bypass grafting or noncoronary vascular interventions, any history of chronic heart failure (defined as New York Heart Association class III or IV), or any history of cerebral infarction or transitory cerebral ischemia. The Simplified Acute Physiology Score (SAPS) II[25] is calculated from 17 baseline variables; scores range from 0 to 163, with higher scores indicating higher severity of disease. A total of 1 or 2 of the 17 variables were missing for 77 patients in the higher-threshold group and for 99 in the lower-threshold group. In this analysis, these missing variables were considered to be within the normal range, thereby not contributing to the composite SAPS II of these patients.

regarding transfusion in critically ill patients.[10] Whether this was due to the use of leukoreduced blood cannot be assessed, but results similar to ours were observed in the FOCUS trial, in which the majority of patients also received leukoreduced blood.[27] The safety of leukoreduced blood was challenged by the results of a trial involving patients with upper gastrointestinal bleeding, which showed increased mortality with liberal transfusion of this product.[11] Ongoing bleeding may have contributed to the increased mortality observed with liberal transfusion in that trial.[11] Thus the effects of leukoreduction on outcome are unclear, as they were a decade ago, as indicated in a 2004 meta-analysis of trial data on leukoreduced versus nonleukoreduced blood.[29]

The strengths of our trial include a low risk of bias, because group assignment at randomization was concealed, and the blinding of the assessors of mortality and the statistician to the assigned intervention. It is reasonable to assume that our results are generalizable, because patients were recruited both in university hospitals and in nonuniversity hospitals, and the majority of patients who underwent screening were included. The trial protocol was pragmatic, so routine practice was maintained except for the hemoglobin thresholds for transfusion. In addition, the characteristics of the patients and the outcome rates were similar to those observed in some recent trials involving patients with septic shock in the ICU.[3,19,30,31]

Our trial has limitations. First, the investigators, clinicians, and patients were aware of the study-group assignments, and we did not assess all the cointerventions. Because the trial was multicenter and large and used stratified randomization, it is unlikely that imbalance in concomitant interventions affected the results. Second, the confidence interval was relatively wide for the point estimate for mortality, so we cannot exclude a 9% relative increase or a 22% relative decrease in mortality at 90 days in the lower-threshold group versus the higher-threshold group. Third, we had limited power to detect differences in some other outcome measures (in particular, the ischemic events) and in some of the subgroup analyses (in particular, the subgroup defined according to the presence or absence of chronic cardiovascular disease).

We recorded only one serious adverse reaction to blood transfusion, but serious adverse reactions are rare events in general, and their frequencies are unknown among patients with septic shock in the ICU. We included some patients who had received a blood transfusion before ICU admission, and some patients had protocol suspensions and violations, which tended to reduce the difference between the two intervention groups. However, we found clear differences between the two groups in the hemoglobin levels and the numbers of transfusions, and the per-protocol analyses, which excluded patients who had protocol suspensions and violations, supported the primary analysis. Protocol suspensions and violations have been difficult to prevent in transfusion trials,[32,33] and when reported they appear to have occurred at frequencies similar to those observed in our trial.

In conclusion, patients with septic shock who underwent transfusion at a hemoglobin threshold of 7 g per deciliter, as compared with those who underwent transfusion at a hemoglobin threshold of 9 g per deciliter, received fewer transfusions and had similar mortality at 90 days, use of life support, and number of days alive and out of the hospital; the numbers of patients with ischemic events and severe adverse reactions to blood in the ICU were also similar in the two intervention groups.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Holst LB, Haase N, Wetterslev J, et al. Lower versus higher hemoglobin threshold for transfusion in septic shock. N Engl J Med. DOI: 10.1056/NEJMoa1406617

Supplementary Appendix for

Lower versus Higher Hemoglobin Threshold for Transfusion in Septic Shock

The TRISS trial investigators

Steering Committee: Lars B. Holst, Nicolai Haase, Jan Wernerman, Anne B. Guttormsen, Sari Karlsson, Për I. Johansson, Anders Åneman, Jørn Wetterslev and Anders Perner (Chair).

Management Committee: Lars B. Holst (Chair), Jørn Wetterslev and Anders Perner.

Scientific Committee: Tim Walsh, Jacques Lacroix and Dean Fergusson

Data Monitoring and Safety Committee: Daniel De Backer (Chair), Kathy Rowan and Jørgen H. Petersen.

Trial Site Investigators (in Denmark unless otherwise specified): *Rigshospitalet*: L.B. Holst, A. Perner, L.H. Andersen, U. G. Pedersen, N. Reiter, J. Wiis, J.O. White, L. Russell, K.J. Thornberg, L. Quist, M. Ibsen, P.B. Hjortrup, R.G. Müller, M.H. Møller, M. Steensen, C. Claudius, M.N. Kjær, K.R. Uhre, V. Christiansen, L.H. Nislev, F. Sjøvall, I.L. Jarnvig, J. Nielsen, H.C. Thorsen, P. Bredahl, *Randers Hospital*: M. Vang, H. Bundgaard, M.A. Thyø, M. Villumsen, *Karolinska University Hospital, Huddinge, Sweden*: J. Wernerman, K. Kilsand, I. Tjäder, S. O. Wernerman, Herning Hospital: R. Winding, D. Lodahl, R. Mærkedahl, N. Dey, S., S. Haubjerg, *Hvidovre Hospital*: L. Nebrich, C. Albeck, A. Christensen, *Aarhus University Hospital*: H. Nibro, D. Keld, K. Larsen, S.E. Dyrskog, D. Illum, *Ålborg University Hospital*: B. Rasmussen, S.N. Granum, *Holbæk Hospital*: H. H. Bülow, J. Elkjær, J. R. M. Lauridsen, *Kolding Hospital*: J. S. Nielsen, *Haukeland University Hosptial, Norway*: A. B. Guttormsen, B. Sjøbø, *Karolinska University Hospital, Solna, Sweden*: A. Oldner, O. Friman, *Helsinki University Hospital, Finland*: V. Pettilä, L. Pettilä, S. Sutinen, *Södersjukhuset, Sweden*: M. Cronhjort, *Næstved Hospital*: B. K. Kristensen, P. Kirkegaard, *Bispebjerg Hospital*: A. Lindhardt, D. G. Strange, *Horsens Hospital*: Elsebeth Haunstrup Krogh, Omar Rian, *Hjørring Hospital*: M. Kruse, S. Aagaard, M.B. Pawlowicz, J. Madsen, *Køge Hospital*: L. M. Poulsen, Vejle Hospital: P. Berezowicz, *Halmstad Hospital, Sweden*: M. Chew, F. Abdeen, W. Müller, G. Sternheden, S. Holmstrøm, *Gentofte Hospital*: K. Thormar, N. Mtchedlishvili, *Stavanger University Hospital, Norway*: K. Strand, *Tampere University Hospital, Finland*: S. Karlsson, S. Varila, A. Kukkurainen, *Glostrup Hospital*: A. Bendtsen, *Joensuu Hospital, Finland*: M. Reinikainen, *Slagelse Hospital*: S. A. Iversen, *Sønderborg Hospital*: S. Leivdal, *Akershus University Hospital, Norway*: P. M. Bådstøløkken, *Ålesund Hospital, Norway*: J. Å. Longva, R. B. Johansen, *Helsingborg Hospital, Sweden*: N. Nielsen, *Växjö Hospital, Sweden*: J. M. Breider

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Trial criteria for septic shock

(1) ***AT LEAST TWO SYSTEMIC INFLAMMATORY RESPONSE SYNDROME (SIRS) CRITERIA****:*[1]

1. ***CORE TEMPERATURE >38°C or <36°C****. (Core temperature was rectal, urinary bladder, central line, or tympanic). If oral, inguinal or axillary temperatures were used, we added 0.5°C to the measured value. Hypothermia <36°C was confirmed by core temperature. We used the most deranged value recorded* ***in the 24 hours before randomization****.*
2. ***HEART RATE ≥90 beats/minute****. If patient had an atrial arrhythmia, we recorded the ventricular rate. If patients had a known medical condition or were receiving treatment that would prevent tachycardia (for example, heart block or beta blockers), they had to meet two of the remaining three SIRS criteria. We used the most deranged value recorded* ***in the 24 hours before randomization****.*
3. ***MECHANICAL VENTILATION*** *for an acute process or respiratory rate ≥ 20 breaths per minute or a $PaCO_2$ < 4.3 kPa (32 mmHg). We used the most deranged respiratory rate or $PaCO_2$ recorded* ***in the 24 hours before randomization****.*
4. ***WHITE BLOOD CELL COUNT ≥12 x 10^9/l or ≤ 4 x 10^9/l****. We used the most deranged value recorded* ***in the 24 hours before randomization****.*

AND

(2) ***SUSPECTED OR VERIFIED FOCUS OF INFECTION*** *as either:*

(i) An organism grown in blood or sterile site

OR

(ii) An abscess or infected tissue (e.g. pneumonia, peritonitis, urinary tract, vascular line infection, soft tissue, etc).

AND

(3) ***HYPOTENSION*** *(Systolic blood pressure ≤ 90 mmHg or mean arterial pressure ≤ 70 mmHg) despite fluid therapy OR* ***VASOPRESSOR/INTROPE*** *infusion to maintain blood pressure.*

Trial exclusion criteria

- Documented wish against transfusion **OR**

- Previous serious adverse reaction with blood products, excl. transfusion-associated circulatory overload **OR**

- Presence of acute myocardial ischemia **OR**
  (defined as: patients diagnosed with ***acute myocardial infarction*** *(ST-elevation myocardial infarction or non-ST elevation myocardial infarction) or* ***unstable angina pectoris*** during current hospital admission, according to the criteria in the clinical setting in question (e.g. elevated biomarkers, ischemic signs on ECG, clinical presence) **AND** the patient has received treatment, initiated during current hospital admission, as a consequence of this (reperfusion strategies (PCI/thromobolysis) or initiation/increased antithrombotic treatment)).

- Life-threatening bleeding **OR**
  (defined as: (1) Presence of hemorrhagic shock, as judged by research or clinical staff. OR (2) the need for surgical procedure, incl. endoscopy to maintain hemoglodin levels).

- Red cell transfusion during current ICU admission **OR**

- Withdrawal from active therapy or brain death **OR**

- Acute burn injury - regardless of degree and burn surface area **OR**

- Lack of informed consent

Trial criteria for serious adverse reactions and ischemic events

Serious adverse reactions after blood transfusion were defined as either

Allergic reactions defined by the clinician on the basis of muco-cutaneous signs and symptoms (e.g. urticaria, pruritus, localized angio-edema) occurring within 6 hours of red cell transfusion

Severe hemolytic complications defined by the clinician on the basis of hemoglobinuria or increased free plasma hemoglobin occurring within 24 hours of transfusion.

Transfusion-associated acute lung injury (TRALI) defined as: I. Acute or worsening hypoxemia ((PaO_2/FiO2 < 40 (PaO_2 in kPa) or <300 (PaO_2 in mmHg) regardless of PEEP) OR > 50% relative increase in FiO_2 AND II. Occurrence within 6 hours after red cell transfusion AND III. Acute or worsening pulmonary infiltrates on frontal chest x-ray OR clinical signs of overt pulmonary edema.

Transfusion-associated circulatory overload (TACO) defined as: I. Acute or worsening hypoxemia (PaO_2/FiO_2 < 40 (PaO_2 in kPa) or <300 (PaO_2 in mmHg) regardless of PEEP) OR > 50% relative increase in FiO_2 AND II. Occurrence within 6 hours after red cell transfusion AND III. Acute or worsening pulmonary infiltrates on frontal chest x-ray OR clinical signs of overt pulmonary edema AND IV. Increased blood pressure AND VI. Positive fluid balance.

Ischemic events were defined as either

Cerebral ischemia defined as any form of cerebral ischemia on a CT- OR MRI scan

Acute myocardial ischemia defined as patient diagnosed with acute myocardial infarction (ST-elevation myocardial infarction or non-ST elevation myocardial infarction) or unstable angina pectoris according to the criteria in the clinical setting in question (e.g. elevated biomarkers, ischemic signs on ECG and clinical presentation) AND the patient received treatment as a consequence of this (reperfusion strategies (PCI/thrombolysis) or initiation/increased antithrombotic treatment).

Intestinal ischemia was defined as ischemia verified by endoscopy OR open surgery.

Limb ischemia defined as clinical signs AND need of open/percutaneous vascular intervention, amputation OR initiation/increased antithrombotic treatment.

Point-of-care testing devices
One of the following point-of-care testing devices was used to assess hemoglobin concentrations in the TRISS trial: ABL 625, 700- and 800-series or ABL90 from Radiometer, Copenhagen, Denmark (31 ICUs) or Cobas b 221 from Roche Diagnostics, Rotkreuz, Switzerland (one ICU).[2, 3]

Protocol suspension criteria
The attending doctor could temporarily suspend the protocol and transfuse an individual patient at a hemoglobin value that differed from the allocated one during any of the following events:

- The presence of life-threatening bleeding defined as the presence of hemorrhagic shock, as judged by research or clinical staff.
- Ischemic events defined as:
    - Acute myocardial ischemia: Defined as ***acute myocardial infarction*** *(ST-elevation myocardial infarction and non-ST elevation myocardial infarction) or* ***unstable angina pectoris***, according to the criteria in the clinical setting in question (e.g. elevated biomarkers, ischemic signs on ECG, clinical presence) AND the patient had received treatment as a consequence of this (reperfusion strategies (percutaneous cardiac intervention/thrombolysis) or initiation/increased antithrombotic treatment).
    - Cerebral ischemia: Verified by CT- or MRI scan
    - Intestinal ischemia: Verified by endoscopy or surgery.
    - Acute limb ischemia: Clinical signs AND need of open or percutaneous vascular intervention, amputation or initiation/increased antithrombotic treatment.
- The use of extracorporeal membrane oxygenation (not originally protocolized, but added during trial)

The attending doctor decided when the patient again was to be transfused at the allocated hemoglobin threshold. Suspension was not considered a breach of protocol, and data collection continued during the suspension. These patients were analyzed according to their assigned group on an intention-to-treat basis.

Trial populations

Intention-to-treat population: All randomized patients except those who
- Withdrew consent for the use of data
OR
- Were not eligible for randomization according to the inclusion/exclusion criteria AND never had the intervention (decision to transfuse based on the allocated hemoglobin threshold)

Per-protocol population no. 1: All randomized patients except patients having one or more major protocol violations defined as:

1. One or more blood transfusions given despite a hemoglobin level above 7.0 g/dl in patients assigned to the lower Hb-threshold group
OR

2. One or more blood transfusion not given within 24 hours after a hemoglobin level less than 9.0 g/dl in patients assigned the higher Hb-threshold group
OR

3. Monitoring revealed that one or more in- or exclusion criteria were violated
OR

4. One or more transfusions of red cell unit(s) destined for another patient
OR

5. One or more transfusions given despite lack of cross-match between donor and recipient. Administration of 0-neg blood without cross-match between donor and recipient was not regarded as a protocol violation
OR

6. Any protocol suspension defined as transfusions administered when hemoglobin level was above the allocated threshold level on days with the presence of ischemic events, life-threatening bleeding events
OR

7. Patients stopped or withdrawn

Per-protocol no. 2: All randomized patients except patients having one or more protocol violations defined as

1. One or more transfusions *given* despite the patients hemoglobin level being *above the threshold* the patient was assigned to
OR

2. *O*ne or more transfusions *not given* within a period of 24 hours after the patient documented a hemoglobin level *below the threshold* that the patient was assigned to

Per-protocol no. 3: All randomized patients except patients having one or more:

1. Bleeding episodes
OR

2. Ischemic episodes

Handling of missing data

SAPS II in the 24 hours prior to randomization
This score is based on 17 variables, which were registered in the baseline case report form from source data. We had missing source data for one or more of the 17 variables in 176 patients. These values were not included in the baseline characteristics and we imputed best/worst case scenarios in the analyses adjusting for design variables (Table S12).

SOFA score in the 24 hours prior to randomization
Missing Glasgow Coma Scale (GCS) score: No missing values, because investigators recorded 15, if the score was missing in source data. If the patient was sedated, the GCS score estimated before sedation was used; if missing the GCS score was registered as 15.

Missing cardiovascular component: Missing values were imputed using data from the screening form (if the patient had a mark for hypotension he/she was given the cardiovascular SOFA score 1 and with a mark for vasopressor or inotropic agent the score 3 was given). After this imputation we had no missing values.

Missing PaO_2/FiO_2-ratio: No missing values.

Missing renal component: No missing values.

Missing platelet count: 17 missing values.

Missing plasma bilirubin: 109 missing values.

We handled the missing SOFA scores as we did for the missing SAPS II.

Missing outcome data
For the primary outcome measure and all mortality endpoints we had full data sets on all 998 patients in the intention-to-treat population.

There were missing data for 21 patients for the following secondary outcome measures, because the patient or the surrogate decision-maker did not want continued data registration: life support at days 5, 14 and 28 after randomization, serious adverse reactions in the ICU, ischemic events in the ICU, percent of days alive without vasopressor/inotropic therapy, mechanical ventilation or renal replacement therapy, and percent of days alive out of hospital.

We did not impute any data for these outcome measures, because they only represented 21/998 (2%) of the patients.

Table S1. Additional baseline characteristics

Characteristic	Lower Hb-threshold (N=502)	Higher Hb-threshold (N=496)
Estimated body weight – kg	75 (64-87)	75 (62-87)
Chronic cardiovascular disease – no. (%)		
Previous myocardial infarction	33 (7)	30 (6)
Previous angina pectoris	26 (5)	7 (1)
Severe chronic heart failure (NYHA group 3-4)	13 (3)	9 (2)
Previous coronary intervention	38 (8)	32 (6)
Previous vascular intervention (non-coronary)	16 (3)	18 (4)
Previous ischemic stroke or transitory cerebral ischemia	40 (8)	43 (9)
Organ failures – no. (%) †		
Cerebral failure ‡	120 (24)	132 (27)
Respiratory failure	456 (91)	442 (89)
Circulatory failure	502 (100)	496 (100)
Hepatic failure	88 (20)	70 (16)
Kidney failure	219 (44)	232 (47)
Coagulation failure	144 (29)	122 (25)
Renal replacement therapy - no. (%)		
Acute	55 (11)	42 (8)
Chronic	13 (3)	11 (2)

Values with ranges are medians (interquartile ranges). NYHA denotes New York Heart Association

† Defined as Sepsis-related Organ Failure Assessment score of 2 or more in the given organ system at randomization except for circulatory failure which was defined as a score of 1 or more (Table S15).[4] Most patients had 2 or more organ failures.

‡ If the patient was sedated, the Glasgow Coma Scale (GCS) score estimated before sedation was used; if missing the GCS score was registered as 15.

Table S2. Protocol suspensions

Suspension	Lower Hb-threshold (N=488)	Higher Hb-threshold (N=489)
Myocardial ischemia – no. (%)	6 (1.2)	0 (0)
Transfusions given during suspensions for myocardial ischemia – no. (%)	10/1545 (0.7)	0/3088 (0)
Other ischemia – no. (%) *	6 (1.2)	0 (0)
Transfusions given during suspensions for other ischemia – no. (%)	16/1545 (1.0)	0/3088 (0)
Life-threatening bleeding – no. (%)	18 (3.7)	9 (1.8)
Transfusions given during suspensions for life-threatening bleeding – no. (%)	52/1545 (3.4)	13/3088 (0.4)
ECMO therapy – no. (%) †	1 (0.2)	2 (0.4)
Transfusions given during ECMO – no.	55	10 and 15

Values with ranges are medians (interquartile ranges). Four patients had 2 suspension periods based on different criteria, all in the lower Hb-threshold group.

* Other ischemia includes cerebral, intestinal and limb ischemia.

† ECMO denotes extracorporeal membrane oxygenation.

Table S3. Blood hemoglobin concentrations before and after randomization

	Lower Hb-threshold (N=488)	Higher Hb-threshold (N=489)
Baseline †		
Lowest hemoglobin – g/dl	8.4 (7.8-8.7)	8.4 (7.7-8.7)
Highest hemoglobin – g/dl	9.8 (9.2-10.5)	9.8 (9.2-10.6)
Day 1 ‡		
Number of measurements	5 (3-8)	6 (4-8)
Lowest hemoglobin – g/dl	8.1 (7.4-8.5)	8.5 (8.1-8.7)
Highest hemoglobin – g/dl	8.7 (8.2-9.2)	9.8 (9.5-10.2)
Day 2		
Number of measurements	8 (6-10)	8 (6-10)
Lowest hemoglobin – g/dl	7.7 (7.1-8.3)	9.0 (8.8-9.5)
Highest hemoglobin – g/dl	8.7 (8.2-9.2)	10.3 (9.9-10.7)
Day 3		
Number of measurements	7 (5-10)	8 (6-10)
Lowest hemoglobin – g/dl	7.7 (7.1-8.2)	9.3 (8.9-9.8)
Highest hemoglobin – g/dl	8.7 (8.1-9.3)	10.3 (9.8-10.8)
Day 4		
Number of measurements	8 (6-9)	7 (5-9)
Lowest hemoglobin – g/dl	7.6 (7.1-8.2)	9.3 (9.0-10.0)
Highest hemoglobin – g/dl	8.5 (8.1-9.2)	10.4 (10.0-11.0)
Day 5		
Number of measurements	7 (5-9)	7 (5-9)
Lowest hemoglobin – g/dl	7.7 (7.3-8.2)	9.3 (9.0-10.0)
Highest hemoglobin – g/dl	8.5 (8.1-9.2)	10.3 (10.0-11.0)
Day 6		
Number of measurements	7 (6-9)	7 (5-9)
Lowest hemoglobin – g/dl	7.7 (7.1-8.2)	9.4 (8.9-9.9)
Highest hemoglobin – g/dl	8.7 (8.2-9.3)	10.5 (10.0-11.0)
Day 7		
Number of measurements	7 (6-9)	7 (5-9)
Lowest hemoglobin – g/dl	7.6 (7.1-8.1)	9.3 (8.9-9.8)
Highest hemoglobin – g/dl	8.5 (8.1-9.1)	10.3 (10.0-11.0)

Values are medians (interquartile ranges).
† In the 24 hours prior to randomization
‡ The first day was from the time of randomization to the next start of the specific ICU's 24-hour observation chart and lasted median 15 (9-19) hours in the lower Hb-threshold group and 14 (9-19) hours in the higher Hb-threshold group

Table S4. Blood transfusion before and after randomization

	Lower Hb-threshold (N=488)	Higher Hb-threshold (N=489)
Baseline †		
No. of patients transfused (%)*	48/487 (10)	58/488 (12)
Number of units per patient	2 (2-4)	2 (1-3)
Volume – ml	600 (395-900)	490 (290-800)
Day 1 ‡		
No. of patients transfused (%)*	81/488 (17)	455/489 (93)
Number of units per patient	1 (1-2)	2 (1-2)
Volume – ml	250 (240-480)	480 (240-512)
Day 2		
No. of patients transfused (%)*	93/472 (20)	208/473 (44)
Number of units per patient	1 (1-2)	1 (1-2)
Volume – ml	250 (240-500)	250 (240-480)
Day 3		
No. of patients transfused (%)*	86/430 (20)	134/419 (32)
Number of units per patient	1 (1-1)	1 (1-1)
Volume – ml	241 (240-295)	240 (240-283)
Day 4		
No. of patients transfused (%)*	64/366 (17)	103/375 (27)
Number of units per patient	1 (1-1)	1 (1-1)
Volume – ml	240 (240-264)	240 (240-270)
Day 5		
No. of patients transfused (%)*	54/328 (16)	72/326 (22)
Number of units per patient	1 (1-1)	1 (1-1)
Volume – ml	240 (240-276)	240 (240-290)
Day 6		
No. of patients transfused (%)*	56/287 (20)	82/288 (28)
Number of units per patient	1 (1-1)	1 (1-1)
Volume – ml	240 (240-286)	240 (240-290)
Day 7		
No. of patients transfused (%)*	45/260 (17)	71/248 (27)
Number of units per patient	1 (1-1)	1 (1-2)
Volume – ml	240 (240-274)	250 (240-480)

Values with ranges are medians (interquartile ranges) of the patients transfused on that day.

† In the 24 hours prior to randomization

* Where the denominator is below all patients allocated to the group this was due to death, ICU discharge or missing source data.

‡ The first day was from the time of randomization to the next start of the specific ICU's 24-hour fluid chart and lasted median 15 (9-19) hours in the lower Hb-threshold group and 14 (9-19) hours in the higher Hb-threshold group.

Table S5. Number of patients stratified by the number of units transfused

	Lower Hb-threshold (N=488)	**Higher Hb-threshold (N=489)**
	no./total no. (%)	
0 units	176/488 (36)	6/489 (1)
1 unit	88/488 (18)	70/489 (14)
2 units	57/488 (12)	85/489 (17)
3 units	47/488 (10)	80/489 (16)
4 units	27/488 (6)	53/489 (11)
5 or more units	93/488 (19)	195/489 (40)

Table S6. Bleeding and surgery after randomization

	Lower Hb-threshold (N=488)	**Higher Hb-threshold (N=489)**
Overt bleeding - no. (%)*	147 (30)	148 (30)
Upper GI tract	36 (7)	34 (7)
Lower GI tract	33 (7)	31 (6)
Lower airway	46 (9)	27 (6)
Urinary tract	17 (3)	9 (2)
Wounds	37 (7)	61 (12)
During surgery	41 (8)	51 (10)
Other	46 (9)	41 (8)
Severe bleeding - no. (%) †	30 (6)	52 (11)
Surgery		
No. of patients having one or more days with surgery (%)	148 (30)	166 (34)
No. of days with surgery in these patients - median (interquartile range)	2 (1-3)	1 (1-3)
No. of patients transfused above their allocated threshold during surgery - (%) ‡	28 (6)	15 (3)

* Any bleeding from any of the anatomical sites given below.

† Overt bleeding and the use of 3 units of red blood cells on that day.

‡ These were not violations as transfusion was not protocolized during surgery.

Table S7. Patients with do not resuscitate orders

	Lower Hb-threshold (N=488)	Higher Hb-threshold (N=489)
No. of patients with one or more days with DNR order – (%)	125/488 (26)	154/489 (31)
No. of days with DNR order for the patients who had these – median (IQR)	2 (1-4)	2 (1-4)
No. of patients transfused on a day with DNR order – (% of patients transfused)	41/312 (13)	73/483 (15)
No. of transfusions given on days with DNR order – (% of transfusions)	127/1545 (8)	210/3088 (7)

DNR denotes do not resuscitate and was defined as any written order limiting cardio-pulmonary resuscitation in the case of cardiac arrest. IQR denotes interquartile range.

Table S8. Use of plasma, platelets and fluids and fluid balances

Variable	Lower Hb-threshold (N=488)		Higher Hb-threshold (N=489)	
	No. receiving / No. at risk (%)*	Volume (ml)	No. receiving / No. at risk (%)*	Volume (ml)
Fresh frozen plasma				
Day -1 ¶	61/487 (13)	0 (0-0)	62/488 (13)	0 (0-0)
Day 1 ‡	41/488 (8)	0 (0-0)	43/489 (9)	0 (0-0)
Day 2	43/472 (9)	0 (0-0)	43/473 (9)	0 (0-0)
Day 3	20/430 (5)	0 (0-0)	19/419 (9)	0 (0-0)
Total §	113/488 (23)	0 (0-0)	127/489 (26)	0 (0-264)
Platelets				
Day -1 ¶	32/487 (7)	0 (0-0)	29/488 (6)	0 (0-0)
Day 1 ‡	26/488 (5)	0 (0-0)	23/489 (5)	0 (0-0)
Day 2	34/472 (7)	0 (0-0)	42/473 (9)	0 (0-0)
Day 3	30/430 (7)	0 (0-0)	24/419 (6)	0 (0-0)
Total §	79/488 (16)	0 (0-0)	96/489 (20)	0 (0-0)
Albumin ‡‡				
Day 1 ‡	132/487 (27)	0 (0-100)	126/489 (26)	0 (0-100)
Day 2	141/472 (30)	0 (0-194)	125/473 (26)	0 (0-100)
Day 3	99/430 (23)	0 (0-0)	98/419 (23)	0 (0-0)
Total §	306/487 (63)	250 (0-1000)	303/489 (62)	250 (0-950)
Synthetic colloids ††				
Day 1 ‡	7/487 (1)	0 (0-0)	5/489 (1)	0 (0-0)
Day 2	5/472 (1)	0 (0-0)	3/473 (1)	0 (0-0)
Day 3	0/430 (0)	0 (0-0)	1/419 (0)	0 (0-0)
Total §	16/487 (3)	0 (0-0)	15/489 (3)	0 (0-0)
Other fluids ¶¶				
Day 1 ‡	479/485 (99)	1944 (977-3430)	486/487 (100)	2027 (990-3403)
Day 2	466/469 (99)	2523 (1590-3726)	467/471 (99)	2351 (1498-3559)
Day 3	425/428 (99)	2017 (1188-3103)	416/417 (100)	2100 (1285-2992)
Total §	438/440 (99)	14128 (6745-27853)	449/449 (100)	14778 (6741-26756)
Fluid balance	No. with data / No. at risk**	Volume (ml)	No. with data / No. at risk**	Volume (ml)
Day 1 ‡	485/488	890 (-46-2156)	487/489	1328 (394-2629)
Day 2	469/472	813 (-223-2183)	467/471	724 (-342-1901)
Day 3	428/430	291 (-629-1281)	417/419	259 (-650-1094)
Total §	431/488	2649 (-195-7021)	447/489	3351 (276-8037)

Values are medians (interquartile ranges) of all patients who had data registered on that day(s).

* No. receiving is those patients who did receive the specific solution on the given day(s). No. at risk is those patients who had registered data on that day(s). Where the no. is below all patients allocated to the group this is due to death, ICU discharge or missing data. We had the following missing data: data on single day forms were missing for one patient for each of the synthetic colloid (starch, dextran and gelatin) and for one patient for each of the albumin solutions (5% and 20%) and data on 1.1% and 1.2% of day forms for total inputs and total outputs (used to calculate other fluids), respectively. Complete cases are given here.

¶ In the 24 hours prior to randomization.

‡ The first day was from the time of randomization to the next start of the specific ICU's 24-hour fluid chart and lasted median 15 (IQR, 9-19) hours in the lower Hb-threshold group and 14 (9-19) hours in the higher Hb-threshold group.

§ Cumulative data for the full trial period in ICU to a maximum of 90 days after randomization

‡‡ Total volumes of 5 and 20% albumin.

†† Total volumes of hydroxyethyl starch, dextran and gelatin solutions.

¶¶ Including crystalloids, dextrose (10% or below), water and fluids given with medications.

** No. with data is those patients where data were registered for that day(s). No. at risk is those patients who were in the ICU on that day(s). Where the no. is below the no. allocated to the group this is due to death or ICU discharge. Data were missing on 1.1% and 1.2% of day forms for total inputs and total outputs (used to calculate fluid balances), respectively. Complete cases are given here.

Table S9. Circulatory parameters from baseline to day 7 after randomization

Variable	Lower Hb-threshold (n=488)		Higher Hb-threshold (n=489)	
	No. assessed†	Value	No. assessed†	Value
Lowest $ScvO_2$ – % ‡				
Baseline	230	69 (58-76)	228	68 (60-76)
Day 1	191	69 (61-75)	185	70 (62-76)
Day 2	175	66 (60-74)	191	71 (64-77)
Day 3	143	69 (61-75)	135	71 (64-76)
Day 4	128	66 (58-72)	107	69 (64-77)
Day 5	88	65 (60-72)	88	71 (65-77)
Day 6	80	67 (60-75)	81	71 (63-76)
Day 7	68	66 (58-74)	67	69 (63-75)
Highest lactate – mmol per liter ‡				
Baseline	485	2.4 (1.6-4.2)	486	2.7 (1.6-4.5)
Day 1	481	2.1 (1.4-3.3)	487	2.1 (1.4-3.3)
Day 2	467	1.9 (1.4-2.9)	469	2.0 (1.4-3.0)
Day 3	419	1.8 (1.3-2.6)	412	1.8 (1.4-2.6)
Day 4	360	1.7 (1.3-2.5)	370	1.7 (1.3-2.5)
Day 5	318	1.7 (1.3-2.4)	318	1.7 (1.3-2.4)
Day 6	282	1.7 (1.3-2.4)	279	1.6 (1.3-2.3)
Day 7	254	1.6 (1.2-2.3)	243	1.7 (1.3-2.4)

Values are medians (interquartile ranges).

$ScvO_2$ denotes central venous oxygen saturation, which was sampled from a central line with the tip in the superior caval vein.

† Number of patients where the measurements were documented in source data.

‡ Where more measurements were documented within the time period the lowest value of $ScvO_2$ and the highest value of lactate were registered.

Table S10. Protocol violations*

Violation	Lower Hb-threshold (N=463) †	Higher Hb-threshold (N=470) †
One or more transfusions *given* despite the patients Hb-level being *above the assigned Hb-threshold* – no. (% of patients)	45/463 (10)	16/470 (3)
No. of transfusions *given* despite the patients Hb-level being *above the assigned Hb-threshold* – (% of transfusions)	80/1323 (6)	18/3005 (1)
Transfusion *not given* on a day where the patient had at least one Hb-measurement *below the assigned Hb-threshold* – no. (% of patients)	42/463 (9)	104/470 (22)
One or more transfusions destined for another patient – no.	0	0
One or more transfusions given despite lack of cross-match between donor and recipient – no.	0	0

* In addition to the transfusions registered as violations, 43 patients were transfused above their allocated threshold during surgery. These patients are not included here; the details appear in Table S6.
Hb denotes hemoglobin

† This population consisted of 933 patients (all 998 patients minus the 62 patients who discontinued the trial protocol (see Fig 1) and the 3 patients who received extracorporeal membrane oxygenation).

Table S11. Results of the unadjusted outcome analyses

Outcome	Lower Hb-threshold	Higher Hb-threshold	Relative Risk* (95% CI)	P-value*
Primary outcome measure	*no./total no. (%)*			
Dead at day 90	216/502 (43.0%)	223/ 496 (45.0%)	0.96 (0.83 - 1.10)	0.54
Secondary outcome measures †	*no./total no. (%)*			
Use of life support ‡				
Day 5	278 / 432 (64.4%)	267/ 429 (62.2%)	1.03 (0.93 -1.15)	0.52
Day 14	140 / 380 (36.8%)	135 / 367 (36.8%)	1.00 (0.83 - 1.21)	0.99
Day 28	53 / 330 (16.1%)	64 / 322 (19.9%)	0.81 (0.58 - 1.12)	0.20
Ischemic events in the ICU ¶	35 / 488 (7.2%)	39 / 489 (8.0%)	0.90 (0.58 - 1.39)	0.64
Severe adverse reactions §	0 / 488 (0.0)	1 / 489 (0.2)	-	1.00

* Results of Chi^2 analyses

† Some patients did not wish a part of the follow-up, so there were missing data for 14 patients in the lower Hb-threshold group and 7 patients in the higher Hb-threshold group for the secondary outcome measures.

‡ Defined as infusion of vasopressor or inotropic agents or use of invasive or non-invasive mechanical ventilation or renal replacement therapy on those days. The total no. declined because of patients dying.

¶ Defined as one or more events of acute myocardial, cerebral, intestinal or limb ischemia (the details appear in Table S13 in the Supplementary Appendix).

§ Defined as either allergic, hemolytic, transfusion-associated acute lung injury or transfusion-associated circulatory overload (Table S13).

Table S12. Results of the adjusted analyses of 90-day mortality

	Partially adjusted analyses (stratification variables)*		**Fully adjusted analyses (stratification and design variables) †**	
Populations			**Lowest possible relative risk**	**Highest possible relative risk**
	Relative risk (95% confidence interval) P value		Relative risk (95% confidence interval) P value	
Intention-to-treat N = 998 N-low = 502 N-high = 496	0.94 (0.78 to 1.09) 0.44		0.94 (0.80 to 1.10) 0.90	1.01 (0.68 to 1.17) 0.87
Per-protocol #1 N = 759 N-low = 402 N-high = 357	0.92 (0.75 to 1.09) 0.34		0.93 (0.75 to 1.12) 0.45	1.02 (0.84 to 1.21) 0.83
Per-protocol #2 N = 769 N-low = 400 N-high = 369	0.92 (0.77 to 1.09) 0.37		0.94 (0.77 to 1.12) 0.51	1.03 (0.85 to 1.21) 0.78
Per-protocol #3 N = 661 N-low = 334 N-high = 327	0.95 (0.78 to 1.14) 0.62		0.96 (0.77 to 1.15) 0.68	1.04 (0.85 to 1.24) 0.66

N = number of patients in the total population
N-low = number of patients in the lower Hb-threshold group
N-high = number of patients in the higher Hb-threshold group

*The primary analyses (Column 1) were adjusted for the stratification variables (hematological malignancy and site (all sites including < 10 patients were grouped into one resulting in 20 site variables instead of 32).

† The secondary analyses were adjusted for stratification and design variables (Columns 2 and 3). The design variables were the following predefined baseline risk factors: (a) previous cardiovascular disease Y/N, (b) surgery during the index hospitalization, but prior to randomization Y/N, (c) Age, (d) hemoglobin value, (e) blood transfusion given 24 hours prior to randomization Y/N, (f) binary Simplified Acute Physiology Score (SAPS) II (threshold 53) and (g) binary Sepsis-related Organ Failure Assessment (SOFA) score (threshold 10).[4, 5] These variables were forced into the model.

We had missing SAPS and SOFA scores for 176 and 115 patients, respectively. Therefore we did sensitivity analyses imputing the missing values to test the maximum range of possible results in the multiple logistic regression analysis. At one end missing values were imputed with parameters resulting in the maximum obtainable scores of SAPS and SOFA in the lower Hb-threshold group and the parameters that resulted in the minimum obtainable scores of SAPS and SOFA in the higher Hb-threshold group (Lowest possible relative risk; results in Column 2) and vice versa giving the other extreme (Highest possible relative risk; results in Column 3). As the results of these two sensitivity analyses were comparable to the primary analyses (Column 1 vs. Columns 2 and 3), we did not perform multiple imputation of the missing SAPS II and SOFA scores.

Table S13. Use of life-support, severe adverse reactions and ischemic events

	Lower Hb-threshold (N=488)	Higher Hb-threshold (N=489)
Use of life support †	*no./total no. (%)*	
Day 5*		
Vasopressor/inotropic agent	153/432 (35.4)	140/429 (32.6)
Mechanical ventilation	242/432 (56.0)	238/429 (55.5)
Renal replacement therapy	109/432 (25.2)	88/429 (20.5)
Day 14*		
Vasopressor/inotropic agent	44/380 (11.6)	45/367 (12.3)
Mechanical ventilation	116/380 (30.5)	109/367 (29.7)
Renal replacement therapy	59/380 (15.5)	46/367 (12.5)
Day 28*		
Vasopressor/inotropic agent	9/330 (2.7)	16/322 (5.0)
Mechanical ventilation	35/330 (10.6)	48/322 (14.9)
Renal replacement therapy	24/330 (7.3)	28/322 (8.7)
Severe adverse reactions to blood in ICU	*no./total no.*	
Allergic reactions ‡	0/488	0/489
Acute hemolysis §	0/488	1/489
Transfusion-associated acute lung injury ¶	0/488	0/489
Transfusion-associated circulatory overload ‖	0/488	0/489
Ischemic events in ICU	*no./total no. (%)*	
Cerebral ††	4/488 (1.0)	10/489 (2.0)
Myocardial **	13/488 (2.7)	6/489 (1.2)
Intestinal ‡‡	11/488 (2.3)	14/489 (2.9)
Limb §§	11/488 (2.3)	11/489 (2.3)

* The total numbers of patients were below the group totals because of patients dying. Beyond that we had no missing data.

† Defined as infusion of vasopressor or inotropic agents or use of invasive or non-invasive mechanical ventilation or any form of renal replacement therapy on those days.

‡ Allergic reactions after blood transfusion was defined by the clinician on the basis of muco-cutaneous signs and symptoms (e.g. urticaria, pruritus, localized angio-edema) occurring within 6 hours of red cell transfusion

§ Severe hemolytic complications after blood transfusion was defined by the clinician on the basis of hemoglobinuria or increased free plasma hemoglobin occurring within 24 hours of transfusion.

¶ Transfusion-associated acute lung injury (TRALI) after blood transfusion defined as: I. Acute or worsening hypoxemia ((PaO_2/FiO2 < 40 (PaO_2 in kPa) or <300 (PaO_2 in mmHg) regardless of PEEP) OR > 50% relative increase in FiO_2 AND II. Occurrence within 6 hours after red cell transfusion AND III. Acute or worsening pulmonary infiltrates on frontal chest x-ray OR clinical signs of overt pulmonary edema.

‖ Transfusion-associated circulatory overload (TACO) after blood transfusion defined as: I. Acute or worsening hypoxemia (PaO_2/FiO_2 < 40 (PaO_2 in kPa) or <300 (PaO_2 in mmHg) regardless of PEEP) OR > 50% relative increase in FiO_2 AND II. Occurrence within 6 hours after red cell transfusion AND III. Acute or worsening pulmonary infiltrates on frontal chest x-ray OR clinical signs of overt pulmonary edema AND IV. Increased blood pressure AND VI. Positive fluid balance.

†† Cerebral ischemia was defined as any form of cerebral ischemia on a CT- OR MRI scan.

** Myocardial ischemia was defined as patient diagnosed with acute myocardial infarction (ST-elevation myocardial infarction or non-ST elevation myocardial infarction) or unstable angina pectoris according to the criteria in the clinical setting in question (e.g. elevated biomarkers, ischemic signs on ECG and clinical presentation) AND the patient received treatment as a consequence of this (reperfusion strategies (PCI/thrombolysis) or initiation/increased antithrombotic treatment).

‡‡ Intestinal ischemia was defined as ischemia verified by endoscopy OR open surgery.

§§ Limb ischemia was defined as clinical signs AND need of open/percutaneous vascular intervention, amputation OR initiation/increased antithrombotic treatment.

Table S14. Post-hoc analyses of number of patients with myocardial ischemia

	Lower Hb-threshold	Higher Hb-threshold	Relative Risk (95% CI)	P-value*
	no./total no. (%)			
Myocardial ischemia †	13 / 488 (2.7)	6 / 489 (1.2)	2.17 (0.83 – 5.67)	0.10
STEMI ‡	4 / 488 (0.8)	1 / 489 (0.2)	4.01 (0.45 – 35.73)	0.18
Non-STEMI/unstable angina §	9 / 488 (1.8)	5 / 489 (1.0)	1.80 (0.61 – 5.34)	0.28

* Analyzed by unadjusted chi^2-testing.
STEMI denotes ST-elevation myocardial infarction

† The number of patients having one or more myocardial ischemic events in ICU. Myocardial ischemia was defined as patient diagnosed with acute myocardial infarction (ST-elevation myocardial infarction or non-ST elevation myocardial infarction) or unstable angina pectoris according to the criteria in the clinical setting in question (e.g. elevated biomarkers, ischemic signs on ECG and clinical presentation) AND the patient received treatment as a consequence of this (reperfusion strategies (PCI/thrombolysis) or initiation/increased antithrombotic treatment).

‡ Defined post-hoc as signs of myocardial ischemia (e.g. symptoms, elevated biomarkers or clinical signs) and ST elevations on ECG.

§ Defined post-hoc as non-ST elevation myocardial infarction or unstable angina pectoris according to the criteria in the clinical setting in question (e.g. elevated biomarkers, ischemic signs on ECG and clinical presentation) AND antithrombotic treatment was initiated/increased).

Table S15. Sepsis-related organ failure assessment (SOFA) scoring in the TRISS trial

Organ System	0	1	2	3	4
GCS score	15	13-14	10-12	6-9	<6
Respiration PaO_2 / FiO_2 (in mmHg)	>400	301 - 400	<301	101 - 200 (with respiratory support*)	≤ 100 (with respiratory support*)
(in kPa)	>53	40 – 53	<40	13 – 27 (with respiratory support*)	≤ 13 (with respiratory support*)
Coagulation Platelets (x 10^9 / l)	>150	101 - 150	51 - 100	21 – 50	≤ 20
Liver Bilirubin (mg / dl)	< 1.2	1.2 – 1.9	2.0 – 5.9	6.0 – 11.9	> 12.0
(μmol / l)	<20	20 - 32	33 - 101	102 - 204	>204
Cardiovascular Hypotension	MAP > 70 mmHg	MAP < 70 mmHg	dopamine ≤ 5.0 (doses are given in μg / kg / minute)	dopamine >5.0 (doses are given in μg / kg / minute)	dopamine >15.0 (doses are given in μg / kg / minute)
			or any dose dobutamine	or adrenaline ≤0.1	or adrenalin >0.1
			or any dose milrinone or any dose levosimendan	or noradrenaline ≤0.1 or any dose vasopressin or any dose phenylephrine	or noradrenaline >0.1
Renal Creatinine (mg / dl)	< 1.2	1.2 – 1.9	2.0 – 3.4	3.5 – 4.9	> 5.0
(μmol/l)	< 110	110 – 170	171 – 299	300 – 440	> 440
OR Urine output				or < 500 ml / day	or < 200 ml / day

GCS denotes Glasgow Coma Scale. If a value was not available, the value of the latest obtained sample was used.*Respiratory support was defined as any form of invasive or non-invasive ventilation including mask CPAP or CPAP delivered through a tracheotomy.

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PAPER III

Restrictive versus liberal transfusion strategy for red blood cell transfusion: systematic review of randomised trials with meta-analysis and trial sequential analysis

Lars B Holst [1], **Marie W Petersen** [1], **Nicolai Haase** [1], **Anders Perner** [1], **Jørn Wetterslev** [2]

[1] Department of Intensive Care 4131, Rigshospitalet, DK-2100 Copenhagen, Denmark

[2] Copenhagen Trial Unit, Centre for Clinical Intervention Research, Copenhagen University Hospital-Rigshospitalet, Blegdamsvej 3, DK-2200 Copenhagen, Denmark

Review team

Lars Broksø Holst, MD, Dept. of Intensive Care 4131, Rigshospitalet, DK-2100 Copenhagen, Denmark.

Marie Warrer Petersen, B.Sc., Dept. of Intensive Care 4131, Rigshospitalet, DK-2100 Copenhagen, Denmark.

Nicolai Haase, MD, Dept. of Intensive Care 4131, Rigshospitalet, DK-2100 Copenhagen, Denmark.

Anders Perner, MD, Dept. of Intensive Care 4131, Rigshospitalet, DK-2100 Copenhagen, Denmark.

Jørn Wetterslev, MD, The Copenhagen Trial Unit (CTU), Centre of Clinical Intervention Research, Rigshospitalet, Dept. 33-44, DK-2100 Copenhagen, Denmark.

Correspondence to: Lars B. Holst, lars.broksoe.holst@regionh.dk

Funding sources/sponsors

Dept. of Intensive Care 4131, Rigshospitalet, DK-2100 Copenhagen, Denmark

The Copenhagen Trial Unit (CTU), Centre of Clinical Intervention Research, Rigshospitalet, Dept. 33-44, DK-2100, Copenhagen, Denmark.

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Abstract

Background

Red blood cells (RBCs) are commonly used in the treatment of haemorrhage and anaemia, but the balance between benefit and harm of restrictive versus liberal transfusion strategies has not been firmly established.

Objective

We performed an up-to-date systematic review comparing benefit and harm of restrictive versus liberal transfusion strategies to guide RBC transfusion.

Design

Systematic review with meta-analyses and trial sequential analyses of randomised clinical trials using predefined haemoglobin or haematocrit levels to guide RBC transfusion.

Data sources

Trials were identified using a systematic search strategy in the following databases: Cochrane Central Register of Controlled Trials (CENTRAL); SilverPlatter MEDLINE (WebSPIRS) (1950 to date); SilverPlatter EMBASE (WebSPIRS) (1980 to date); Science Citation Index Expanded (SCI-EXPANDED) (1900 to present). Reference lists of identified trials and other systematic reviews were assessed, and authors and transfusion experts were contacted to identify additional trials.

Trial selection

Published and unpublished randomised clinical trials evaluating a restrictive versus a liberal transfusion strategy in adults or children were considered for inclusion, irrespective of language, blinding procedure, publication status, or sample size.

Data extraction

Two authors independently screened titles and abstracts of trials identified and relevant trials were evaluated in full text for eligibility. Subsequently, two reviewers independently extracted data on methods, interventions, outcomes, and risk of bias from included trials. Risk ratios and mean differences with 95% confidence intervals were estimated with random effects models accounting for clinical heterogeneity.

Results

31 trials with a total of 9813 randomised patients were included. Restrictive versus liberal transfusion strategy were not associated with the relative risk (RR) of death (0.89 (95% confidence interval (CI) 0.76 to 1.05, 5607 patients in eight lower risk of bias trials), overall morbidity (RR 0.98, 95% CI 0.85 to 1.12, 4517 patients, six lower risk of bias trials), fatal or non-fatal myocardial infarction (RR 1.32, 95% CI 0.61 to 2.83, 4630 patients in six lower risk of bias trials). Results were not affected by the inclusion of trials with unclear or high risk of bias. Trial sequential analysis on mortality and myocardial infarction showed that the required information size have not been reached but in the analysis of overall morbidity the possibility of a 15% RR reduction or increase with restrictive transfusion strategies could be excluded. However, the proportion of patients receiving RBCs (RR 0.54, 95% CI 0.47 to 0.63, 8923 patients in 24 trials) and the number of RBC units transfused (RR -1.43, 95% CI -2.01 to -0.86) were lower with the restrictive transfusion strategies.

Conclusion

In conventional meta-analyses, restrictive compared with liberal transfusion strategies reduced the number of RBCs used and the number of patients being transfused but were not shown to be associated with altered mortality, overall morbidity, or myocardial infarction. A restrictive transfusion strategy may be safe in most clinical settings, and a liberal transfusion strategy has not been shown to convey any benefit to patients, but a potential for harm. Further trials are warranted to guide transfusion strategies especially in patients with myocardial infarction or acute brain injury.

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Introduction

Transfusion of red blood cells (RBCs) are frequently used to treat anaemia or bleeding in a variety of patient groups.[1–3] Recent published randomised clinical trials (RCTs)[4–8] have questioned the use of RBCs by favouring restrictive transfusion strategies and elucidating the potential harm with liberal strategies. A Cochrane review compared the effects of different transfusion thresholds and identified 19 RCTs including 6264 patients.[9] The majority of data on mortality were from the Transfusion Requirements in Critical Care (TRICC) trial[4] (52%) and Transfusion Trigger Trial for Functional Outcomes in Cardiovascular Patients Undergoing Surgical Hip Fracture Repair(FOCUS) trial (23%)[10] underlining that the evidence base for guiding the use of RBC is somehow limited.[11]

Data from several newly published RCTs[12–16] warrant an up-to-date review to inform on benefit and harm of different transfusion strategies guiding RBC transfusion. The objective of the present work was to provide a systematic review including latest published RCTs using conventional meta-analysis to compare the effects of different transfusion strategies on important outcomes in various patient groups. It was of particular interest to examine whether the evidence may support a restrictive strategy without harming the patients.

Methods

The present systematic review was conducted according to the protocol previously published in the PROSPERO register (www.crd.york.ac.uk/PROSPERO, registration no. CRD42013004272). The methodology and reporting were based on recommendations from the Cochrane Collaboration[17] and the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) statement,[18] and evaluated according to the GRADE recommendations.[19]

Eligibility Criteria

Prospective RCTs were eligible for inclusion if RBC transfusions were administered on the basis of a clear transfusion “trigger” or “threshold”, defined as a specific haemoglobin (Hb) or haematocrit (Hct)-level. Comparator group patients were required to be either transfused at higher Hb or Hct levels than the intervention group or transfused in accordance with current transfusion practices. Trials including surgical and/or medical patients and adults and/or children were considered for inclusion, whereas trials conducted on neonates and children with low birth weight were excluded. All RCTs irrespective of language, blinding, publication status, or sample size were eligible. Quasi-randomised trials were excluded regarding assessment of benefit, but were considered for inclusion regarding assessment of harm. Trial flow and exclusions are presented in Figure 1.

Search strategy

Relevant RCTs were identified through an up-to-date systematic search strategy used in a published Cochrane review,[9] with no language or date restrictions in the Cochrane Central Register of Controlled Trials (CENTRAL); SilverPlatter MEDLINE (WebSPIRS) (1950 to October 2014); SilverPlatter EMBASE (WebSPIRS) (1980 to October 2014); Science Citation Index Expanded (SCI-EXPANDED) (1900 to October 2014). Main authors of included trials and experts in this field were contacted to identify any planned, unreported, or ongoing trials. References of included trials were reviewed to identify additional trials. Moreover, ongoing clinical trials and unpublished trials were identified via Current Controlled Trials, ClinicalTrials.gov, and www.centerwatch.com.For detailed information regarding search strategies see supplementary appendix 1.

Trial selection

Authors (LB, MWP and NH) independently reviewed all titles and abstracts identified through the systematic search strategy. Trials not fulfilling the eligibility criteria were excluded and the remaining trials were evaluated in full text. Disagreements were resolved with JW. For a detailed description of search results, included, and excluded trials, see Figure 1.

Data extraction

The author, institution and the publication source of trials were not masked for the authors at any time. Authors (LBH, NH or MWP) independently extracted trial characteristics (single or multicentre, country), baseline characteristics of patients (age, sex, disease severity), inclusion- and exclusion criteria, description of intervention (thresholds, duration), and outcomes using pre-made extraction forms. Corresponding authors of trials were contacted in case of any unclear or missing information.

Predefined primary outcomes were mortality and overall morbidity (one or more complication(s) as defined by authors). Secondary outcomes were adverse events (transfusion reactions, cardiac events (e.g. myocardial infarction, cardiac arrest, acute arrhythmia a ngina), renal failure, thromboembolic events, infections, haemorrhagic events, stroke or transitory cerebral ischemia). We also registered the proportion of patients transfused with allogeneic and/or autologous RBCs, and the number of allogeneic and autologous blood units transfused. Hb- or Hct-levels during intervention and length of hospital stay were regarded as process variables and thus reported as trial characteristics, see Table 1.

Risk of bias assessment

The major sources of bias domains (random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessors, incomplete outcome data, selective outcome reporting, baseline imbalance, financial and academic) were reviewed in all trials according to recommendations from the Cochrane Collaboration.[17]

Any assessment of the overall risk of bias in the present review involved consideration of the relative importance of the different domains. As blinding of transfusion trials are generally not feasible we characterised trials with low risk of bias in all other domains than blinding as trials with lower risk of bias in the assessment of overall risk of bias. All other trials were assessed as unclear or high risk of bias. For details regarding blinding of the included trials, see Table 2.

Grading the quality of evidence

The quality of evidence were assessed using GRADE (Grading of Recommendation, Assessment, Development and Evaluation) methodology.[19] The quality of evidence for mortality, overall morbidity and fatal- and non-fatal myocardial infarction were assessed regarding risk of bias, inconsistency, indirectness, imprecision and publication bias and classified as very low, low, moderate or high. Summary of findings for trials with lower risk of bias and for all trials are presented in Table 3 and 4, respectively.

Statistical analysis

All statistical analyses were performed using Review Manager (RevMan) version 5.3.3 (The Nordic Cochrane Centre, The Cochrane Collaboration, 2014) and Trial sequential analysis (TSA) program version 0.9 beta (www.ctu.dk/tsa).[20] For all included trials, relative risks (RR) and mean differences (MD) with 95% confidence intervals (CI) were reported for dichotomous- and continuous outcomes, respectively. These measures were pooled in meta-analyses.

We used a random-effects model[20] and a fixed-effect model[21] for meta-analysis in the presence of two or more trials included in analysis of an outcome. Heterogeneity among trials was quantified with inconsistency factor (I^2) or (D^2) statistics.[22] Results from both models are reported in case of discrepancy between the two otherwise we report results from the random effects model. Heterogeneity was explored by chi-squared test with significance set at *p*-value 0.10. Sensitivity analyses included application of continuity adjustment in trials with zero events.[17]

Pre-defined subgroup analyses were performed regarding risk of bias (lower vs. high or unclear) and we chose to emphasize the results from the trials with lower risk of bias,[17] patient population (adult vs children; surgical vs medical), length of follow-up (90 days or less vs more than 90 days) and transfusion product (leuko-reduced vs non-leuko-reduced RBC suspensions). Only subgroup analyses showing statistical significant test of interaction ($p<0.05$) were considered to provide evidence of an intervention effect pending the subgroup. We pre-planned exploration of moderate to high heterogeneity using meta-regression including mean age and fraction of men as covariates if possible. However it was not feasible due to missing values of the covariates in the included trials, but we performed a post hoc subgroup analysis stratifying trials according to clinical setting. There were no data to support the predefined subgroup analysis of randomised trials of patients with sepsis vs patients without sepsis.

Meta-analyses may result in type-I errors due to an increased risk of random error when sparse data are analysed[23] and due to repeated significance testing when a cumulative meta-analysis is updated with new trials.[20,24] TSA was applied to cumulative meta-analysis to assess the risk of type-I errors. TSA combines information size estimation (cumulated sample size of included trials) with an adjusted threshold for statistical significance[20,25] in the cumulative meta-analyses.[26] The latter, called trial sequential monitoring boundaries, adjust the confidence intervals and reduce type-I errors. When the cumulative Z-curve crosses the trial sequential monitoring boundary, a sufficient level of evidence for the anticipated intervention effect may have been reached and no further trials are needed. If the Z-curve does not cross any of the boundaries and the required information size has not been reached, there is insufficient evidence to reach a conclusion. Information size was calculated as a diversity adjusted required information size,[27] suggested by the diversity of the intervention effect estimates among the included trials.
The required information size was calculated based on a relative risk reduction (RRR) of 15% in mortality and overall morbidity and a RRR of 50% in myocardial infarction. All TSAs were appropriately adjusted for heterogeneity (diversity adjustment) according to an overall type-I error of 5% and a power of 80% considering early and repetitive testing.

Results

Trial selection

The updated systematic search strategy identified an additional 1930 records of which 38 records were assessed in full-text for eligibility to supplement the former 19 published RCTs. We found 33 eligible records published between October 1986 and October 2014 resembling 31 trials on 9813 patients.[4–8,10,12,13,15,16,28–46] Three identified records provided data from the same trial.[46–48] All trials

were published in English. A total of 26 records were excluded from the systematic review, [47–72] the primary reasons being lack of well-defined Hb or Hct levels guiding the intervention (six trials),[49–53,55] the inclusion of preterm or very low birth weight neonates (seven trials),[54,56–61] and secondary publications or subgroup analyses (nine records).[47,48,62–68] Three records related to ongoing trials.[69–71] Results of the search strategy is summarised in Figure 1.

Characteristics of trials

Both single (17 trials)[6,7,14,15,28–31,33,35,36,39,40,43,44,73] and multicentre (14 trials)[4,5,8,10,12,13,16,32,34,37,38,41,45,46] RCTs were included in the systematic review. Population size ranged from 25[35] to 2016[10] patients, and eight trials included more than 500 patients.[4–7,10,12,16,46] The clinical settings of the majority of RCTs were trauma (2 trials),[29,44] perioperative and acute blood loss (21 trials),[7,10,14–16,28,30–33,35–37,39–41,43,46,74] and critical care (seven trials)[4,5,8,12,13,34,38] and one trial included patients with leukaemia undergoing stem cell transplantation.[45] Table 1 summarises characteristics of included trials.

Intervention

In 24 trials,[4,6,7,12,13,15,16,28,30–38,41,42,44–46,73–75] patients received allogenous RBCs, and among these two trials also allowed the use of autologous transfusion.[39,40] The remaining five trials did not provide information on the type of RBCs used.[14,15,28,41,43] Leuko-reduced RBCs were transfused in 12 trials,[5,12,13,33,34,36,37,41,46,73,76] and partially leuko-reduced RBCs were administered in two trials.[8,10] Non-leuko-reduced RBCs were used in five trials,[4,7,38–40] and information was not provided in the remaining 12 trials.[14–16,28–32,35,43,44,74]

The intervention trigger value varied between trials and the restrictive transfusion triggers ranged from Hb 7.0 to 9.7 g/dl, Hct 24 to 30% or symptoms of anaemia as defined by authors. The liberal transfusion trigger values ranged from Hb 9 to 13 g/dl and Hct 30 to 40%.

Risk of bias assessment

Detailed information regarding blinding is provided in Table 2. Overall, 11 RCTs were judged as lower,[4–6,10,12,16,32,34,36,73,76] 14 as unclear,[8,14,15,28,29,33,35,38–41,43,44,74] and six as high risk of bias trials;[7,13,30,31,37,46] Figure 2 and 3 summarises risks of bias.

Clinical outcomes

Mortality

Data on mortality were provided in 23 trials (8321 patients)[4–8,10,12–15,29,30,32–34,37,38,41,43,44,73,74], but few trials followed the patients for 90 days or more.[8,12,13,15,38,41]

A total of eight trials with 5607 randomised patients were included in the analysis of mortality in trials with lower risk of bias (Figure 4),[4,5,8,12,32,34,73] showing an RR of 0.89 (95% CI 0.76 to 1.05; p=0.16; I^2=25%); the GRADE quality was judged to be low (Table 3). The trial sequential analysis adjusted 95% confidence interval was 0.68 to 1.17 (Figure 5).

Restrictive versus liberal transfusion strategies did not affect the RR of death (0.95, 95% CI 0.81 to 1.11; p=0.52; I^2=27%) including all trials despite risk of bias (Figure 6); the GRADE quality was judged to be low (Table 4). The trial sequential analysis adjusted 95% confidence interval was 0.74 to 1.21 (Figure 7).

Subgroup analyses of mortality

None of the predefined subgroup analyses showed differences in the intervention effect between subgroups (see table 5 and figure 8).

Overall morbidity

A total of six trials with lower risk of bias including 4517 patients were included in the meta-analysis of overall morbidity (Figure 9).[4–6,8,10,34] Overall morbidity did not differ between the restrictive and liberal transfusion strategy (RR 0.98, 95% CI 0.85 to 1.12; p=0.75; I^2=60%) and the trial sequential analysis adjusted 95% confidence interval was 0.81 to 1.19. Future trials are unlikely to show an association with a 15% RRR in favour of the restrictive or the liberal strategy as the boundary for futility was crossed (Figure 10). The GRADE quality of evidence was judged to be very low (Table 3). A total of 12 trials with 5975 randomised patients were included in the meta-analysis of overall morbidity regardless of risk of bias (RR 1.06, 95% CI 0.93 to 1.21; p=0.36; I^2=58%).[4–8,10,34,36,38,40,46,74]

Fatal or non-fatal myocardial infarction

Six trials assessing fatal or non-fatal myocardial infarction including 4630 patients were defined as trials with lower risk of bias.[4,5,10,12,34,73] Restrictive transfusion strategies were not associated with RR reduction or increase in fatal or non-fatal myocardial infarction (RR 1.32, 95% CI 0.61 to 2.83; p=0.48; I^2= 44%) (Figure 11) and the trial sequential analysis adjusted 95% confidence interval was 0.28 to 6.21 (Figure 12). The GRADE evidence profile was judged to be very low (Table 3). A total of 16 trials with 6.501 randomised patients were included in the meta-analysis of fatal or non-

fatal myocardial infarction regardless of risk of bias (RR 1.05, 95% CI 0.82 to 1.36; p=0.70; I^2= 6%); the GRADE quality of evidence was judged to be low (Table 4).[4,5,7,8,10,12,13,30,31,34,37,39–42,73]

Other adverse events

A total of eight trials defined as lower risk of bias with 5107 patients were included in the meta-analysis on infectious complications. Our analysis showed an association in favour of using a restrictive transfusion strategy (RR 0.73, 95% CI 0.55 to 0.98, p=0.03, I^2= 53%) (appendix 2).[4–6,10,16,32,36,73] The inclusion of all 15 trials with 7217 patients, regardless of risk of bias, did not alter the result (RR 0.79, 95% CI 0.64 to 0.97, p=0.03, I^2= 40%).[4–8,10,16,31,32,36,37,41,42,46,73] Our analysis showed no association of restrictive versus liberal transfusion with other adverse events (cardiac complications, renal failure, thromboembolic, stroke or transitory ischaemic insult, or haemorrhage) (Supplementary appendix 3 and 4).

Number of patients and units transfused

A total of 24 trials with 8923 patients were included in the meta-analysis of the proportion of patients receiving RBCs (RR 0.54, 95% CI 0.47 to 0.63; p<0.001; I^2= 95%) and a total of 12 trials with 4022 patients were included in the meta-analysis of the number of units transfused (mean difference -1.43, 95% CI -2.01 to -0.86; p<0.001; I^2= 96%) both showing lower numbers associated with restrictive vs liberal transfusion strategies (Supplementary appendix 5 and 6).

Discussion

We did not find any association with mortality, overall morbidity or myocardial infarction when comparing restrictive vs liberal transfusion strategies; however the overall quality of evidence was low. We performed TSA to account for sparse data and repetitive testing on accumulating data and found that the 95% CIs of the point estimates widened, but the results of the conventional meta-analyses were unchanged. In our analysis of all-cause mortality, the cumulative z-curve did not cross any boundaries with only 38% of the required information size being reached (5607 of 14.762 patients) indicating that further trials are needed to establish firm evidence. In our analysis of all trials, the TSA indicated that it is unlikely that future trials will show overall harm with restrictive transfusion strategy. Regarding overall morbidity, we showed no association with benefit or harm between groups but the TSA indicated that future trials on this outcome will be futile. We found that the TSA on pooled risk of fatal or non-fatal myocardial infarction was inconclusive, because only 26% of the required information size has been obtained. Regarding infectious complications our analysis indicated possible association between restrictive transfusion strategy and reduced rate of infection across different clinical settings.

Relation to other reviews

Well-conducted systematic reviews with meta-analysis on RBC transfusion have been published. A Cochrane review indicated that restrictive transfusion strategies were not associated with the rate of adverse events (i.e. mortality, cardiac events, stroke, pneumonia and thromboembolism) as compared to liberal transfusion strategies. Restrictive transfusion strategies were associated with a reduction in hospital mortality (RR 0.77, 95% CI 0.62-0.95) but not in 30 day mortality (RR 0.85, 95% CI 0.70 to 1.03).[9]

Salpeter and colleagues published a review in 2014 including 6936 patients from 19 trials assessing the impact of RBC transfusion.[77] Pooled data from three trials (2364 patients) using restrictive Hb-transfusion triggers of 7 g/dl showed reductions in in-hospital mortality (RR 0.74, 95% CI 0.60 to 0.92), total mortality (RR 0.80, 95% CI 0.65 to 0.98), re-bleeding (RR 0.64, 95% CI 0.45 to 0.90), acute coronary syndrome (RR 0.44, 95% CI 0.22 to 0.89), pulmonary oedema (RR 0.48, 95% CI 0.33 to 0.72), and bacterial infections (RR 0.86, 95% CI 0.73 to 1.00) as compared to liberal transfusion. In contrast pooled data including trials with less restrictive transfusion thresholds did not show associations with any of the predefined outcomes.

Rhode and colleagues recently published a systematic review with meta-analysis including 18 RCTs reporting data on in-hospital infections.[78] Restrictive transfusion strategies were associated with a reduced risk of infections among hospitalised patients as compared with liberal strategies (RR 0.88, 95 % CI 0.78-0.99). Our analysis showed comparable result with a possibility of lowering the rate of infections using restrictive transfusion strategies. We also included data on non-health-care associated infections but our results may be influenced by multiple testing and sparse data.

We included data from the recent TRISS trial randomising 1005 patients with septic shock in the ICU, not showing a difference in mortality or morbidity with the use of pre-storage leukocyte-reduced RBCs at a transfusion trigger of 7 g/dl.[12] In accordance with the Cochrane review we did not find evidence of harm with the use of restrictive as compared with liberal transfusion strategies. However, our TSAs were inconclusive regarding the assessment of mortality and myocardial infarction due to insufficient information.

Strengths and limitations of this review

Applications of the Cochrane methodology is a major strength of this systematic review comprising a pre-published protocol, non-restricted up-to-date literature search, independent data extraction

by at least two authors, and risk of bias assessment leading to GRADE evaluations of important outcomes. TSA was performed to explore the risk of random error due to sparse data and repetitive testing in order to increase the robustness of the meta-analyses and distinguish the current information size from the required information size. We did not show associations between the interventions and the outcomes in any of the analysis performed, and the predefined subgroup analyses all supported the primary findings.

Our systematic review also has limitations. The RCTs included in the primary analysis addressed different indications for transfusion by randomising a variety of patient groups (e.g. paediatric and adult patients) in different clinical settings (e.g. elective surgery and critical illness). Thus, the risk of introducing potentially important heterogeneity is imminent. To get a clinical applicable result, we excluded trials of neonates and infants with very low birth weight. None of the included trials were blinded as this is not feasible. This may introduce both performance and detection bias. However, the primary outcome of all-cause mortality is less prone to be influenced by lack of blinding.[79] Transfusion triggers varied between trials with some trials using a liberal transfusion threshold equal to the restrictive one in other trials, introducing clinical heterogeneity. Both clinical heterogeneity and inadequate follow-up increases the risk of type-II error. Bias in the included trials, loss to follow-up and incomplete reporting of outcome measures are additional limitations in this review. The definitions of overall morbidity and adverse events were very heterogeneous and should be taken into account when interpreting these data.

Unanswered questions

Weather the overall use of RBC should be guided by a restrictive or a liberal strategy is still in question. Patients with coronary artery disease and in particularly patients with ongoing cardiac ischemia might require a higher Hb level to sustain oxygen delivery to the myocardial cells. However, RBC transfusion could worsen patient outcome due to increased risk of circulatory overload and increased thrombogenicity with higher Hct levels. Results from the FOCUS trial showed no association with the primary composite outcome of morbidity and mortality 60 days postoperatively or the incidence of coronary syndrome when comparing two transfusion strategies (8 g/dl (or symptoms of anaemia) vs 10 g/dl).[10] Two small RCTs evaluating a restrictive transfusion trigger of Hb < 8 g/dl in patients with symptomatic coronary artery disease have been published;[8,34] pooled data from these two trials randomising a total of 155 patients did not show an association between restrictive transfusion strategy and cardiac events or mortality as compared to a liberal strategy. A meta-analysis including observational studies on transfusion in patients with myocardial infarction indicates that the rates of subsequent myocardial infarction and all-cause

mortality may be associated with blood transfusions compared with standard supportive interventions, after adjustment for possible confounding variables.[80] Large RCTs of restrictive vs liberal transfusion are warranted in patients with myocardial infarction.

Due to the lack of ability for the injured brain to compensate for decreased oxygen delivery, might also require more liberal transfusion strategies to prevent secondary cerebral ischaemic insults.[81] One RCT using a factorial design compared the effects of erythropoietin and two different Hb-thresholds for RBC transfusion (7 vs 10 g/dl) in 200 patients with a closed head injury and showed no difference in neurological outcome at six months.[41] Also in patients with acute brain injury data from high-quality RCTs are needed to guide transfusion practice.

Conclusions

Restrictive transfusion strategies were associated with reduced numbers of RBC units transfused and reduced proportions of patients receiving transfusions. We did not find any association with mortality, overall morbidity, and fatal or non-fatal myocardial infarction with the use of restrictive as compared with liberal transfusion strategies in various clinical settings, but the required information sizes have not been reached except for overall morbidity where a 15% relative risk reduction or increase with restrictive transfusion strategies may be refuted. Analyses of all trials, regardless of risk of bias, demonstrated similar findings. We found possible associations between restrictive transfusion strategies and reduced number of infectious complications.

Restrictive transfusion strategies may be safe in most clinical settings, and liberal transfusion strategies have not shown to confer any benefit to patients, but have a potential for harm. Further trials are warranted to guide transfusion strategies especially in patients with myocardial infarction and acute brain injury.

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Contributors: LBH developed the protocol, was responsible for the searches, selected trials, extracted data, assessed the risk of bias of trials, did the data analysis, and developed the final review. MWP developed the protocol, selected trials, extracted data, assessed the risk of bias of trials, and developed the final review. NH developed the protocol, selected trials, extracted data, assessed the risk of bias of trials, and developed the final review. AP developed the protocol, analysed data, and developed the final review. JW developed the initial idea for the review, developed the protocol, selected trials, advised on statistical methods, analysed data and resolved any disagreements during data extraction and bias assessment, and developed the final review. All authors read and approved the final manuscript. LBH and JW are the guarantors and affirm that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

Competing interests: All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: AP was principal investigator for Transfusion Requirements In Septic Shock (TRISS) trial and LBH, NH and JW were members of the steering committee. AP is head of research in his intensive care unit, which receives research grants from CSL Behring, Fresenius Kabi, and Cosmed.

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What is already known on this topic
Red blood cells are commonly used in the treatment of haemorrhage and anaemia and recent trials have shown potential harm with this intervention Recent meta-analysis indicates no harm with the use of a restrictive transfusion strategy
What this trial adds
This review includes new data from five recently published randomised trials of restrictive versus liberal transfusion strategies, and the review includes data on more than 9000 patients. The pooled analyses did not show harm with restrictive transfusion strategies (no increased risk of mortality, overall morbidity or acute myocardial infarction) but transfusion numbers and rates were reduced as compared to liberal strategies. Liberal strategies have possible associations with harm (risk of infectious complications) and further large trials with lower risk of bias are needed to establish firm evidence to guide transfusion in subgroup of patients in particular those with myocardial infarction and acute brain injury.

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Tables 1-5 - paper III

Table 1 Trial characteristics

Trial ID	Source	Country	Trial characteristics*	Inclusion period	Clinical setting	RBCs (type/suspension /leukodepletion)	Storage age**	Protocol violations¶	Intervention trigger value†
Almeida 2013	Critical Care	Brazil	198/1	Jan 2012 - Dec 2012	Cancer pts undergoing major abdominal surgery requiring postoperative ICU care	NA/NA/NA	NA	NA	R: 7, L: 9
Blair 1986	Brirish Journal of Surgery	UK	50/1	NA	Surgical pts with GI bleeding	Allogen/citrate/NA	NA	NA	R: 8 or persistant shock, L: 2 units
Bracey 1999	Transfusion	USA	428/1	Feb 1997 - Nov 1997	First time elective CABG surgery	Allogen/NA/NA	NA	NA	R: 8 or predefined clinical condition, L: 9
Bush 1997	American Journal of Surgery	USA	99/1	Aug 1995 - Nov 1996	Elective aortic or infrainguinal artierial reconstruction	Allogen/NA/NA	NA	R. 3, L: 2	R: 9, L: 10
Carson 1998	Transfusion	USA/ Scotland	84/4	Mar 1996 - Mar 1997	Primary hip fracture pts	Allogen/NA/NA	NA	R: 4/42, L: 1/42	R: 8 or symptoms of anemia, L: 10
Carson 2011	New England Journal of Medicine	USA/ Canada	2016/47	May 2003 - Oct 2009	Primary hip fracture pts with CVD or risk of CVD	Allogen/NA/leukod epleted (R: 88.6%, L: 90.2%)	R: 22.1 (9.9), L: 22.0 (9.5)	R: 56/1007, L: 91/1006	R: 8 or symptoms of anemia, L: 10
Carson 2013	American Heart Journal	USA	110/8	Mar 2010 - May 2012	Pts with coronary syndrome or stable coronary artery disease undergoing catheterisation	Allogen/NA/leukod epleted (R: 92%, L: 95%)	R: 24.6 (9.1), L: 23.4 (10.9)	R: 1/55, L: 5/55	R: 8 or symptoms of anemia, L: 10
Cholette 2011	Pediatric Crit Care	USA	60/1	Aug 2006 - Sep 2009	Infants and children with single ventricle physiology undergoing cavapulmonary bypass	Allogen/NA/leukod epleted	NA	R: 0/30, L: 0/30	R: 9 with symptoms, L: 13
Cooper 2011	American Journal of Cardiology	USA	45/2	May 2003 – Oct 2009	Acute MI	Allogen/NA/leukod epleted	NA	NA	R: hct < 24 (24-27)%, L: 30 (30-33)%
Gast-Bakker 2013	Intensive Care Medicine	Netherlands	107/1	Apr 2009 - Jan 2012	Infants and children undergoing elective heart surgery for congenital heart defect	Allogen/NA/leukod epleted	R: 9.8 (6.8), L: 9.8 (7.2)	R: 3/53, L: 4/54	R: 8.0, L: 10.8
Gregersen 2013	NA	Denmark	160/1	NA	Hip fracture pts	NA/NA/NA	NA	NA	R: 9.7, L: 11.3
Fortune 1987	Journal of Trauma	USA	25/1	NA	Traumatic pts with haemorrhagic shock (class 3-4)	Allogen/NA/NA	NA	NA	R: hct < 30%, L: < 40%

Foss 2009	Transfusion	Denmark	120/1	Feb 2004 - Jul 2006	Primary hip fracture pts	Allogen/NA/leukodepleted	NA	NA	R: 8.0, L: 10.0
Grover 2005	Vox Sanguinis	UK	260/3	NA	Elective total knee or hip arthroplasty	Allogen/NA/leukodepleted	NA	NA	R: 8 and maintained between 8.0-9.5, L: <10 and maintained between 10.0-12.0
Hajjar 2010	Journal of the American Medical Association	Brazil	502/1	Feb 2009 - Feb 2010	Elective CABG and/or valve replacement	Allogen/citrate/non-leukodepleted	NA	R: 0/255, L: 1/257	R: hct < 24%, L: < 30%
Hebert 1995	Journal of the American Medical Association	Canada	69/25	Mar 1993 - Jan 1994	Euvolemic, critically ill pts	Allogen/NA/non-leukodepleted	NA	R: 2/33, L: 2/36	R: 7, L: 9
Hebert 1999	New England Journal of Medicine	Canada	838/25	Nov 1994 - Nov 1997	Euvolemic, critically ill pts	Allogen/citrate/non-leukodepleted	NA	R: 6/418 (Cross over: 4/418), L: 18/420, (Cross over: 11/420)	R: 7.0 (7.0-9.0), L: 10.0 (10.0-12.0)
Holst 2014	New England Journal of Medicine	Scandinavia	1005/32	Dec 2011 - Dec 2013	Septic shock	Allogen/SAGM/leukodepleted	NA	R: 45/463, L: 16/470	R: 7, L: 9
Johnson 1992	Journal of Thoracic and Cardiovascular surgery	USA	39/1	NA	Elective revascularisation	Allogen and autologous/NA/non-leukodepleted	NA	R: 1/21, L: 0/18	R: hct < 25%, L: < 32%
Lacroix 2007	New England Journal of Medicine	Canada/ USA/UK/ Belgium	648/19	Nov 2001 - Aug 2005	Stable, critically ill infants and children	Allogen/NA/leukodepleted	R: 16.0 (10.5), L: 15.7 (10.3)	R: 1/320, L: 10/317	R: 7 (8.5-9.5), L: 9.5 (11.0-12.0)
Lotke 1999	Journal of Arthroplasty	USA	152/1	NA	Total knee arthroplasty	Allogen and autologous/NA/non-leukodepleted	NA	NA	R: 9.0, L: 2 units
Parker 2013	Injury	England	200/1	NA	Primary hip fracture pts	NA/NA/NA	NA	NA	R: Symptoms of anemia[§], L: 10
Prick 2013	British Journal of Obstetricians and Gynaecologists	Netherlands	521/37	May 2004 - Feb 2011	Pts with sustained post partum haemorrhage	Allogen/NA/NA	NA	R: 33, L: 7	R: Symptoms of anemia[‡], L: 8.9
Robertson 2014	Journal of the American Medical Association	USA	200/2	May 2006 - Aug 2012	Pts with closed head injury	NA/NA/leukodepleted	NA	R: 4/99, L: 0/101	R: 7, L: 10
Shehata 2012	Transfusion	Canada	50/1	Jan 2007 - Jun 2010	Elective cardiac surgery pts	Allogen/NA/NA	NA	R: 16%, L: 59%	R: 70 g/L peri- and 75 g/L postoperatively, L: 95 g/L peri- and 100 g/L postoperatively
So-Osman 2010	Vox Sanguinis	Netherlands	619/3	2001 - 2003	Primary elective hip or knee replacement	Allogen/NA/leukodepleted	NA	NA	R: New transtrictive transfusion policy related to

									including center and time since surgery, L: Standard of care
Villanueva 2013	New England Journal of Medicine	Spain	921/1	NA	Pts with upper GI bleeding	Allogen/NA/leukodepleted	NA	R: 39/444, L: 15/445	R: 7, L: 9
Walsh 2013	Critical Care Medicine	England	100/6	Aug 2009 - Dec 2010	Mechanically ventilated pts in ICU	Allogen/SAGM/leukodepleted	NA	R: 2/51, L: 3/49	R: 7, L: 9
Webert 2008	Transfusion	Canada	60/4	Mar 2003 - Oct 2	Pts with acute leukemia undergoing stem cell transplantation	Allogen/AS-3/leukodepleted	NA	R: 24/29, L: 28/31	R: 80 g/L, L: 120 g/L
Wu 2011	ESICM 24th Annual Congress 2011	China	226/1	NA	Orthotopic liver transplantation	NA/NA/NA	NA	NA	R: 7 (7-9), L: 10 (10-12)
Zygun 2009	Neurological Critical Care	England	30/1	Jan 2003 - Jul 2005	Severe traumatic brain injury	Allogen/NA/NA	NA	NA	R: 8.0 (2 units transfused), L1: 9.0 (2 units transfused), L2: 10.0 (2 units transfused)

Legend for table 1:

RBC = red blood cells; pts = patients; ICU = intensive care unit; NA = not available; R = restrictive; L = liberal; GI = gastrointestinal; CABG = coronary artery bypass graft; CVD = cardiovascular disease; MI = myocardial infarction;
* Number of patients included in trial/number of trial sites
** Values are means (standard deviations) unless otherwise specified
¶ Values are proportions of patients unless otherwise specified
† Haemoglobin levels are reported in g/dl unless otherwise specified
§ Symptoms of anaemia included recurrent vasovagal episodes on mobilisation, chest pain of cardiac origin, congestive cardiac failure, unexplained tachycardia, hypotension or dyspnoea due to anaemia, decreased urine output unresponsive to fluid replacement, and any other symptoms felt appropriate by the medical staff looking after the patient
‡ Symptoms of anaemia defined as dyspnoea or syncope

Table 2 Summary of reported blinding procedure in included trials*

Trial ID	Patient	Clinical/trial personnel	Outcome assessor
Almeida 2013	NA†	NA	NA
Blair 1986	NA	NA	NA
Bracey 1999	Not blinded	Not blinded	Not blinded
Bush 1997	NA	Surgeons/anaesthesiologists not blinded; clinical staff NA	NA
Carson 1998	NA	NA	Study nurses obtaining subjective (functional status, place of residence) and objective outcomes (60 day survival status) were blinded for intervention during follow up pr telephone
Carson 2011	Not blinded	Not blinded	Study nurses obtaining subjective (functional status, place of residence) and objective outcomes (60 day survival status) were blinded for intervention during follow up pr telephone
Carson 2013	NA	NA	Composite outcome of death and MI; Study nurses obtaining subjective (functional status, place of residence) and objective outcomes (MI, unstable angina, 60 day survival status) were blinded for intervention during follow up pr telephone
Cholette 2011	Not blinded	Operation personnel blinded peri-operatively; clinical staff not blinded post-operatively	Outcome assessor NA; DSMC blinded
Cooper 2011	Not blinded	Not blinded	NA
Fortune 1987	NA	NA	NA
Foss 2009	Blinded	NA	Physiotherapist assessing ambulation blinded
Gast-Bakker 2013	Not blinded	Not blinded	Not blinded
Gregersen 2013	NA	NA	NA
Grover 2005	Blinded	Surgeons/anaesthesiologists not blinded; clinical staff NA	Holter monitor assessor blinded
Hajjar 2010	Blinded	Anaesthesiologists/ICU clinicians blinded; surgeons NA	Outcome assessor blinded; DSMC NA
Hebert 1995	Not blinded	Not blinded	NA
Hebert 1999	Not blinded	Not blinded	Outcome assessor NA; DSMC blinded
Holst 2014	Not blinded	Not blinded	Outcome assessor; Statisticians and DSMC blinded
Johnson 1992	NA	Surgeons/anaesthesiologists not blinded; clinical staff NA	NA
Lacroix 2007	Not blinded	Not blinded	Outcome assessor NA; Statisticians and DSMC blinded
Lotke 1999	NA	NA	Blinded
Parker 2013	NA	NA	Study nurses assessing Mobility Score blinded
Prick 2013	NA	NA	NA
Robertson 2014	NA	Not blinded	Trial investigators not blinded; Outcome assessors

Shehata 2012	NA	NA	NA
So-Osman 2010/2013	Not blinded	Surgeons/clinicians not blinded	Assessor and study investigators blinded; Study nurses not blinded
Villanuaeva 2013	Not blinded	Not blinded	Not blinded
Walsh 2013	Blinded	Not blinded	NA
Webert 2008	Not blinded	Not blinded	Study personnel assessing bleeding and adjudication commitee blinded
Wu 2011	NA	NA	NA
Zygun 2009	NA	NA	NA

Legend for table 2:

* To supplement the ROB table (figure 2.) on the blinding procedure, not assessed in the overall evaluation of trial bias domains due to feasibility issues.
† When blinding issue were not reported in trials, not accounted for (NA) are registered.

Table 3. Summary of findings for all trials with lower risk of bias

Should restrictive or liberal transfusion strategy be used for guiding red blood cell transfusion?

Settings: Intensive care unit, perioperative, and trauma

Intervention: Restrictive red blood cell transfusion

Comparison: Liberal red blood cell transfusion

No of Participants (studies)	**No of Participants Restrictive**	**No of Participants liberal**	**Relative Risk (95% CI)**	**Absolute effect**	**Quality of the evidence (GRADE)**	**Quality assessment domains**
All cause mortality, longest follow up, low risk of bias trials						
5607 (8)	426/2809 (15%)	469/282 (16.6%)	RE: 0.89 (0.76 to 1.05); I^2 = 25%) TSA adjusted 95% CI of a RR of 0.89 is 0.68 to 1.17	18 fewer per 1000 (from 40 fewer to 8 more)	⊕⊕⊖⊖ **low** Critical importance	Inconsistency: No serious[1] Indirectness: No serious Imprecision: Serious[2] Reporting bias: Reporting bias[3]
Overall morbidity - lower risk of bias trials						
4517 (6)	858/2261(37.9%)	897/2256(39.8%)	RE: 0.98 (0.85 to 1.12); I^2 = 60%) TSA adjusted 95% CI for a RR of 0.98 is 0.81 to 1.19	8 fewer per 1000 (from 60 fewer to 48 more)	⊕⊖⊖⊖ **very low** Critical importance	Inconsistency: Serious[4] Indirectness: No serious Imprecision: Serious[2] Reporting bias: Reporting bias[1]
Fatal and non-fatal myocardial infarction in lower risk of bias trials						
4630 (6)	57/2318 (%)	41/2312 (%)	RE: 1.32 (0.61 to 2.83); I^2 = 44%) TSA adjusted 95% CI of the RR of 1.32 is 0.28 to 6.21	6 more per 1000 (from 7 fewer to 32 more)	⊕⊖⊖⊖ **very low** Critical importance	Inconsistency: Serious[5] Indirectness: No serious Imprecision: Very serious[6] Reporting bias: Reporting bias[3]

1) I square =17% and p value for heterogeneity is 0.29

2) Anticipation of a 15% RRR results in TSA adjusted confidence intervals including more than a 25% relative risk reduction or more than a 25% relative risk increase. However, less than a 15% RRR or RRI may also be considered clinically relevant and these are apparently not excluded in any analyses

3) According to funnel plot a possibility for publication bias regarding smaller trials showing benefit for restrictive transfusion strategy is apparent

4) Two trials showing no effect and appreciable harm with restrictive transfusion strategy

5) I square = 55% and p value for heterogeneity is 0.06. Variance in point estimates from 0.54 to 2.95

6) Four of five trials showing no effect and appreciable harm with restrictive transfusion strategy

CI: **Confidence interval;** RR: **Risk Ratio;** RE**: Random effects model.**

GRADE Working Group grades of evidence
High quality: **Further research is very unlikely to change our confidence in the estimate of effect.**
Moderate quality: **Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.**
Low quality: **Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.**
Very low quality: **We are very uncertain about the estimate.**

Table 4. Summary of findings for all trials despite risk of bias

Should restrictive or liberal transfusion strategy be used for guiding red blood cell transfusion?

Settings: Intensive care unit, perioperative, and trauma

Intervention: Restrictive red blood cell transfusion

Comparison: Liberal red blood cell transfusion

No of Participants (studies)	**No of Participants** **Restrictive**	**No of Participants** **liberal**	**Relative Risk (95% CI)**	**Absolute effect**	**Quality of the evidence (GRADE)**	**Quality assessment domains**
All cause mortality, longest follow up, all trials						
8321 (23)	558/4167 (13.4%)	586/4154 (14.1%)	RE: 0.95 (0.81 to 1.11); I^2 = 27%) TSA adjusted 95% CI of a RR of 0.95 is 0.74 to 1.21	7 fewer per 1000 (from 27 fewer to 16 more)	⊕⊖⊖⊖ **very low** Critical importance	Risk of bias: Very serious[1] Inconsistency: No serious[2] Indirectness: No serious Imprecision: Serious[3] Reporting bias: Reporting bias
Overall morbidity - all trials						
5975 (12)	1070/2982 (35.9%)	1084/2993 (36.2%)	RR: 1.06 (0.93 to 1.21); I^2 = 58%	22 more per 1000 (from 25 fewer to 76 more)	⊕⊖⊖⊖ **very low** Critical importance	Risk of bias: Very serious[4] Inconsistency: No serious[5] Indirectness: No serious Imprecision: Serious[6] Reporting bias: Reporting bias[7]
Fatal and non-fatal myocardial infarction, all trials						
6501 (16)	145/3259 (4.4%)	137/3248 (4.2%)	RR: 1.05 (0.82 to 1.36); I^2 = 6%)	2 more per 1000 (from 8 fewer to 15 more)	⊕⊕⊖⊖ **low** Critical importance	Risk of bias: Very serious[8] Inconsistency: No serious[9] Indirectness: No serious Imprecision: Serious[10] Reporting bias: None

1) Overall 8 lower, 10 unclear and 5 high risk of bias trials. Limitations for more than one criterion. No blinded trials. Assessor outcome not important for all cause mortality so only one level downgrade

2) I statistics 27% , p value for heterogeneity p=0.12, overlap of confidence intervals

3) Anticipation of a 15% RRR results in TSA adjusted confidence intervals including more than a 25% relative risk reduction or more than a 25% relative risk increase. However, less than a 15% RRR or

RRI may also be considered clinically relevant and these are apparently not excluded in any analyses

4) Overall 6 lower, 4 unclear and 2 high risk of bias trials. Limitations for more than one criterion. Possible assessment bias as all trials are unblinded

5) I square = 58% and p value for heterogeneity is 0.006

6) Five of 12 trials showing no effect and appreciable harm with restrictive transfusion strategy

7) According to funnel plot a possibility for publication bias regarding smaller trials showing benefit for restrictive transfusion strategy is apparent

8) Overall 5 lower, 5 unclear and 5 high risk of bias trials

9) I square = 11% and p value for heterogeneity is 0.33

10) 10 trials showing no effect and appreciable benefit or harm with restrictive transfusion strategy

CI: **Confidence interval;** RR: **Risk Ratio;** RE**: Random effects model**

GRADE Working Group grades of evidence
High quality: **Further research is very unlikely to change our confidence in the estimate of effect.**
Moderate quality: **Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.**
Low quality: **Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.**
Very low quality: **We are very uncertain about the estimate.**

Table 5 Subgroup analyses of mortality

Subgroups*	Number of trials	Restrictive events/total	Liberal events/total	Effect Estimate Relative Risk (RR), Confidence Interval (CI)	Heterogeneity (test[II], I^2)
By follow-up time					p=0.85[§]
≤90 days	18	479/3830	503/3813	RR 0.96, 95% CI 0.80 to 1.15	p=0.15, I^2=26%
>90 days	5	82/337	88/341	RR 0.93, 95% CI 0.70 to 1.23	p=0.32, I^2=16%
By leuko-reduction					p=0.53[§]
Reduced	12	367/2993	404/2985	RR 0.85, 95% CI 0.70 to 1.03	p=0.23, I^2=22%
Non-reduced	3	123/698	134/708	RR 0.94, 95% CI 0.75 to 1.17	p=0.36, I^2=2%
By patient age					p=0.87[§]
< 18 years	2	14/350	15/347	RR 0.94, 95% CI 0.46 to 1.90	p=0.51, I^2=0%
≥ 18 years	15	412/3087	424/3078	RR 1.00, 95% CI 0,78 to 1.29	p=0.02, I^2=48%
By surgical setting					p=0.65[§]
Surgical	14	175/2648	181/2650	RR 1.04, 95% CI 0.74 to 1.46	p=0.07, I^2=39%
Medical	7	153/918	165/907	RR 0.94, 95% CI 0.70 to 1.26	p=0.22, I^2=28%
By clinical setting‡					p=0.74[§]
Trauma	2	3/46	2/34	RR 0.19 (0.05 to15.82)	p=0.91, I^2=50%
Perioperative	13	175/2622	179/2626	RR 1.06 (0.76 to1.49)	p=0.73, I^2=41%
Critical care	8	380/1499	405/1494	RR 0.92 (0.80 to1.06)	p=0.36, I^2=10%

Legend for table:

* Predefined subgroup analyses
‡ Post hoc subgroup analysis
§ Test for subgroup differences
II Test for heterogeneity

Figures 1-12 - paper III

Figure 1 PRISMA flow diagram of records through review

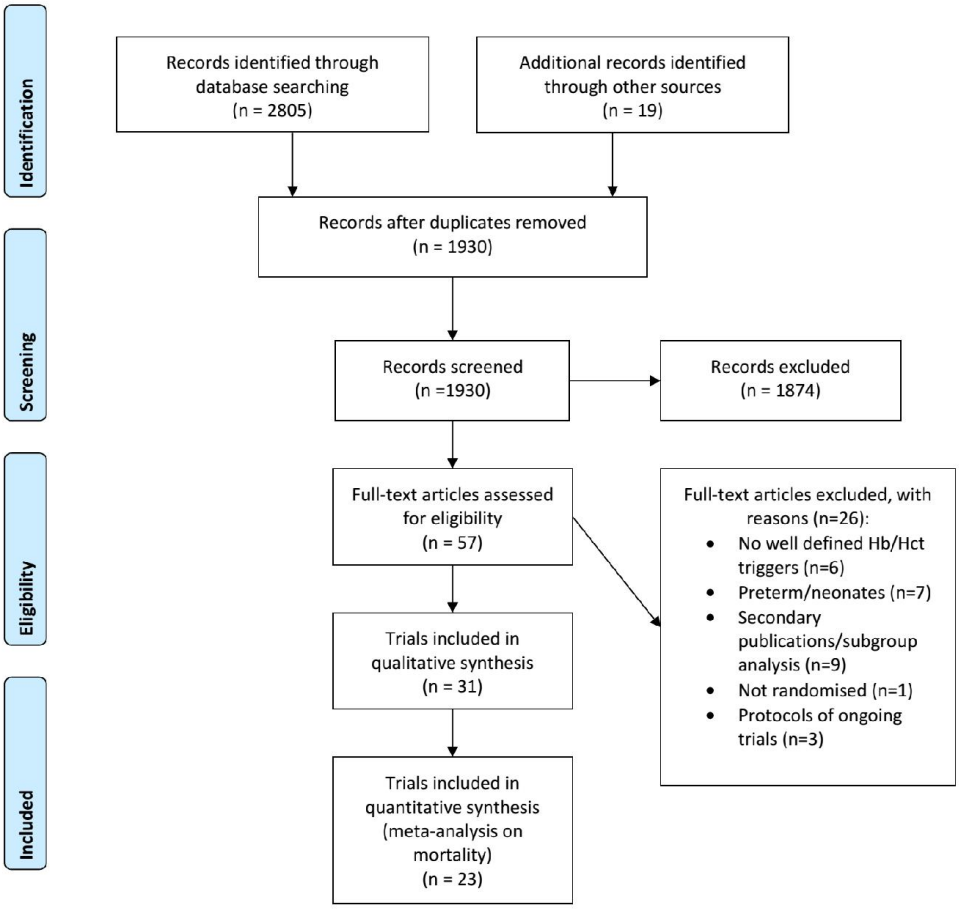


Figure 2 Risk of bias summary for all included records

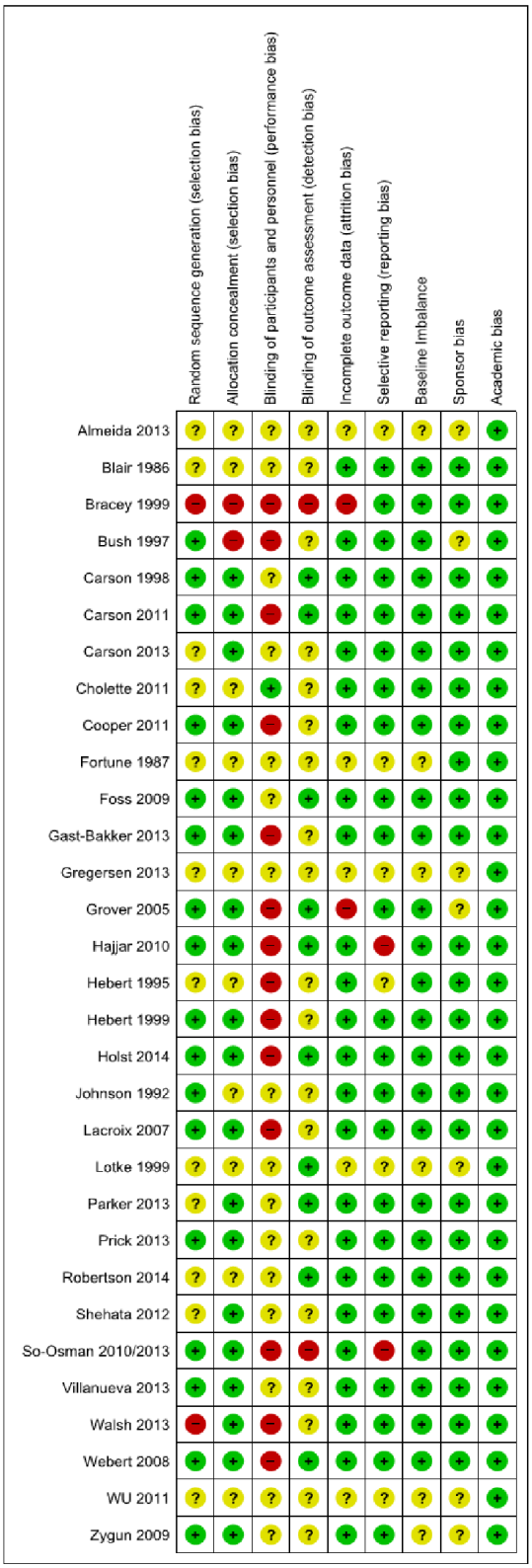


Figure 3 Risk of bias graph

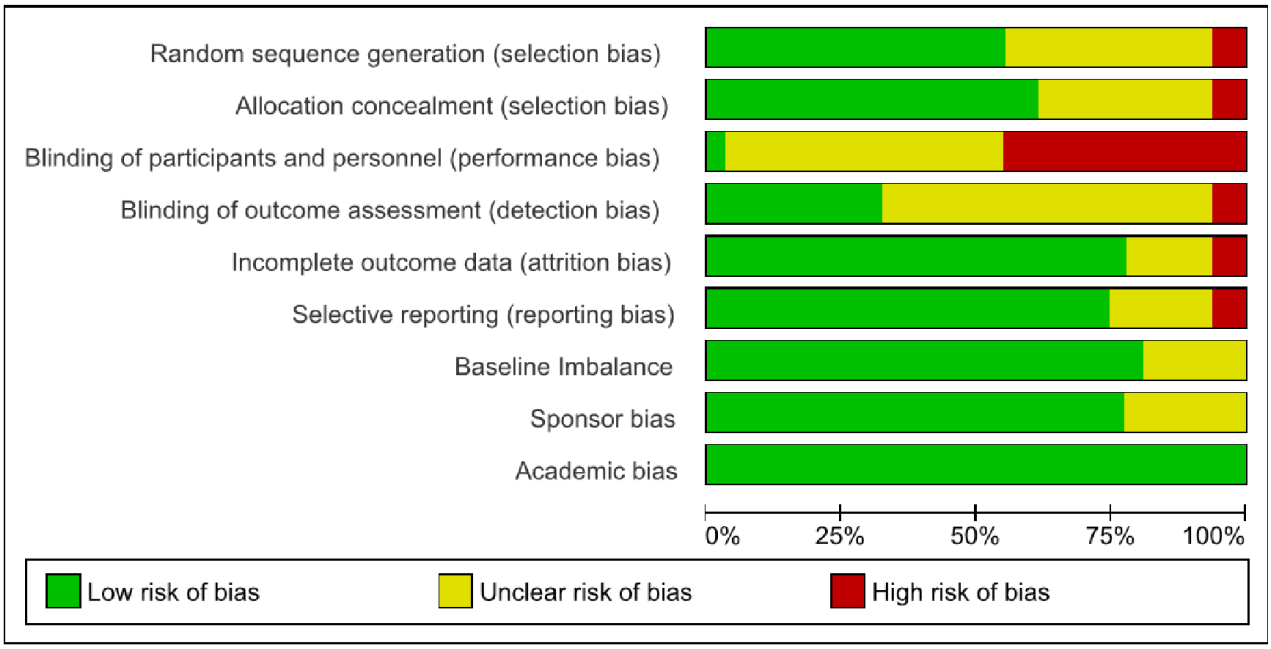


Figure 4 Forest plot of mortality in lower risk of bias trials. Size of squares for risk ratio reflects weight of trial in pooled analysis. Horizontal bars represent 95% confidence intervals

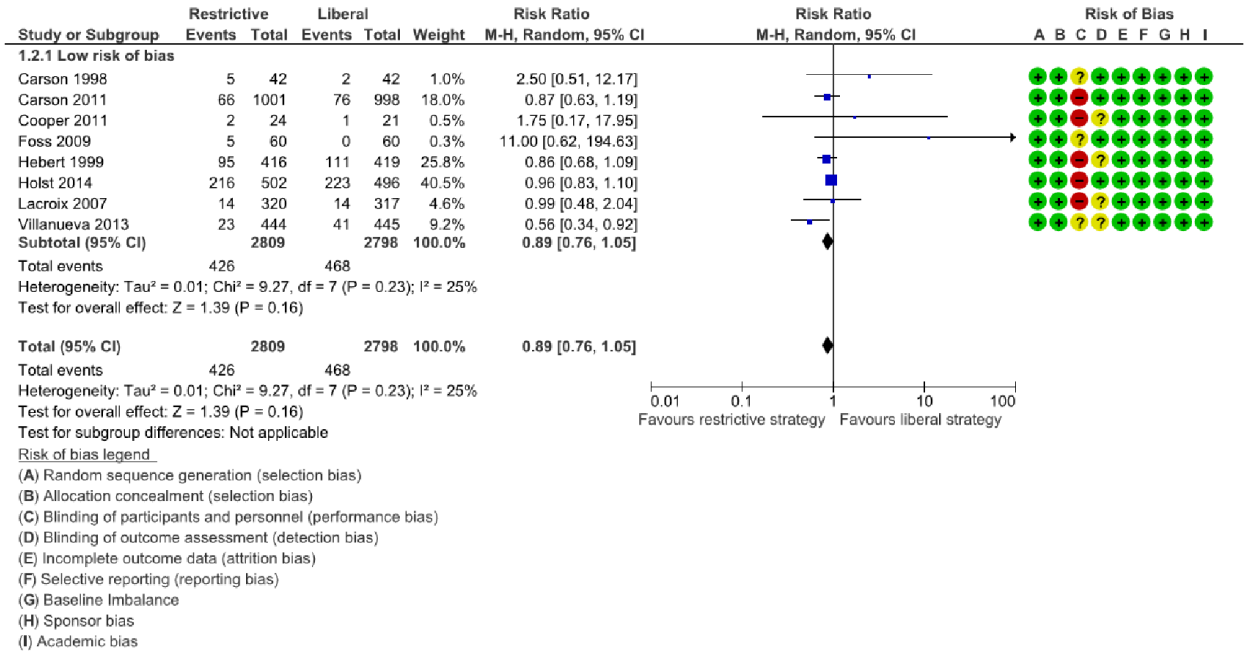
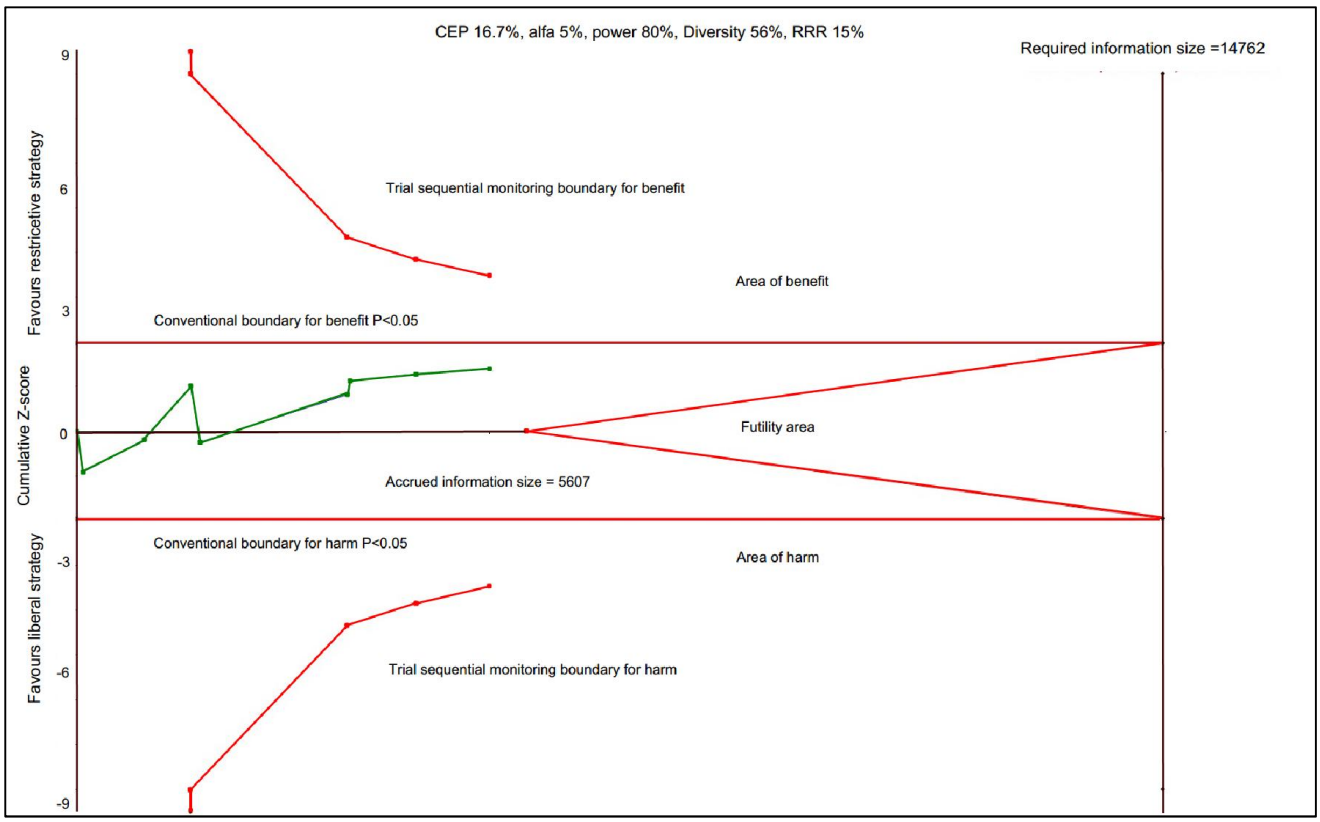
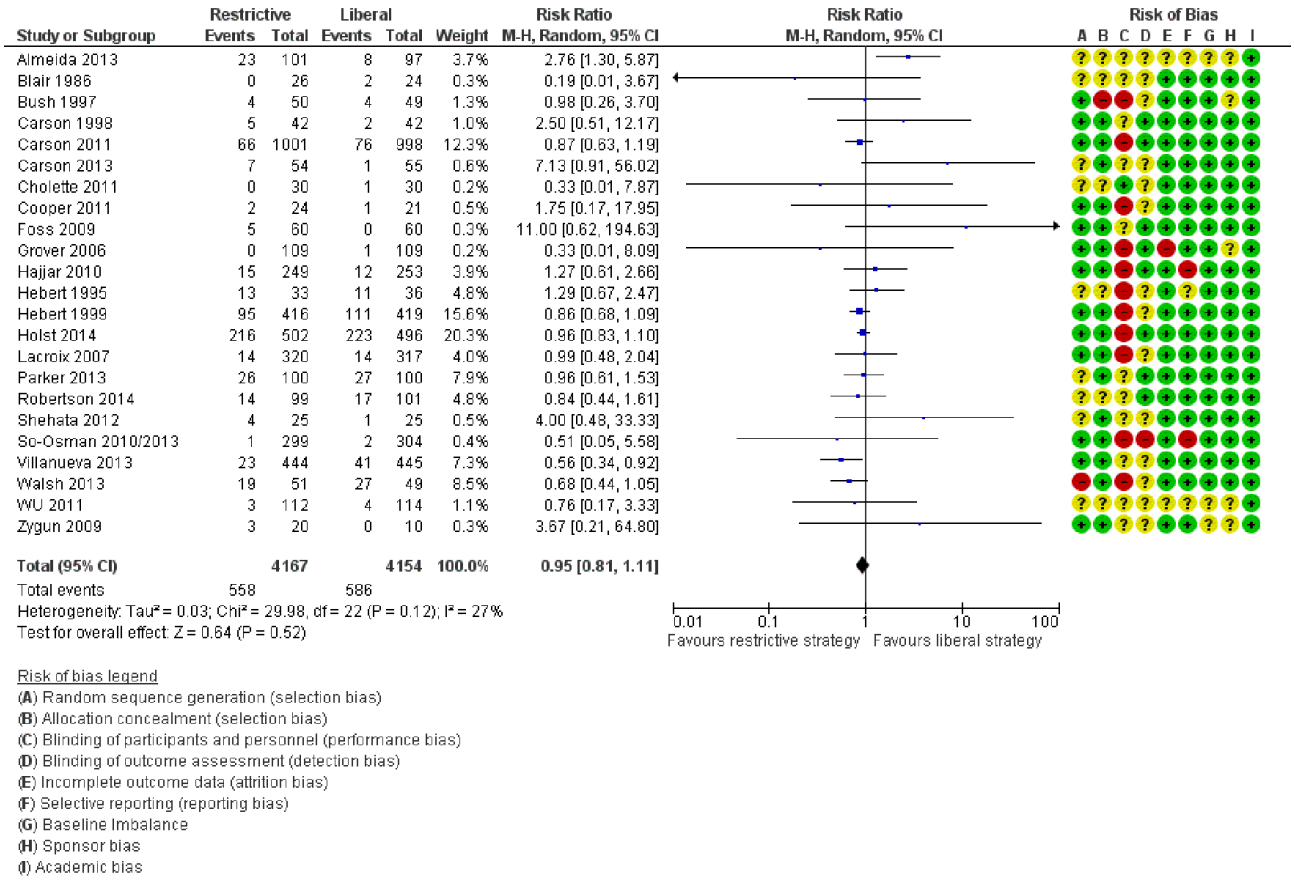


Figure 5 TSA of trials with lower risk of bias reporting data on mortality



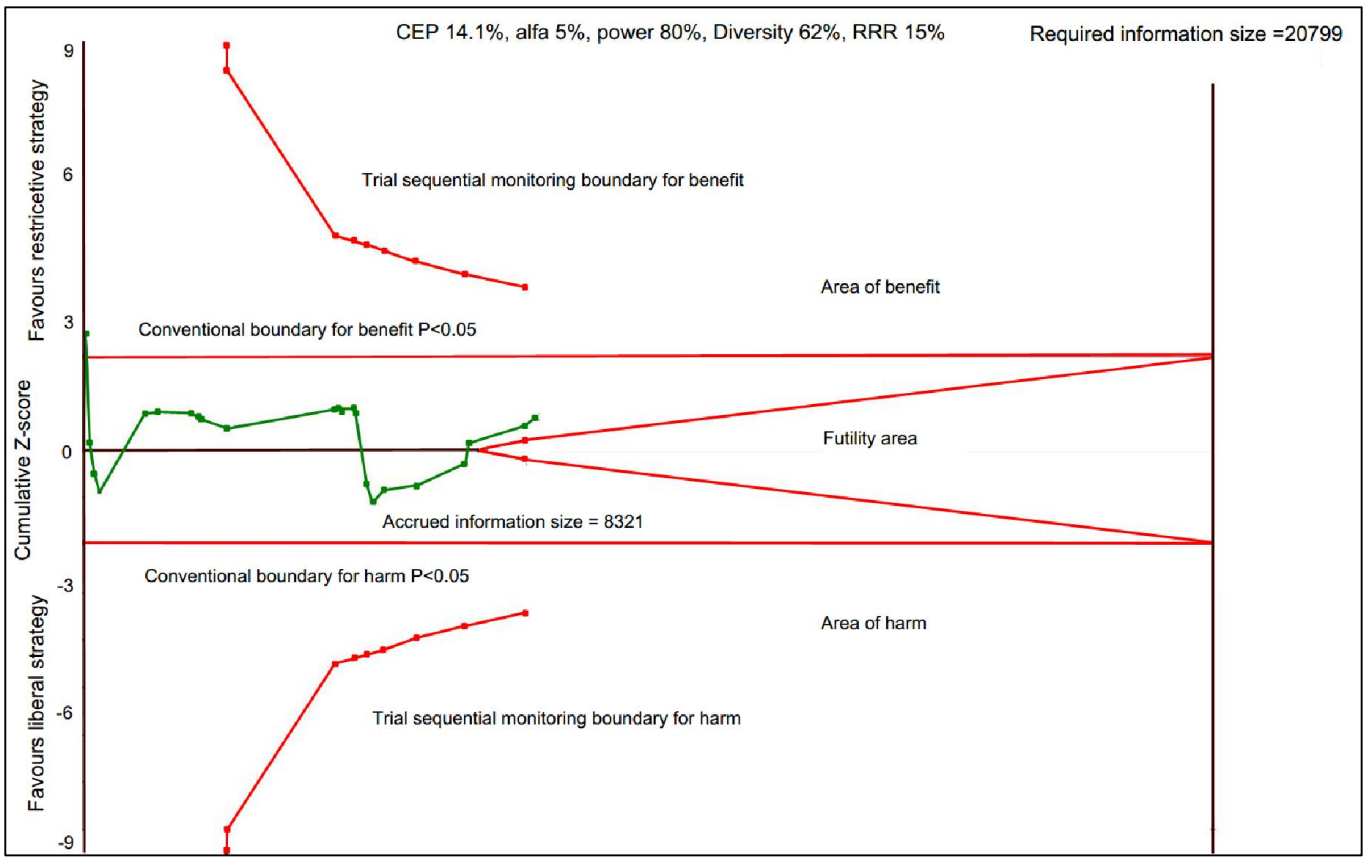
TSA of 8 trials with lower risk of bias reporting all cause mortality, control event proportion of 16.7%, a Diversity of 56%, alfa of 5%, power of 80%, and a RRR of 15%. The required information size of 14,762 has not been reached and none of the boundaries for benefit, harm, or futility has been crossed leaving the meta-analysis inconclusive of a 15% relative risk reduction. The TSA adjusted 95% CI of a RR of 0.89 is 0.68 to 1.17

Figure 6 Forest plot of mortality despite risk of bias. Size of squares for risk ratio reflects weight of trial in pooled analysis. Horizontal bars represent 95% confidence intervals



Risk of bias legend
(A) Random sequence generation (selection bias)
(B) Allocation concealment (selection bias)
(C) Blinding of participants and personnel (performance bias)
(D) Blinding of outcome assessment (detection bias)
(E) Incomplete outcome data (attrition bias)
(F) Selective reporting (reporting bias)
(G) Baseline Imbalance
(H) Sponsor bias
(I) Academic bias

Figure 7 TSA of all trials reporting data on mortality



TSA of 23 trials (despite risk of bias) reporting all cause mortality, control event proportion of 13.7%, a Diversity of 62%, alfa of 5%, power of 80%, and a RRR of 15%. The required information size of 20,799 is far from being reached and none of the boundaries for benefit, harm, or futility has been crossed leaving the meta-analysis inconclusive of a 15% relative risk reduction. The TSA adjusted 95% CI of a RR of 0.95 is 0.74 to 1.21

Figure 8 Forest plot of mortality in trials stratified by clinical setting. Size of squares for risk ratio reflects weight of trial in pooled analysis. Horizontal bars represent 95% confidence intervals

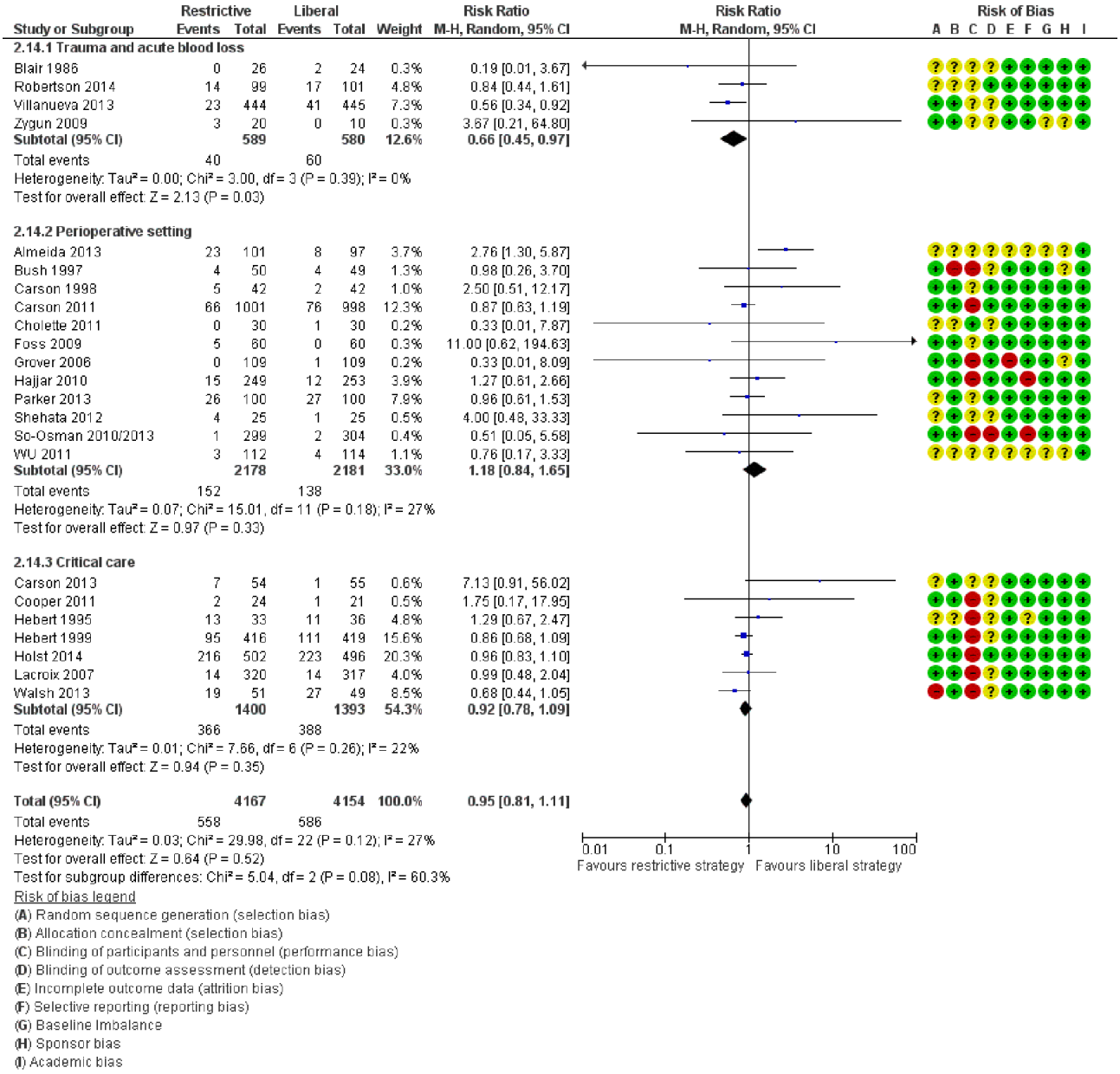


Figure 9 Forest plot of overall morbidity in low risk of bias trials. Size of squares for risk ratio reflects weight of trial in pooled analysis. Horizontal bars represent 95% confidence intervals

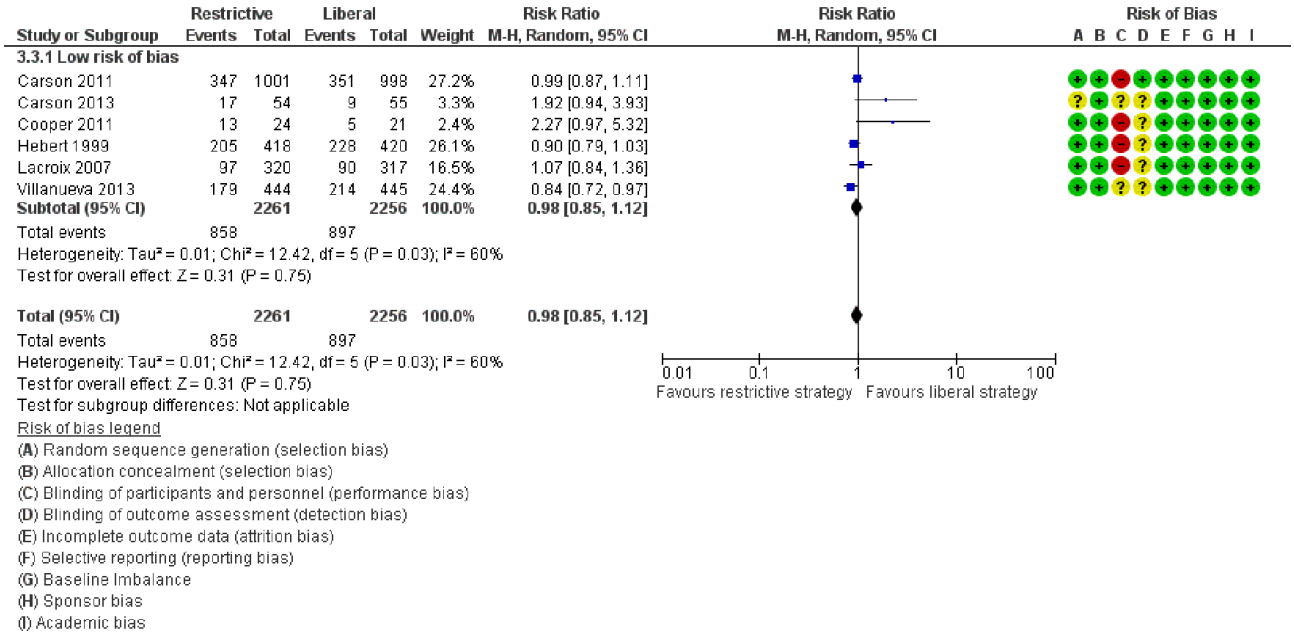
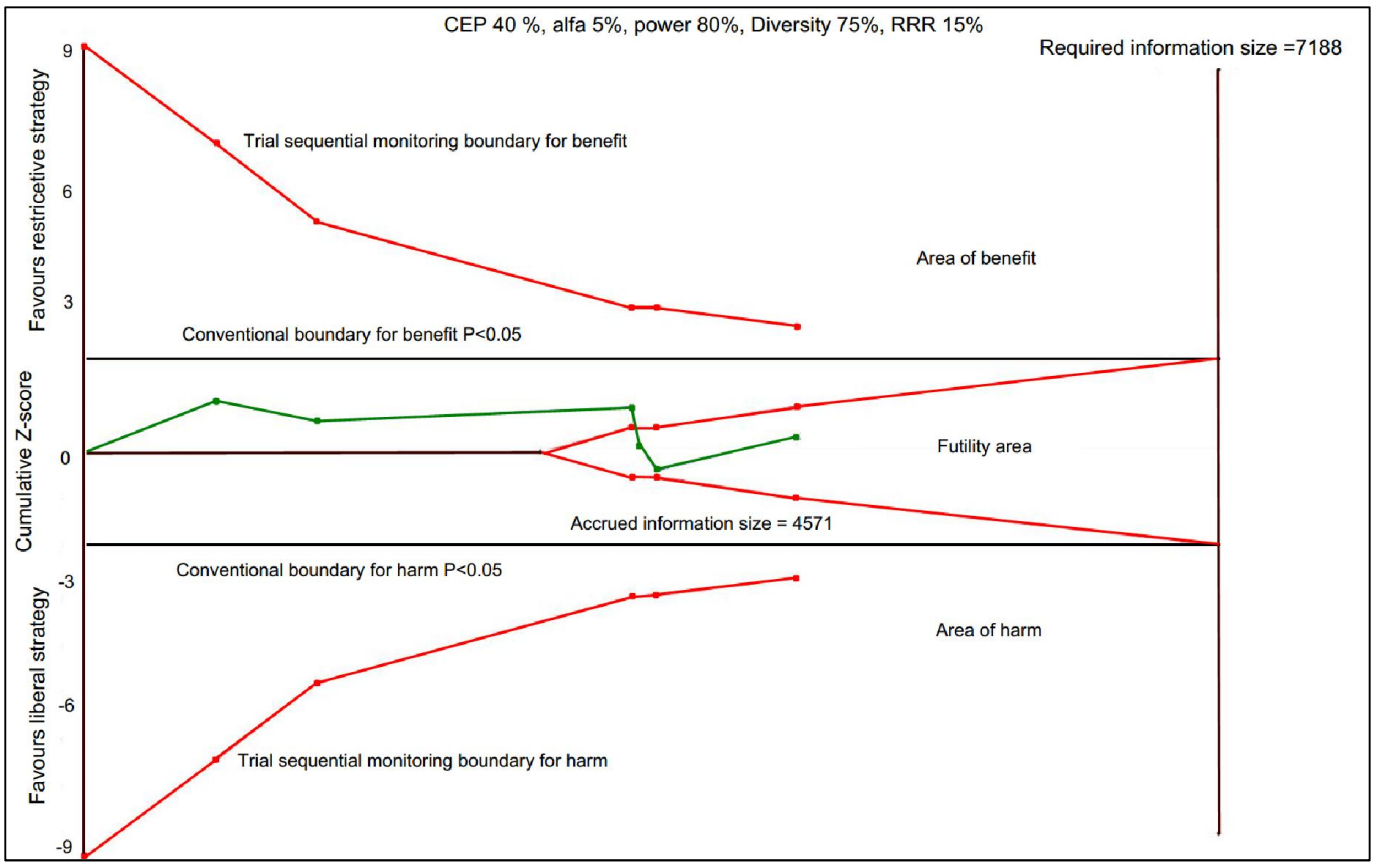


Figure 10 TSA of trials with lower risk of bias reporting data on overall morbidity



TSA of 6 trials reporting overall morbidity, a control event proportion of 40%, a Diversity of 75%, an alpha of 5%, a power of 80%, and a RRR of 15%. The required information size of 7,188 is not reached but the boundaries for futility of showing a 15% RRR has been crossed leaving out the possibility of a 15% relative risk reduction. The TSA adjusted 95% CI for a RR of 0.98 is 0.81 to 1.19

Figure 11 Forest plot of myocardial infarctions in low risk of bias trials. Size of squares for risk ratio reflects weight of trial in pooled analysis. Horizontal bars represent 95% confidence intervals

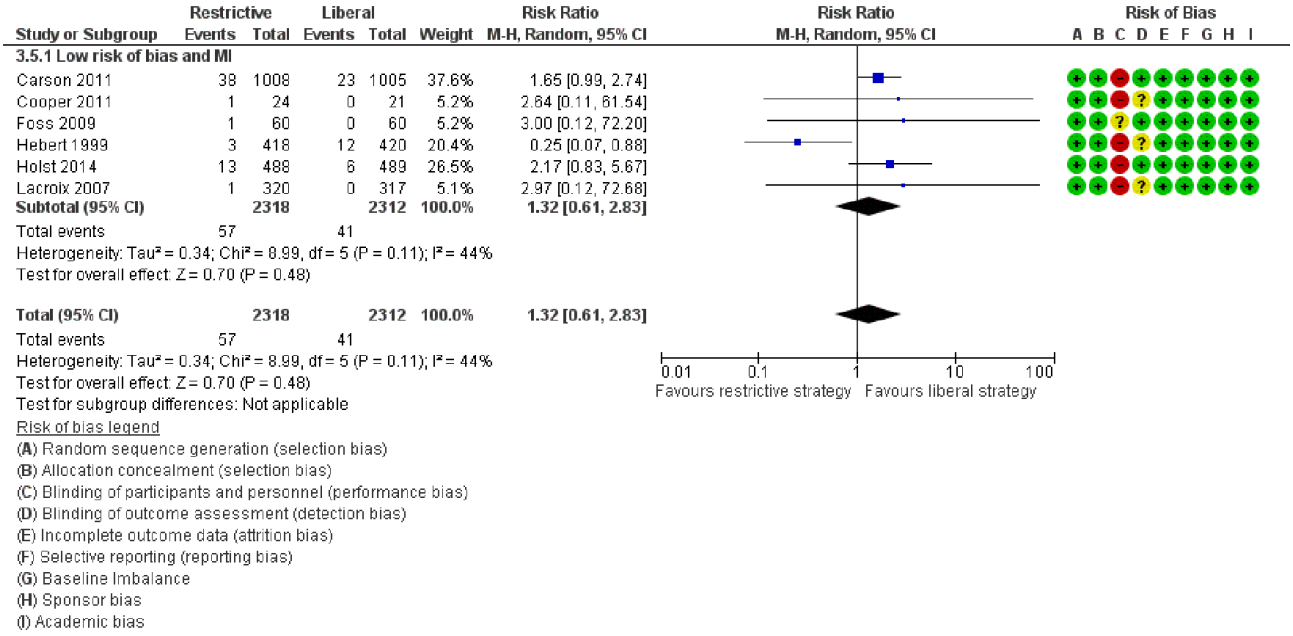
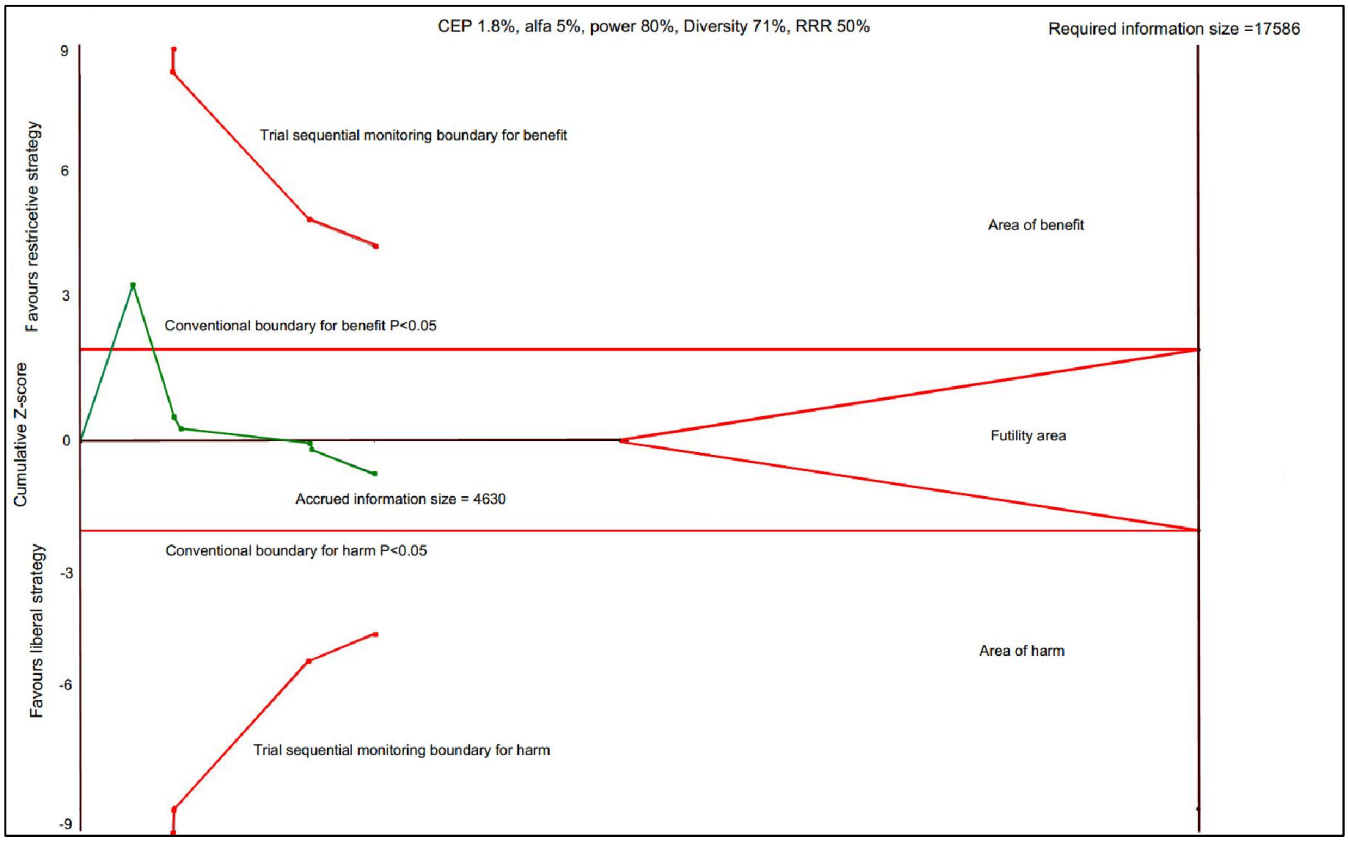


Figure 12 TSA of trials with lower risk of bias reporting data on myocardial infarction



TSA of 6 trials reporting myocardial infarction with a control event proportion (CEP) of 1.8%, a Diversity of 78%, an alpha of 5%, a power of 80%, and a RRR of 50%. The diversity adjusted required information size of 17,586 is far from being reached and none of the boundaries for benefit, harm, or futility has been crossed leaving the meta-analysis inconclusive of even a 50% relative risk reduction. The TSA adjusted 95% CI of the RR of 1.32 is 0.28 to 6.21

Supplementary appendix 1-6 - paper III

Appendix 1:

Search strategies for
Transfusion thresholds and other strategies for guiding allogeneic red blood cell transfusion
Updated searches from 2011 to October 1st 2014

Cochrane Central Register of Controlled Trials (CENTRAL)(Issue 8 of 12, 2014) in *The Cochrane Library* (148 hits in CENTRAL)

#1 MeSH descriptor: [Blood Transfusion] explode all trees and with qualifiers: [Methods - MT, Standards - ST]
#2 transfus* near/5 (polic*or practic* or protocol* or trigger* or threshold*or indicator* or strateg* or criteri* or standard* or requir*)
#3 (red blood cell* or RBC) near/5 (polic*or practic* or protocol* or trigger* or threshold*or indicator* or strateg* or criteri* or standard*) and (therap* or transfus*)
#4 (h?emoglobin or h?emocrit or HB or HCT) near/5 (polic*or practic* or protocol* or trigger* or threshold*or indicator* or strateg* or criteri* or standard*)
#5 transfus* near/5 (restrict* or liberal*)
#6 (blood transfus*) near/3 (management or program*)
#7 (#1 or #2 or #3 or #4 or #5 or #6) from 2011 to 2014

MEDLINE (Ovid SP)(2011 to September 2014)(605 hits)

1. exp Blood Transfusion/
2. ((Red blood cell* or RBC) adj3 (therap* or transfus*)).mp. [mp=protocol supplementary concept, rare disease supplementary concept, title, original title, abstract, name of substance word, subject heading word, unique identifier]
3. 1 or 2
4. exp Reference Standards/
5. standards.fs.
6. methods.fs.
7. 4 or 5 or 6
8. 3 and 7

9. (transfus* adj5 (polic*or practic* or protocol* or trigger* or threshold*or indicator* or strateg* or criteri* or standard* or requir*)).mp. [mp=protocol supplementary concept, rare disease supplementary concept, title, original title, abstract, name of substance word, subject heading word, unique identifier]
10. ((red blood cell* or RBC) adj5 (polic*or practic* or protocol* or trigger* or threshold*or indicator* or strateg* or criteri* or standard*)).mp. [mp=protocol supplementary concept, rare disease supplementary concept, title, original title, abstract, name of substance word, subject heading word, unique identifier]
11. ((h?emoglobin or h?emocrit or HB or HCT) adj5 (polic*or practic* or protocol* or trigger* or threshold*or indicator* or strateg* or criteri* or standard*)).mp. [mp=protocol supplementary concept, rare disease supplementary concept, title, original title, abstract, name of substance word, subject heading word, unique identifier]
12. (transfus* adj5 (restrict* or liberal*)).mp. [mp=protocol supplementary concept, rare disease supplementary concept, title, original title, abstract, name of substance word, subject heading word, unique identifier]
13. ((blood or transfus*) adj3 (management or program*)).mp. [mp=protocol supplementary concept, rare disease supplementary concept, title, original title, abstract, name of substance word, subject heading word, unique identifier]
14. 8 or 9 or 10 or 11 or 12 or 13
15. randomized controlled trial.pt.
16. controlled clinical trial.pt.
17. clinical trials as topic.sh.
18. random*.ab,ti.
19. trial.ti.
20. placebo.ab.
21. 15 or 16 or 17 or 18 or 19 or 20
22. (animals not (humans and animals)).sh.
23. 21 not 22
24. 14 and 23
25. limit 24 to yr="2011 -Current"

EMBASE (Ovid SP)(2011 to September 2014)(1075 hits)

1. exp blood transfusion/
2. ((Red blood cell* or RBC) adj3 (therap* or transfus*)).mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer]

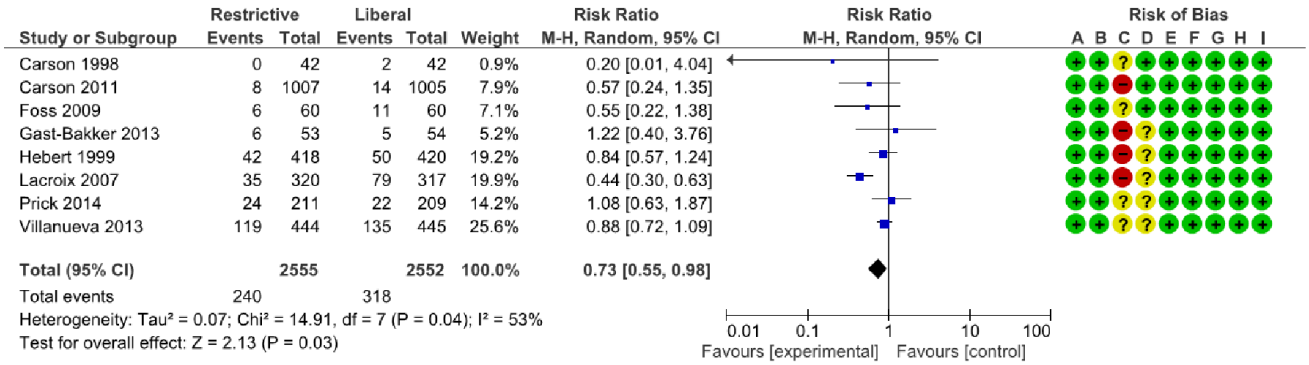
3. 1 or 2
4. exp STANDARD/
5. 3 and 4
6. (transfus* adj5 (polic*or practic* or protocol* or trigger* or threshold*or indicator* or strateg* or criteri* or standard* or requir*)).mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer]
7. ((red blood cell* or RBC) adj5 (polic*or practic* or protocol* or trigger* or threshold*or indicator* or strateg* or criteri* or standard*)).mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer]
8. ((h?emoglobin or h?emocrit or HB or HCT) adj5 (polic*or practic* or protocol* or trigger* or threshold*or indicator* or strateg* or criteri* or standard*)).mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer]
9. (transfus* adj5 (restrict* or liberal*)).mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer]
10. ((blood or transfus*) adj3 (management or program*)).mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer]
11. 5 or 6 or 7 or 8 or 9 or 10
12. exp controlled clinical trial/
13. random*.ab,ti.
14. trial.ti.
15. placebo.ab.
16. 12 or 13 or 14 or 15
17. exp animal/ not (exp human/ and exp animal/)
18. 16 not 17
19. 11 and 18
20. limit 19 to yr="2011 -Current"

Science Citation Index Expanded (SCI-EXPANDED)(2011 to Septembers 2014)(155 hits)

#7 121 #6 AND #1
#6 22,416 #5 AND #4
#5 282,080 TS=(human*)
#4 216,088 #3 OR #2
#3 26,919 TS=((singl* OR doubl* OR trebl* OR tripl*) SAME (blind* OR mask*))
#2 210,011 TS=(random* OR ((controlled OR clinical) AND trial) OR placebo)

#1 3,259 TS=((blood OR (red blood cell*) OR RBC OR hemoglobin* OR haemoglobin* OR haemocrit OR hemocrit OR HB OR HCT) AND transfus* AND (polic* OR practice OR protocol* OR trigger* OR threshold* OR indicator* OR strateg* OR criteri* OR standard* OR restrict* OR liberal* OR management OR program*))

Appendix 2 Forest plot of infectious complications in lower risk of bias trials. Size of squares for risk ratio reflects weight of trial in pooled analysis. Horizontal bars represent 95% confidence intervals



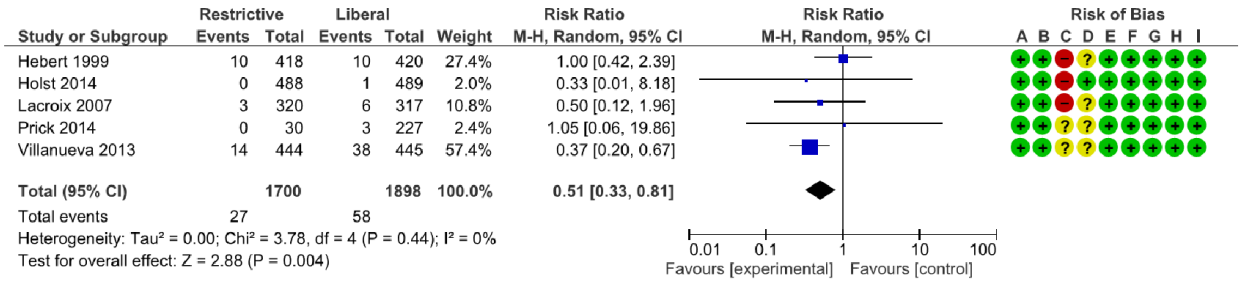
Risk of bias legend

(**A**) Random sequence generation (selection bias)
(**B**) Allocation concealment (selection bias)
(**C**) Blinding of participants and personnel (performance bias)
(**D**) Blinding of outcome assessment (detection bias)
(**E**) Incomplete outcome data (attrition bias)
(**F**) Selective reporting (reporting bias)
(**G**) Baseline Imbalance
(**H**) Sponsor bias
(**I**) Academic bias

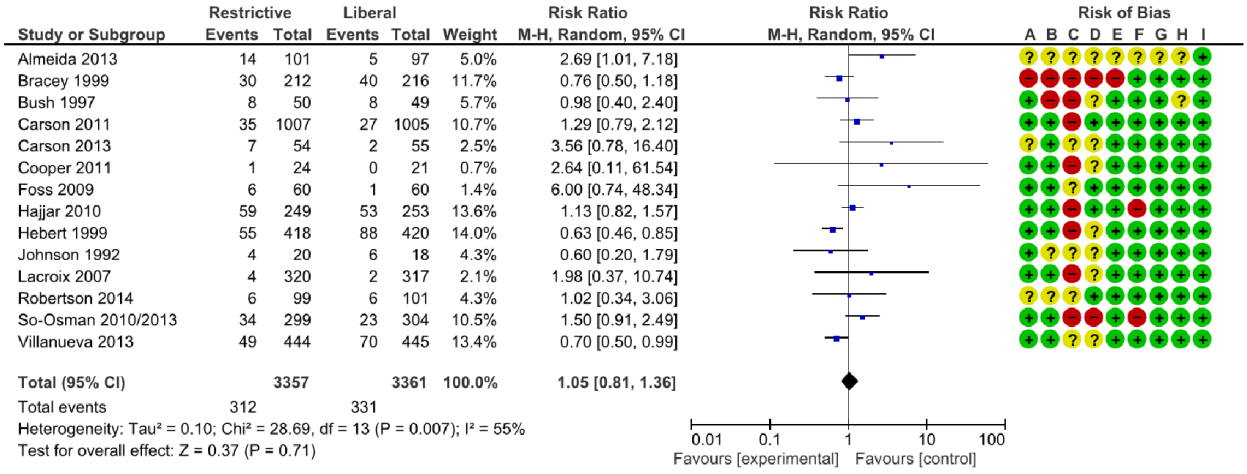
Appendix 3: Other adverse events, all trials

Forest plots of number of other adverse events (transfusion reactions, cardiac complications, renal failure, infectious disease, thromboembolic, events, stroke/TCI, haemorrhagic events). Size of squares for risk ratio reflects weight of trial in pooled analysis. Horizontal bars represent 95% confidence

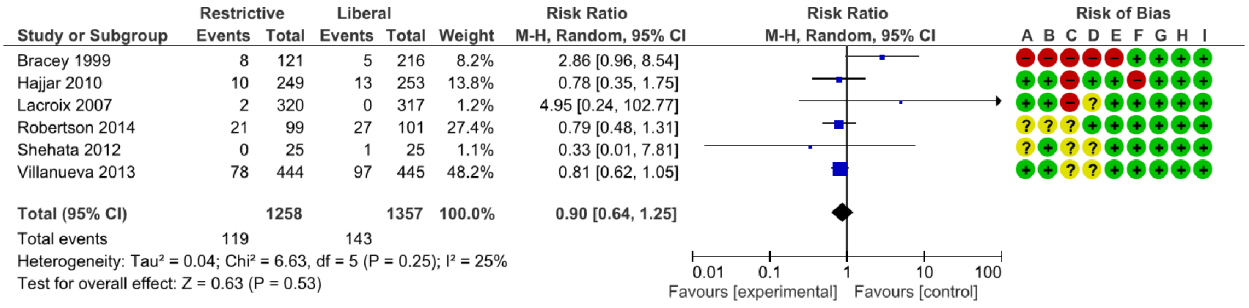
Transfusion reactions



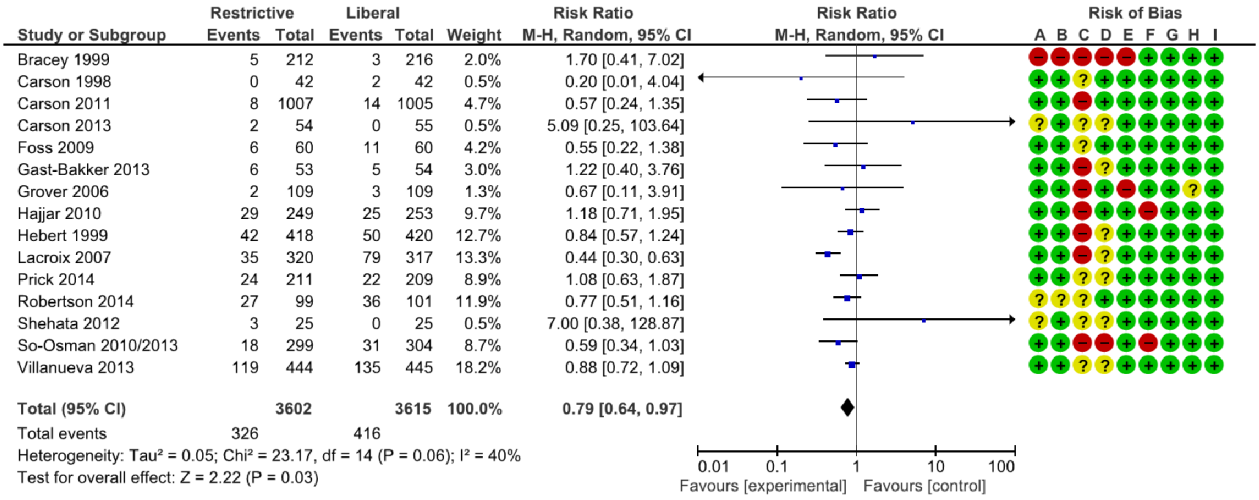
Cardiac complications (not including fatal and non-fatal myocardial infarction)



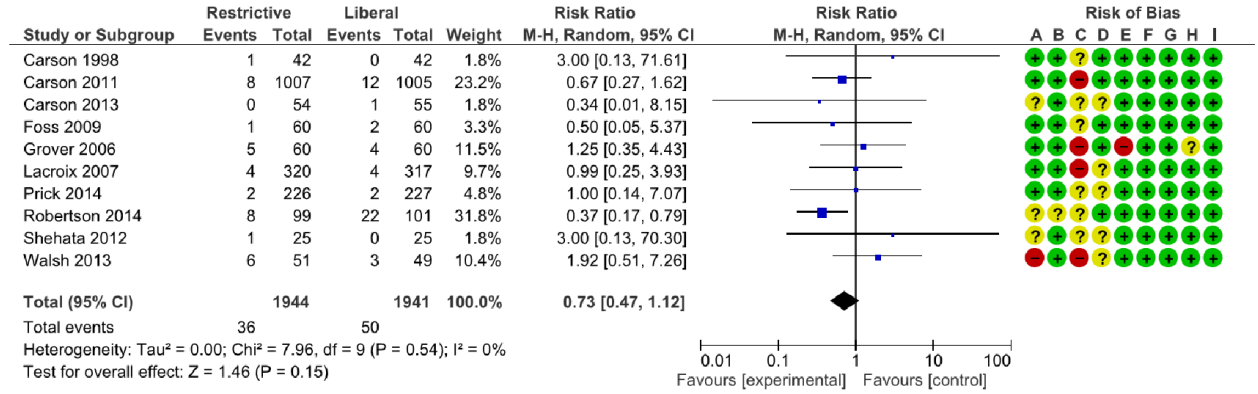
Renal failure



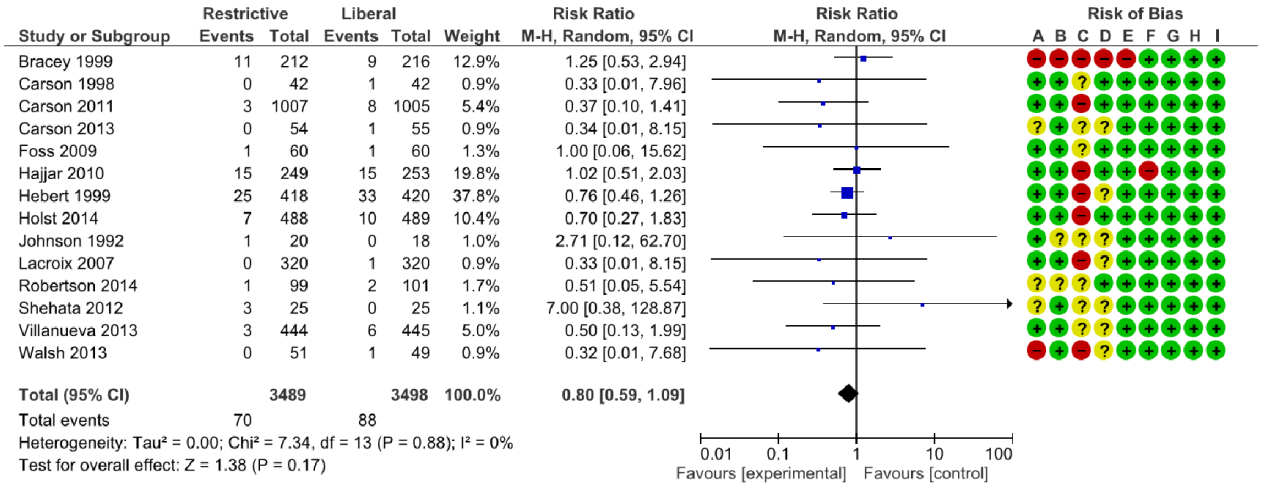
Infectious Complications



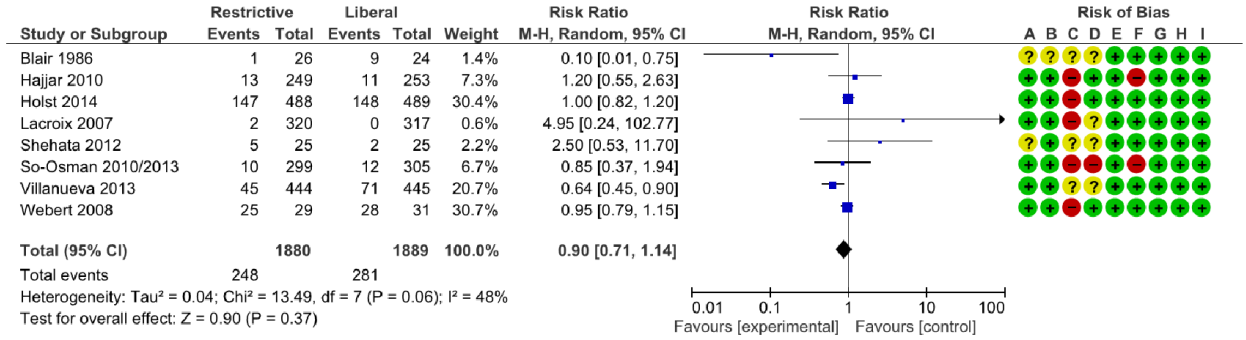
Thromboembolic events



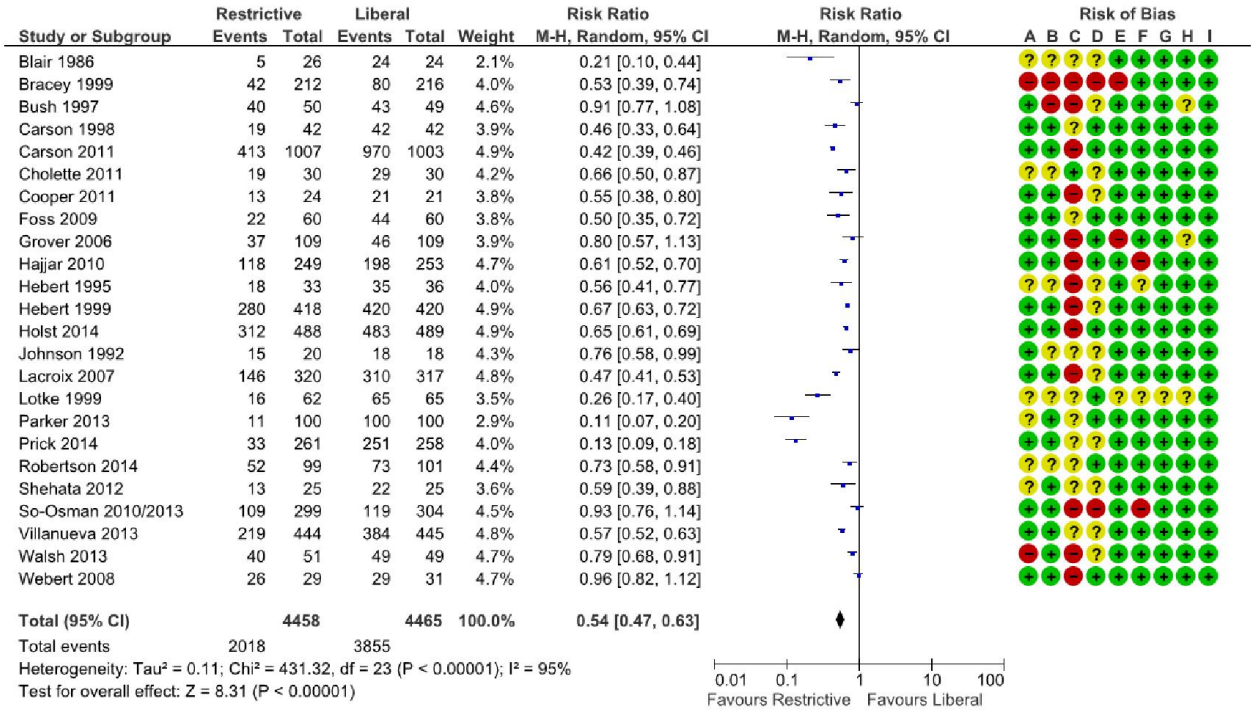
Stroke or Transitory cerebral ischemia



Haemorrhagic events



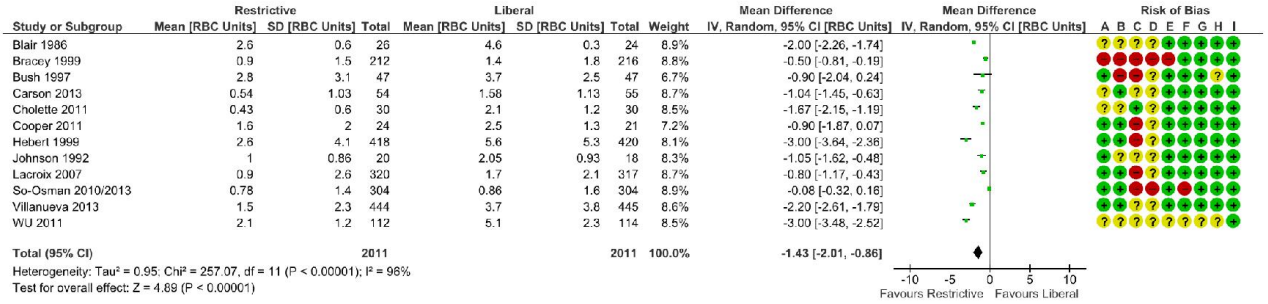
Appendix 5 Forest plot of proportion of patients at risk transfused. Size of squares for risk ratio reflects witght of trial in pooled analysis. Horizontal bars represent 95% confidence interval



Risk of bias legend

(**A**) Random sequence generation (selection bias)
(**B**) Allocation concealment (selection bias)
(**C**) Blinding of participants and personnel (performance bias)
(**D**) Blinding of outcome assessment (detection bias)
(**E**) Incomplete outcome data (attrition bias)
(**F**) Selective reporting (reporting bias)
(**G**) Baseline Imbalance
(**H**) Sponsor bias
(**I**) Academic bias

Appendix 6 Forest plot of number of red blood cell units transfused. Size of squares for risk ratio reflects witght of trial in pooled analysis. Horizontal bars represent 95% confidence intervals



Risk of bias legend
(**A**) Random sequence generation (selection bias)
(**B**) Allocation concealment (selection bias)
(**C**) Blinding of participants and personnel (performance bias)
(**D**) Blinding of outcome assessment (detection bias)
(**E**) Incomplete outcome data (attrition bias)
(**F**) Selective reporting (reporting bias)
(**G**) Baseline imbalance
(**H**) Sponsor bias
(**I**) Academic bias

Appendix 4 Other adverse events.

Trial ID*	Mortality	Morbidity Overall	Transfusion reactions	Myocardial infarction	Cardiac complications	Renal failure	Infectious complica-tions	Thromboembolic complica-trions	Stroke/ TCI	Haemor-rhagic events	Other adverse outcomes
Almeida 2013	60 day, R: 23/101, L: 8/97	NA	NA	NA	Cardiac complications, R: 14/101, L: 5/97	NA	NA	NA	NA	NA	NA
Blair 1986	Unspecified follow up, R: 0/26, L: 2/24	NA	NA	NA	NA	NA	NA	NA	NA	Re-bleeding, R:1/26, L: 9/24	NA
Bracey 1999	During hospitalization for surgery (reported for included and excluded patients), R: 3/215, L: 6/222	NA	NA	MI, R: 1/212, L: 0/216	Atrial arrhythmia, R: 30/212, L: 40/216; Ventricular arrhythmia, R: 13/212, L: 9/216	Renal failure, R: 8/212, L: 5/216	Infection, R: 5/212, L: 3/216	NA	Neurolo-gic event[§], R: 11/212, L: 9/216	NA	Pulmonary complications[§§] R: 57/212, L: 64/216
Bush 1997	30 day, R: 4/50, L: 4/49	NA	NA	Intraoperative MI, R: 1/50, L: 3/49	Q waves on ECG, arrhythmia or MI, R: 8/50, L: 8/49	NA	NA	NA	NA	NA	NA
Carson 1998	60 day, R: 5/42, L: 2/42	NA	NA	MI, R: 0/42, L: 0/42	NA	NA	Pneumonia, R: 0/42, L: 2/42	Thrombo-embolism, R: 1/42, L: 0/42	Stroke, R: 0/42, L: 1/42	NA	Death or inability to walk 10 ft. without assistance, R: 16/42, L:19/42
Carson 2011	60 day all cause, R: 66/1001, L: 76/998	Death and inability to walk 10 ft. independently at day 60, R: 347/1001, L: 351/998	NA	MI, R: 38/1008, L: 23/1005	Congestive heart disease, R: 35/1007, L: 27/1005	NA	Wound infection, R: 8/1007, L: 14/1005	DVT or pumonary embolism, R: 8/1007, L: 12/1005	Stroke or TCI diagnosed by physcician , CT or MRI, R: 3/1007, L: 8/1005	NA	
Carson 2013	30 day, R: 7/54, L: 1/55	Death/MI/unscheduled revascularisation/unschedule	NA	MI, R: 7/54, L: 5/55	Congestive heart failure R: 7/54, L: 2/55	NA	Pneumonia R: 2/54, L: 0/55	Thromboembolic events, R: 0/54, L: 1/55	Stroke, R: 0/54, L: 1/55	NA	Unscheduled hospital admission, R:17/54, L: 9/55;

		d cardiac admission, R: 17/54, L: 9/55			Unscheduled coronary revascularisation, R: 2/54, L: 0/55						
Cooper 2011	30 day, R: 2/24, L: 1/21	Death/recurrent MI, or new/worsening HF, or recurrent ischemia, R: 13/24, L: 5/21	NA	Recurrent in-hospital ischemia, R: 1/24, L: 0/21	NA	NA	NA	NA	NA	NA	In-hospital death, recurrent MI, or new or worsening HF at day 30, R: 5/24, L: 13/21
Gast-Bakker 2013	NA	Overall complications in ICU, R: 15/53, L: 11/54	NA	NA	NA	NA	Respiratory tract infections, R: 6/53, L: 5/54	NA	NA	NA	Cost per patient, R: €316.27, L: €438.45 Mechanical ventilation time (hrs), R: 20 (9-52)** , L: 16 (9-27) LOS PICU (days): R: 2(1-4)** , L: 2 (1-5) LOS Hospital (days): R: 8 (7-11), L: 9 (7-14)
Gregersen 2013	90 day, R: 35%, L: 20%	NA	NA	NA	NA	NA	NA	NA	NA	NA	
Cholette 2011	At discharge, R: 0/30, L: 1/30	NA	NA	NA	NA	NA	NA	NA	NA	NA	Mean arterial lactate levels (mM), R: 1.4 (0.5), L: 1.4 (0.4); Peak arterial lactate levels (mM), R: 3.1 (1.5), L: 3.2 (1.3)

Fortune 1987	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Foss 2009	30 day, R: 5/60, L: 0/60	NA	NA	MI, R: 1/60, L: 0/60	Any cardiovascular event, R: 6/60, L: 1/60; Acute arrythmia, R: 3/60, L: 1/60;	NA	Any infectious complications, R: 6/60, L: 11/60; Sepsis, R: 1/60, L: 1/60	Thromboem-bolic event, R: 1/60, L: 2/60	Cerebro-vascular event, R: 1/60, L: 1/60	NA	Pulmonary edema/congestive heart disease, R: 1/60, L: 1/60;
Grover 2005	3 day (?), R: 0/109, L: 1/109	NA	NA	Silent MI, R: 21/109, L: 26/109; MI, R: 0/109, L: 1/109;	LBBB‡, R: 2/109, L: 1/109; Ventricular tachycardia, R: 2/109, L: 0/109;	NA	Wound infection, R: 2/109, L: 3/109; Chest infection, R: 2/109, L: 3/109	DVT, R: 5/60, L: 4/60; Pulmonary embolism, R: 2/109, L: 1/109;	NA	NA	
Hajjar 2010	30 day, R: 15/249, L:12/253	Any cardiac event4, R: 59/249, L: 53/253	NA	NA	Any cardiac event‡‡, R: 59/249, L: 53/253; Cardiogenic shock, R: 22/249, L: 12/253	AKI requiring dialysis or haemofiltration, R:10/249, L: 13/253	Infectious complications, R: 29/249, L: 25/253	NA	Neurologic complica-tions, R: 15/249, L: 15/253	Bleeding, R: 13/249, L: 11/253	ARDS R: 5/249, L: 2/253
Hebert 1995	120 day, R: 13/33, L: 11/36; 30 day, R: 8/33, L: 9/36	Multiple organ failure (>3 organs), R: 9/33, L: 6/36	NA	NA	NA	NA	NA	NA	NA	NA	Hospital LOS, R: 38 (25-62)**, L: 31 (13-64); Mean organ dysfunction score, R: 9.3 (3.6), L: 10.0 (3.8)
Holst 2014	90 day all cause, R: 216/502, L: 223/496	NA	Acute haemolytic reaction, R: 0/488, L: 1/489	MI, R: 17/488, L: 6/489	NA	Days alive without RRT, R: 85, L: 83	NA	Limb: R:14/488, L: 11/489; Intestinal: R:14/488, L: 14/489	Cerebral ischemia: R: 7/488, L: 10/489	Haemorhagic events, R: 147/488, L: 148/489	Days alive without vasopressors, R: 73, L: 75; Days alive without mechanical ventilation, R: 65,

											L: 67
Hebert 1999	60 day, R: 95/416, L:111/419	Any adverse event, R: 205/418, L: 228/420	Hematological complication[†] R: 10/418, L: 10/420	MI, R: 3/418, L: 12/420	Any cardiac complication[††], R: 55/418, L: 88/420	NA	Infectious complication[¶], R: 42/418, L: 50/420	NA	Cerebro-vascular accidents and encepha-lopathies, R: 25/418, L: 33/420	NA	Gastrointestinal complications[¶¶] R: 13/418, L: 19/420; Pulmonary complications[‖] R: 106/418, L: 50/420; Non-septic shock, R: 205/418, L: 228/420
Johnson 1992	NA	NA	NA	Perioperative MI, R: 0/20, L: 1/18	Arrhythmias, R: 4/20, L: 6/18	NA	NA	NA	Cerebro-vascular accident, R: 1/20, L: 0/18	NA	Pneumothorax, R: 2/20, L: 0/18; Pulmonary edema, R: 0/20, L: 1/18; Postpericardiotomy syndrome, R: 1/18, L: 1/20
Lacroix 2007	28 day, R: 14/320, L: 14/317	Adverse events, R: 97/329, L: 90/317	Reactions to RBC transfusion, R: 3/320, L: 6/317; Transfusion reactions, R: 2/320, L: 0/317	ST-elevation MI, R: 1/320, L: 0/317	Arrhythmia, R: 4/320, L: 2/317	Renal failure, R:2/320, L: 0/317	Nosocomiale infections, R: 65/320, L: 79/317; Sepsis, R: 3/320, L: 2/317	Thrombosis, R: 4/320 L: 4/317	Cerebral infarction, R: 0/320, L: 1/317	Cardio-vascular bleeding, R: 2/320, L: 0/317; Lower GI-bleeding, R: 2/320, L: 0/317; Upper-GI bleeding, R: 2/320, L: 2/317	New or progressive MODS, R: 38/320, L: 39/317; No. of dysfunctional organs, R: 1.6 (1.4), L: 1.5 (1.2); Duration of mechanical ventilation, R: 6.2 (5.9), L: 6.0 (5.4); Fluid overload, R: 1/320, L: 3/317; DIC, R:1/320, L: 0/317; Number of pts with one or more

											serious adverse events, R: 19/320, L: 19/317
Lotke 1999	NA	R: 16/62, L: 5/65	NA	MI, R: 1/62, L: 0/65	Dysarrhythmia, R: 1/62, L: 0/65	NA	NA	NA	NA	NA	Mental confusion, R: 7/62, L: 2/65; Lethargy, R: 4/62, L: 1/65; Orthostatic hypotension, R: 3/62, L: 2/65
Parker 2013	365 day, R: 26/100, L: 27/100; 30 day mortality, R: 5/100, L: 3/100; 90 mortality, R: 11/100, L: 10/100; 120 day mortality, R: 11/100, L: 13/100	NA	NA	NA	NA	NA	NA	NA	NA	NA	Total hospital LOS, R: 23.3, L: 21.8; Mean change in morbility score, R: 2.0, L: 2.4
Prick 2012	NA	NA	R: 0/30, L: 3/227	NA	NA	NA	Total infectious complications, R: 24/211, L: 22/209; Infected surgery wound, R: 1/46, L: 0/41; Infected surgery spisiotomy, R: 6/145, L: 6/137	Thromboembolic event, R: 2/226, L: 2/227	NA	NA	Endometritis, R: 3/225, L: 5/228
Robertson 2014	180 day, R: 14/99, L: 17/101	NA	NA	Acute MI, R: 1/99, L: 1/101	Cardiac arrest, R: 2/99, L: 2/101; Other cardiac complications,	Acute renal failure, R: 21/99,	Infectious complications, R: 27/99, L: 36/101;	Any tromboembolic event, R: 8/99,	Stroke, R: 1/99, L: 2/101	NA	ARDS, R: 16/99, L: 25/101

					R: 6/99, L: 6/101	L: 27/101	Pneumonia, R: 13/99, L: 20/101; Sepsis R: 3/99, L: 5/101	L: 22/101; Pulmonary embolus, R: 1/99, L: 6/101			
Shehata 2012	30 day all cause, R: 4/25, L: 1/25	Composite score‖‖, R: 14/25, L: 8/25	Transfusion reactions, R: 0/25, L: 0/25°	Post operative MI, R:1/25, L: 0/25	NA	Dialysis, R: 0/25, L: 1/25; >50% increase in S-Creatinine, R: 6/25, L: 5/25	Sepsis, R: 3/25, L: 0/25; Pneumonia, R: 4/25, L: 0/25	Pulmonary embolus/DVT, R: 1/25, L: 0/25	Stroke, R: 3/25, L: 0/25	Haemor-rhage, R: 1/25, L: 2/25	Use of inotropes >24 hours, R: 4/25, L: 2/25; MOF, R: 1/25, L: 0/25; Reintubation, R: 4/25, L: 1/25; Intubation >48 hours, R: 5/25, L: 2/25; Hospital LOS >11 days, R: 9/25, L: 5/25
So-Osman 2010	(?), R: 1/299, L: 2/304	Composite complications, R: 99/299, L: 104/304	NA	NA	Cardiovascular complications, R: 34/299, L: 23/304	NA	Infections, R: 18/299 L: 31/304	NA	NA	Haemorrhage, R: 10/299, L: 12/305	Respiratory complications, R: 6/299, L: 15/304; Neuropsychiatric complications, R: 11/299, L: 13/304; Delayed mobilization, R: 22/299, L: 36/304
Villanueva 2013	45 day, R: 23/444, L: 41/445	Any adverse event, R: 179/444, L: 38/445	Fever, TACO or allergic reactons R: 14/444, L: 38/445	NA	ACS, pulmonary edema or arrhythmias, R: 49/444, L: 70/445	AKI, R: 78/444, R: 97/445	Bacterial infections, R: 119/444, L: 135/445	NA	Stroke/ TCI , R: 3/44, L: 6/445	Further bleeding, R: 45/444, L: 71/445	Pulmonary Complications, R: 48/444, L: 53/445
Walsh 2013	180 day, R: 19/51, L: 27/49; 30 day,	NA	NA	ACS, R: 2/52, L: 2/49	NA	NA	NA	Any thrombotic event R: 6/51,	Cerebral infarct, R: 0/51,	NA	ICU LOS, R: 24 (14-40)**, L: 23 (15-33);

	R: 12/51, L: 16/49							L: 3/49; Pulmonary embolus, R: 0/51, L: 0/49; DVT, R: 1/51, L: 0/49; Other thrombotic events, R: 5/51, L: 2/49	L: 1/49		Hospital LOS, R: 34 (13-64)**, L: 31 (19-42); Ventilation-free days at day 60, R: 38 (1-52)**, L: 27 (1-48); Antibiotic free days, R: 47 (36-53)**, L: 48 (41-53); SF-12 physical function score at day 180, R: 30 (24-40)**, L: 31 (24-39); SF-12 mental component score at day 180, R: 51 (31-57)**, L: 39 (34-57); Rivermead Mobility index 189, R: 13 (7-15)**, L: 10 (5-12)
Webert 2008	NA	NA	NA	NA	NA	NA	NA	NA	NA	Clinically significant bleeding, R: 22/29, L: 22/31; Any bleeding event, R: 25/29, L: 28/31	
WU 2011	30 day all cause, R: 3/112, L: 4/114	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Zygun 2009	ICU, R: 3/20, L: 0/10	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Legend for table:

Values are means (standard deviations) unless otherwise specified and blue text coloring denotes outcomes included in meta-analysis. R = restrictive; L = liberal; NA = not available; MI = myocardial infarction; ARDS = acute respiratory distress syndrome; TCI = transient cerebral ischaemia; DVT = deep venous thrombosis; hrs = hours; LOS = lenght of stay; AKI = acute kidney injury; RRT = renal replacement therapy; RBC = red blood cells; MOF = multiple organ failure; ACS = acute coronary syndrome,

** Values are medians (interquartile ranges)

§ Defined as cerebrovascular attack, TCI or paralysis

§§ Defined as pneumothorax, pneumonia, tracheobronchitis, pulmonary edema, purulent sputum, O2 dependence after ICU discharge, and delayed chest tube removal (>48 hours after surgery)

‡ Left bundle branch block

‡‡ Defined as shock, tachyarrhythmia or ischemia

† Defined as transfusion reaction, haemolytic anemia, DIC and other blood dyscrasias

†† Defined as MI, pulmonary edema, angina and cardiac arrest

¶ Defined as bacteraemia, catheter related sepsis and septic shock

¶¶ Defined as bleeding, thrombosis and ischemia

‖ Defined as ARDS and pneumonia

‖‖ Defined as any neurological event, RRT dependent renal failure, >50% creatinine increase, prolonged output state and MI

¤ Zero event trial so not included in meta-analysis on this outcome