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

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# **ORIGINAL PAPERS**

This thesis is based on the following papers:

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

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.

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 metaanalysis and trial sequential analysis. Submitted to BMJ October 7th 2014.

#### 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 transfusions2 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 guidelines9 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 ABO 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 (O2) from the pulmonary to the peripheral capillaries and return carbon dioxide (CO2) 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 (DO2) to the body tissues is defined by21:

Cellular hypoxia develops when oxygen consumption (VO2) in the tissues exceeds DO2 and below this threshold (DO2crit) 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 DO2 is between three and four times greater than global VO2 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 (O2ER) the body

will preserve DO2 above the critical level in otherwise healthy anaemic patients.21,23

#### Tolerance of 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 O2ER 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 DO2crit 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 DO2 and VO2 in different subgroups of critically ill patients, including patients with septic shock, have been difficult to predict as global DO2 increases with RBC transfusion but without a corresponding increase in oxygen consumption VO2.30,31

Red blood cell storage lesion and leukocyte depletion One explanation for the lack of increase in VO2 following increase in DO2 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 2.3diphosphorglycerate (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





19 Higgs 134

5th day of storage

42<sup>nd</sup> day of storage

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 VO2 with DO2 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 DO2.17,38

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).



# **Risks of anaemia**

Low DO<sub>2</sub> Ischemia Organ dysfunction Multiple organ dysfunction Infectious Non-infectious Storage-lesion Immunomodulation Cost

**Risks of transfusion** 

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 guestionable.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 patient 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 (ScvO2), 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 6 g/dl (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 studies3,4 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-trial68 and the SAFE TRIPS2 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 of one randomised trial69 assessing the effects of early goal-directed therapy (EGDT) were adopted by the Surviving Sepsis Campaign70 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 (ScvO2<70%). Trial results showed a significant reduction in mortality (RR 0.58, 95% Cl 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 was 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 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 decreased47,72,73and 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 (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, (paper III) we aimed at comparing our results with those of other randomised trials and subsequently perform an up-to-date systematic review with metaanalysis 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 threshold 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 Trial5 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 a 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 including excluding TACO), presence of acute myocardial ischaemia, lifethreatening 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-ofcare 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 trial68 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	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.Aotal 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 pe	rcent 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 patients in the lower threshold group did not receive transfusions as compared to 6 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 (Cl) 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 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 RE-VIEW 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 collaboration80. A review protocol was prepublished 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 "trigger" or "threshold", described as haemoglobin or haematocrit HCT 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, EM-BASE, 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 trials5,6,65–67,71,83–107 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 leukore-duced 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					
Lowerrisk 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) DE-SIGN

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 setting5 an 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 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 guestioned 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 But 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 population128 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 II134 score and SOFA135 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 RE-VIEW)

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) recommendations82 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 toward lower mortality with the use of restrictive transfusion strategy should be assessed in a larger trial. All together five RCTs5,67,71,97,104 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 trials113,114 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.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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# 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.

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