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FACULTY OF HEALTH AND MEDICAL SCIENCES



# Red blood cell transfusion and long-term outcomes among patients with septic shock

PhD Thesis

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Front cover:

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# 1 Preface

The basis for this PhD thesis is the Transfusion Requirements In Septic Shock trial, which became possible due to the hard work of the TRISS trial group, site investigators, research and clinical staff, participants and their relatives. I am grateful to the trial group, the principal investigator, Professor Anders Perner, and coordinating investigator, Lars Broksø Holst, for entrusting me with the important task of completing the follow-up of the trial.

I have been employed as a research fellow at the Department of Intensive Care, 4131, Rigshospitalet, during the work of the studies in the thesis, from January 2015 and until the end of 2018. I wish to give a special thanks to the Chief of the department, Jan Bonde, for inviting me in to the department, for expressing your support and for all the small, pleasant conversations we have had.

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## 2 Original papers

The present PhD thesis is based on the following papers:

- I. Higher vs. lower haemoglobin threshold for transfusion in septic shock: subgroup analyses of the TRISS trial. *Acta Anaesthesiol Scand.* 2017; 61 (2), 166-75
- II. Long-term outcomes in patients with septic shock transfused at a lower versus a higher haemoglobin threshold: the TRISS randomised, multicentre clinical trial. *Intensive Care Medicine* 2016; 42 (11), 1685-94
- III. Effects of red blood cell storage time on transfused patients in the ICU - protocol for a systematic review. *Acta Anaesthesiol Scand.* 2017; 61 (10), 1384-97
- IV. Effects of shorter versus longer storage time of transfused red blood cells in adult ICU patients: a systematic review with meta-analysis and Trial Sequential Analysis. *Intensive Care Medicine* 2018; 44 (2), 204-17
- V. Storage time of red blood cells in patients with septic shock. *In manuscript*

## 3 Summary

### 3.1 Background

Red blood cell (RBC) transfusion is a common treatment for anaemia and tissue hypoxia for patients with septic shock. The Transfusion Requirements In Septic Shock (TRISS) trial was a randomised, clinical trial (RCT) that aimed to investigate the effect and safety of two different haemoglobin thresholds for RBC transfusion; a lower of 7 g/dl (4.3 mmol/L) and a higher of 9 g/dl (5.6 mmol/L). More than 1000 patients were included in 32 Scandinavian intensive care units (ICUs) in a two-year period from December 2011 to December 2013. The primary results showed no difference in mortality at day 90 between the two treatment groups, however, the number of patients transfused, and the number of transfusions given were reduced in the lower threshold group. With the studies that constitute this thesis, we sought to further investigate the effects of the applied transfusion thresholds among different subgroups of patients in the trial, the long-term consequences of the intervention, and the perspectives of red blood cell storage time, to allow more qualified recommendations for RBC transfusion for patients with septic shock.

### 3.2 Methods

We conducted four different studies. In Study I, we investigated if certain subgroups of patients in the TRISS trial responded differently to the intervention. The subgroups investigated were: patients with comorbidities (chronic lung disease, haematological malignancy or metastatic cancer), patients undergoing surgical procedures and patients who would have fulfilled the new clinical criteria for septic shock (lactate above 2 mmol/L and vasopressor use) developed after the TRISS trial. Study II was a long-term follow-up of the TRISS trial, where we assessed the mortality among all patients at one year after the last patient was included in the trial and the health-related quality of life (HRQoL) among the Danish patients at one year after randomisation. As Study III, we conducted a systematic review of the effects of RBC storage time on outcomes among patients in the ICU. Lastly, we investigated, in a descriptive cohort study (Study IV), the RBC transfusions given (the storage time, the amount, and timing), and the mortality of patients with septic shock in the TRISS trial and in a second cohort of patients with septic shock from 8 different ICUs in Denmark in the period before the TRISS trial.

### 3.3 Results

**Study I:** The effect of the two transfusion thresholds on 90-day mortality did not differ between the pre-defined subgroups and the complementary groups, and the tests of interaction showed no statistically significant subgroup effect on the intervention effect. The number of patients with haematological malignancy and metastatic cancer were very small, which resulted in wide confidence intervals of the effect estimates.

**Study II:** There were no statistically significant differences in the mortality at 1 year (relative risk (RR) 0.97, 95% confidence interval (CI) 0.85-1.09) or in the survival at a median of 21 months after randomisation between the lower and the higher haemoglobin threshold groups (hazard ratio (HR) of 0.88, 95% CI 0.75-1.03). We included 81 percent of the Danish patients in the HRQoL analysis. The patients who were dead at one-year follow-up were assigned the worst possible score (zero) of physical and mental health. We found no difference between the treatment groups in HRQoL, however, a high degree of missingness should be noted as a limitation.

**Study III:** We included seven RCTs with a total of 18,283 ICU patients allocated to transfusions with fresher versus either older or standard issue RBC units. Two RCTs were judged as having overall low risk of bias and



the primary analysis showed no statistically significant difference in mortality at day 90 between the fresher and the older blood group (relative risk 1.04; 95% CI 0.97-1.11; 7349 patients; Trial sequential Analysis-adjusted CI 0.93-1.15). A Trial Sequential Analysis rejected that a more than 10 percent change in relative risk of death is likely when comparing fresher versus older blood for RBC transfusion.

**Study IV:** In the Danish cohort of patients with septic shock from 8 different ICUs, 33 percent of the patients were transfused in the 14 days prior to their ICU admission. Seventy-seven percent and 36 percent were given at least one RBC transfusion during and after their ICU stay, respectively. Only small fractions of patients, in both the Danish cohort and the TRISS cohort, received exclusively blood that was either less than 7 days old or more than 24 days old, with no obvious increased mortality.

### 3.4 Conclusion

With the studies of this thesis we investigated further the effects of transfusion strategy among patients with septic shock and the consequences of RBC unit storage time. The restrictive transfusion strategy of a lower haemoglobin threshold for RBC transfusions in patients with septic shock appears safe also in subgroups of patients with septic shock, and regarding long-term survival and HRQoL. Among adult patients in the ICU, we may reject a clinically meaningful effect of fresher versus older blood for transfusion on mortality, however, further data are needed on the effect of very fresh or very old blood, and the effect of storage time on outcomes in transfused patients with septic shock. Patients with septic shock received a large part of their RBC transfusions both before the ICU admission and after their ICU stay, but the majority was given while in the ICU. They were exposed to RBC units with varying storage times and only small fractions of patients were exposed exclusively to very fresh or exclusively very old blood. Based on the knowledge from the studies of this thesis, a restrictive transfusion strategy for patients with septic shock appear safe for most patient types, also regarding long-term outcomes. Additionally, a continued practice of the blood banks, issuing the oldest compatible RBC units first, appears safe.

## 4 Summary in Danish (Dansk resumé)

### 4.1 Baggrund

Transfusion med røde blodceller (blodtransfusion) er en hyppig behandling af anæmi (blodmangel) og vævsiltmangel hos patienter med septisk shock (blodforgiftningsshock). Transfusion Requirements In Septic Shock (TRISS) forsøget var et klinisk lodtrækningsforsøg, der havde til formål at undersøge sikkerheden ved og effekten af to forskellige hæmoglobin-niveauer som tærskel for blodtransfusion; lav blodprocent (4.3 mmol/L) kontra høj blodprocent (5.6 mmol/L). Mere end 1000 patienter blev inkluderet på 32 skandinaviske intensivafdelinger (ITAer). Forsøgets primære resultater viste at der ikke var forskel i dødeligheden efter 3 måneder mellem de to behandlingsgrupper, men at færre fik blodtransfusioner og der samlet set blev givet mindre blod i gruppen med en lavere blodprocent som tærskel. Med delprojekterne i denne afhandling ønskede vi yderligere at undersøge effekten af tærskelværdierne blandt forskellige typer af patienter i forsøget, tærskelværdiernes konsekvenser for langtidsoverlevelsen og livskvaliteten, samt effekten af blodets opbevaringstid, før muliggøre stærkere anbefalinger om blodtransfusioner til patienter med septisk shock.

### 4.2 Metode

Vi udførte fire forskellige delprojekter. I delprojekt I undersøgte vi om bestemte undergrupper af patienter i TRISS forsøget reagerede forskelligt på at få blodtransfusion ved lav kontra høj blodprocent. Undergrupperne var patienter med kroniske sygdomme (kronisk lungesygdom, blod- og lymfekræft (hæmatologisk kræft) samt kræft med spredning til andre organer (metastatisk kræft)), patienter som var blevet opereret under indlæggelsen og patienter som ville have opfyldt de nye kliniske kriterier for septisk shock (højt laktatniveau i blodet og behov for blodtryksunderstøttende medicin). Delprojekt II var en opfølgning af TRISS forsøget, hvor vi undersøgte dødeligheden 1 år efter at den sidste patient blev inkluderet i forsøget samt helbreds-relateret livskvalitet blandt de danske patienter et år efter inklusionen. Vi udførte desuden en systematisk oversigtsartikel (systematisk review, delprojekt III) om betydningen af blodets opbevaringstid for udfaldet hos patienter på ITA. Og endelig udførte vi en beskrivende kohorteundersøgelse (delprojekt IV) hvor vi brugte viden om blodtransfusioner givet i TRISS forsøget samt viden om blodtransfusioner givet til en anden kohorte af danske patienter med septisk shock behandlet på en ITA i perioden før TRISS forsøget. I dette delprojekt undersøgte vi mængden af blodtransfusioner, blodets opbevaringstid, timingen af blodtransfusionerne og dødeligheden blandt patienterne i de to undersøgelsesgrupper.

### 4.3 Resultater

**Delprojekt I:** Effekten af tærskelværdien for dødeligheden på dag 90 var ikke forskellig mellem de undergrupper af patienter og de modsatte undergrupper patienter, der ikke havde de forudbestemte karakteristika. Der var ingen statistisk betydelig påvirkning af undergrupperne på effekten af blodtransfusion ved lav kontra høj blodprocent. Antallet af patienter i grupperne med hæmatologisk kræft og metastatisk kræft var meget lille, og resultaterne for disse undergrupper var derfor forbundet med en vis usikkerhed.

**Delprojekt II:** Det var ingen statistisk betydelig forskel i dødeligheden ved 1 år (relativ risiko (RR) for død: 0.97, 95% konfidens interval (KI) 0.85-1.09) eller i overlevelsen mediant 21 måneder efter inklusion i forsøget (Hazard ratio: 0.88, 95% KI 0.75-1.03) mellem patienterne behandlet ved den lavere blodprocent kontra patienter behandlet ved den højere blodprocent. Vi kunne inkludere 81 procent af de danske

patienter i undersøgelsen af livskvalitet (de patienter som var døde ved 1 år fik tildelt den værste mulige scoring (nul) for fysisk og mentalt helbred). Der var ingen forskel i livskvalitet mellem behandlingsgrupperne.

**Delprojekt III:** Vi inkluderede syv kliniske lodtrækningsforsøg med et totalt antal af 18.283 patienter på ITA som var tilfældigt fordelt til behandling med frisk kontra ældre blod (kort eller længere opbevaringstid). To af forsøgene blev vurderet til at være i overordnet lav risiko for systematiske fejl (bias), og den primære analyse viste ingen statistisk betydelig forskel i dødelighed ved dag 90 mellem patienterne i gruppen med frisk blod kontra patienter i gruppen med ældre blod (RR 1.04; 95% KI 0.97-1.11; 7349 patienter; Trial Sequential Analyse-justeret 95% CI 0.93-1.15). En supplerende analyse (Trial Sequential Analysis) viste, at vi kunne udelukke en større end 10 procent ændring i den relative risiko for død ved sammenligning af frisk kontra ældre blod til patienter på ITA.

**Delprojekt IV:** I den danske kohorte fik 33 procent af patienterne en blodtransfusion i løbet af de 14 dage før intensivindlæggelse. Under intensivindlæggelse fik 77 procent blodtransfusioner og efter udskrivelse modtog 36 procent blodtransfusioner. En meget lille del af patienterne i både den danske og i TRISS kohorten fik blodtransfusioner som var udelukkende meget frisk (under 7 dage gammelt) eller meget gammelt (mere end 24 dage gammelt). Der var ikke umiddelbart nogen højere dødelighed blandt disse patienter

#### 4.4 Konklusion

Med delprojekterne i denne afhandling undersøgte vi yderligere følgevirkningerne af tærskelværdier for blodtransfusion blandt patienter med septisk shock, samt betydningen af blodets opbevaringstid. Behandlingen ved den lavere blodprocent virker sikker, også for undergrupper af patienter med septisk shock, og med hensyn til langtidsoverlevelse og livskvalitet. Blandt patienter på intensivafdeling kan vi formentlig afvise en klinisk meningsfuld betydning af frisk kontra ældre blod, men vi har brug for mere viden om betydningen af meget frisk og meget gammelt blod, samt om betydningen af blodets opbevaringstid for patienter med septisk shock. Patienter med septisk shock fik en betydelig andel af deres blodtransfusioner både før og efter intensivindlæggelsen, men størstedelen under intensivindlæggelsen. Kun en lille andel af patienterne blev udsat for udelukkende meget frisk eller udelukkende meget gammelt blod. Med viden fra disse projekter må det vurderes at blodbankerne trygt kan fortsætte deres nuværende praksis, hvor det ældste blodprodukt udleveres først. Det må ligeledes vurderes at en restriktiv transfusionsstrategi, hvor en lavere blodprocent bruges som tærskel for blodtransfusioner virker sikker for de undersøgte patienttyper og i forhold til langtidsvirkningerne blandt patienter med septisk shock.

## 5 List of abbreviations

<i>2,3-DPG</i>	2,3-diphosphoglycerate (2,3-bisphosphoglycerate)
<i>CI</i>	Confidence interval
<i>FWER</i>	Family wise error rate
<i>HTR</i>	Haemolytic transfusion reaction
<i>HR</i>	Hazard ratio
<i>HRQoL</i>	Health-related quality of life
<i>ICU</i>	Intensive care unit
<i>IQR</i>	Inter-quartile range
<i>ITT</i>	Intention-to-treat
<i>MA</i>	Meta-analysis
<i>MCS</i>	Mental component summary
<i>MD</i>	Mean difference
<i>NO</i>	Nitric oxide
<i>PCS</i>	Physical component summary
<i>RR</i>	Relative risk
<i>RRI</i>	Relative risk increase
<i>RRR</i>	Relative risk reduction
<i>RCT</i>	Randomised clinical trial
<i>SAPS</i>	Simplified acute physiology score
<i>SIRS</i>	Systemic inflammatory response syndrome
<i>TACO</i>	Transfusion-associated circulatory overload
<i>TRALI</i>	Transfusion-related acute lung injury
<i>SD</i>	Standard deviation
<i>SF-36</i>	Medical Outcomes Study 36-item short form health survey
<i>SIRS</i>	Systemic inflammatory response syndrome
<i>SOFA</i>	Sequential (sepsis-related) organ failure assessment
<i>TRALI</i>	Transfusion-related acute lung injury
<i>TSA</i>	Trial Sequential Analysis
<i>WHO</i>	World Health Organisation

## 6 Introduction

Sepsis, or *blood poisoning* in layman's terms, is a syndrome caused by an infection of any pathogen – bacteria, viruses, fungi or parasites – of any site of origin, though, with the lungs, abdomen and urinary system being the most frequent sites. Even though the term *blood poisoning* implies that there are pathogens in the blood stream, this is far from always the case, and the syndrome is not just caused by pathogens gone astray. The syndrome is rather a person's own body reacting or responding to the infection in a dysregulated and untoward fashion, and it is the interaction between the host and the pathogen – and several nuances in both – that eventually causes the syndrome. Because of the complex aetiology, the management of patients with sepsis is equally complex. The condition can among some patients progress into a more life-threatening and severe state, called septic shock, where the patient needs multiple organ-supporting therapies performed at an intensive care unit (ICU).

One of the key problems in sepsis and septic shock is that of compromised oxygen delivery to organs because of circulatory failure. Moreover, patients with sepsis and septic shock often suffer from anaemia. This is a reduction in the number of red blood cells (RBCs) or a reduction of the oxygen-carrying molecule, haemoglobin, within the RBC, particularly because of blood sampling and dilution. The need for oxygen-delivery to the tissues and a state of anaemia have led to an extensive use of RBC transfusions as a treatment for patients with septic shock.

The optimal haemoglobin level as a threshold for RBC transfusion among patients with septic shock had until recently not been investigated in a randomised clinical trial (RCT). The effectiveness and safety of a lower haemoglobin threshold or restrictive strategy as compared to the more liberal standard practice of a higher haemoglobin threshold for RBC transfusion among patients with septic shock were investigated in the Transfusion Requirements in Septic Shock (TRISS) trial. The lower threshold did not cause any worse outcomes but resulted in fewer patients being transfused and fewer RBC units transfused.

This thesis is the report of a follow-up study of the TRSS trial. In the four sub-studies we investigated (I) if subgroups of patients responded differently to the intervention, (II) the long-term outcomes of the patients, (III) the consequences of RBC storage time on outcomes among critically ill patients, and (IV) the description of RBC properties and timing of transfusions for patients with septic shock. Before strong and more qualified recommendations can be made to guide clinicians in the treatment of patients with septic shock, we need to know more regarding all these aspects of the transfusion strategy.

## 7 Background

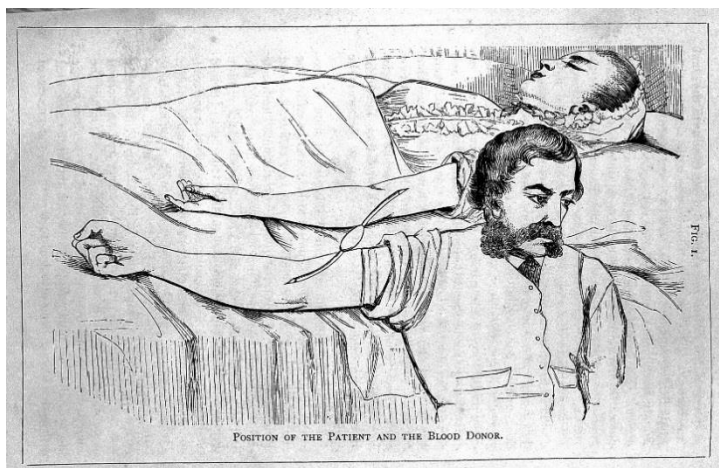
### 7.1 Historical perspective – sepsis and blood transfusions

Sepsis was first described in a medical context by Hippocrates in antiquity. The Greek meaning of the word sepsis is *putrefaction* and Hippocrates described the syndrome as a malodourous decay of the body, which he made attempts to treat with wine and vinegar.<sup>1</sup> In the centuries following, a theory of external organisms initiating the syndrome was both proposed and refuted, and more than 2000 years passed before this theory was proven.

The idea of blood transfusion was also first described by the ancient Greeks.<sup>2</sup> The main theory at this time and for the next two thousand years was that the blood contained the strength, health and even personality of a person or animal, and by blood transfusion one could transfer these properties to another individual. Documented experiments with animal-to-human blood transfusion took place in the 17<sup>th</sup> century, leading to a ban of the treatment by legislation – due to dangerous and often fatal outcomes, but also because the medical establishment had great resistance toward this treatment.<sup>3</sup>

Throughout the 19<sup>th</sup> century the understanding of microbiological infections and the usefulness of blood transfusion evolved. In a maternity ward setting the Austrian physician, Ignaz Semmelweis, found a connection between maternal sepsis (puerperal sepsis) and medical students going directly from performing an autopsy and assisting at child delivery. He introduced the procedure of handwashing and with this lowered the maternal deaths.<sup>1</sup> Along with Semmelweis' discoveries, John Snow, Louis Pasteur and Robert Koch all helped advance the modern understanding of infectious diseases with development of the epidemiological investigation of the cause of diseases, and revealing the mechanisms of the associated germ theory.

The beginning of human-to-human transfusions also dates to the 19<sup>th</sup> century and interestingly, the scene was again the maternity ward. In 1818, Dr James Blundell, a British obstetrician, performed the first known transfusion from human to human.<sup>4</sup> His rationale of using blood transfusion was to replace the blood lost from patients suffering from severe bleeding. After witnessing women bleeding to death when giving birth, he tried changing the fatal outcome by using the treatment which by that time had been banned for nearly one and a half centuries. He transfused blood to a woman immediately after extracting it from the donor's vein and the woman survived (Figure 1). His following experiments with the treatment had both similar successful but also fatal outcomes and the scepticism in the medical world continued.



**Figure 1.** An example of a direct donor-to-recipient transfusion. Illustration from J. H. Aveling, *Immediate transfusion in England* *Obstetrics Journal*, 1873; 1, 303. Credit: Wellcome Collection. CC BY. (May be used without restriction under copyright law).

Almost a hundred years passed during which the use of blood transfusion was limited by practical problems of blood clotting and a missing link in the understanding of transfusion reactions.<sup>5</sup> In 1901 an important and, to the safety of transfusion medicine, essential discovery was made, when the Austrian physician, Karl Landsteiner, discovered three blood types, A, B and C – later renamed O – and a year after colleagues of his found the fourth blood type – AB. Some years later the anticoagulant 0.2% sodium citrate was developed, and indirect transfusion made possible. Further developments occurred before the Second World War as antibiotics were discovered and blood donor services with blood banks were established. It then became possible to treat infections more effectively and to replace large blood losses, both of which played important roles saving the lives of soldiers during the war.<sup>6</sup>

In the 1940's, when the oxygen-carrying capacity of haemoglobin was identified, it became standard practice to use a threshold of 10 g/dl for RBC transfusion – and this strategy was maintained for several decades.<sup>7</sup>

Important discoveries took place in the last part of the twentieth century, that enhanced the understanding of the pathophysiology in sepsis. Endotoxins from the bacteria, cytokines and the role of the immune system, the coagulation system and the coagulopathy in sepsis, and the role of nitric oxide (NO) in endothelial dysfunction during sepsis became recognized and understood.<sup>1</sup> The cellular and systemic changes during sepsis could now be described, and targeted therapies were on the rise. The understanding of tissue hypoxia in sepsis together with anaemia provided the rationale for using RBC transfusion in the treatment of patients with septic shock.<sup>8</sup>

## **7.2 Modern definition of sepsis and septic shock**

The definition of the sepsis syndrome has for more than 25 years been standardized internationally in a consensus definition first published in 1992.<sup>9</sup> Sepsis was then defined as a suspected or proven infection with two or more signs of the systemic inflammatory response syndrome (SIRS), and the syndrome was categorised into three stages: sepsis, severe sepsis and septic shock. This definition was not optimal, as it was shown to be unspecific<sup>10</sup> – but also not sensitive enough.<sup>11</sup>

In 2016 the new consensus definition of sepsis and septic shock was published, called Sepsis-3.<sup>12</sup> The syndrome is now described as a dysregulated host-response to an infection, which carries a significant risk of death because of organ-dysfunction. The syndrome is identified clinically by a suspected or proven infection with a change in the Sepsis-related Organ Failure Assessment (SOFA) score of two or more points.<sup>12</sup>

Septic shock is defined as a state with severe metabolic, cellular and circulatory dysfunctions associated with a significantly higher risk of death, than with sepsis alone.<sup>12,13</sup> To identify this subset of patients with sepsis, the clinical criteria of a lactate of more than 2 mmol/L and the need for vasopressors to maintain a mean arterial pressure (MAP) of 65 mmHg despite sufficient fluid resuscitation, was proposed in the Sepsis-3 definition.<sup>12,13</sup>

### 7.3 Epidemiology of sepsis

Differing definitions, no decisive diagnostic tests for sepsis and the lack of sepsis-code registration complicate the task of counting sepsis, but increasing awareness and international collaboration have improved the knowledge of sepsis epidemiology.<sup>14,15</sup> There are probably more than 30 million sepsis cases and more than 5 million deaths from sepsis per year world-wide.<sup>16</sup> The World Health Organization (WHO) has suggested that sepsis is an underreported but leading cause of death,<sup>17</sup> and it is especially a burden in low and middle-income countries.<sup>18</sup> In the USA there are approximately 3 sepsis cases per 1000 population per year and sepsis has been shown to be the leading cause of hospital deaths.<sup>19,20</sup>

### 7.4 Pathophysiology and clinical presentation

The host-response of sepsis covers both pro-inflammatory and anti-inflammatory mechanisms, which help fight the infection and protect the local tissue from damage, but which also cause injuries to organs and increase the risk of secondary infections.<sup>21</sup> Properties of the pathogen and properties of the host both affect the immune-system and the degree of the response.

Some degree of coagulopathy is present in sepsis and septic shock, which can develop into disseminated intravascular coagulation, where coagulation is continuously activated and clots are formed in the small capillaries resulting in compromised blood flow and also an increased risk of bleeding.<sup>22</sup> The connection between endothelial cells are weakened and leakage of fluid from the vessels to the surrounding tissue causes oedema in the interstitium. The red blood cells undergo changes in shape and function. The RBCs are depleted of the metabolite 2,3-diphosphoglycerate (2,3-DPG) and the deformability of the RBCs are reduced, both properties prevent the RBC from delivering oxygen to peripheral tissue.<sup>23</sup> All the above, together with universal vasodilatation and failure of the circulatory system result in hypoperfusion and hypoxia of the tissue, eventually causing failure of organs.<sup>24</sup>

### 7.5 Anaemia

Approximately two-thirds of the general critically ill patients in the ICU have a lower than normal haemoglobin level (<12 g/dl / 7.5 mmol/L) upon admission.<sup>25,26</sup> During the ICU stay the blood haemoglobin level decreases,<sup>27</sup> the average decline being 0.5 g/dl per day for the first three days, and the decline may continue for patients with sepsis and septic shock.<sup>28,29</sup> The prevalence of anaemia at discharge from the ICU has been observed to be around 80 percent,<sup>30</sup> and one study reported half of the patients who were discharged with anaemia, were still found to be anaemic at 6 months after the ICU stay.<sup>31</sup>

The blood haemoglobin level and development of anaemia in critically ill patients are affected by patient characteristics – namely comorbidity and baseline haemoglobin level – and the circumstances leading to the critical illness – e.g. trauma or surgery. Multiple factors affect the decline in blood haemoglobin level during an ICU stay: loss of blood due to blood drawing for diagnostic testing and overt or occult bleeding;<sup>32,33</sup> inadequate RBC production due to absolute iron- and nutrient-deficiency,<sup>34,35</sup> functional iron deficiency and a blunted erythropoietin response;<sup>33,36</sup> and increased destruction of RBCs caused by immuno-mediated haemolysis, mechanical destruction by haemodialysis and shortened life-span of the circulating RBCs.<sup>37</sup> Patients with septic shock receive large volumes of resuscitation fluids resulting in blood dilution, further lowering the haemoglobin level.<sup>38</sup> Because anaemia, on top of the circulatory collapse, aggravates the tissue oxygen-insult of septic shock, there is an urge to minimize the degree of and to correct anaemia.



## 7.6 Alternatives to blood transfusion

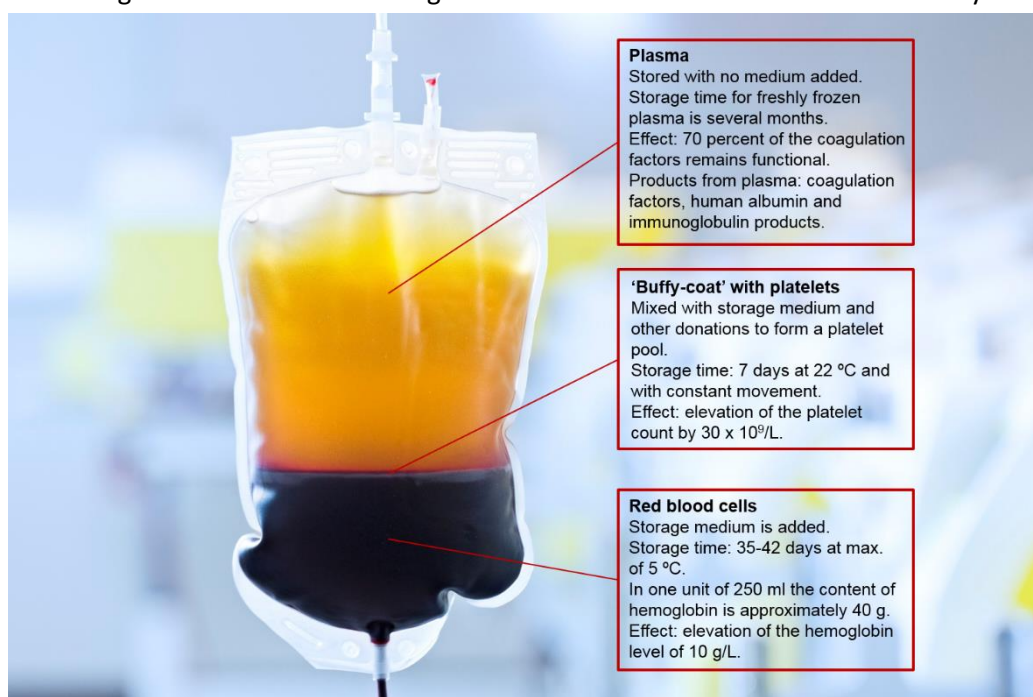
Alternative therapies to RBC transfusion have been tested in critically ill patients. Correction of the inadequate supply of iron needed to produce RBCs have been investigated in a few trials of IV iron versus placebo.<sup>39,40</sup> RCTs testing erythropoiesis-stimulating agents as an adjuvant treatment to patients in the ICU with anaemia did not show any benefit regarding patient-important outcomes, and only a small decrease in the number of RBC transfusions, but with an additional increase in thrombo-embolic events.<sup>41–43</sup> Another potential beneficial treatment is the use of anti-hepcidin.<sup>44</sup> Heparin regulates the iron metabolism and in the type of acutely developed anaemia seen in patients in the ICU, there is an excess of hepcidin.<sup>45</sup> To date, however, no trials have investigated this therapy. Experiments with artificial substitutes for blood, i.e. cell-free haemoglobin-based oxygen carriers, have not shown promise either, but instead increased risk of death and myocardial infarction.<sup>46</sup>

Alternative therapies to RBC transfusion have not shown superiority by minimizing the additional use of RBC transfusion, increasing the haemoglobin level sufficiently or to otherwise improve the outcome of patients.<sup>47–49</sup> Furthermore, the safety and efficacy of the alternative therapies have not been tested in patients with septic shock and are not recommended in the Surviving Sepsis Campaign.<sup>50</sup> Therefore, RBC transfusion remains the only treatment to rapidly and effectively correct anaemia in septic shock.

## 7.7 Red blood cell transfusion

Red blood cell transfusion begins with the voluntary donation of approximately 500 mL of whole blood by healthy donors, the separation of the whole blood into red blood cells, platelets and plasma (Figure 2), and storage of the products in blood banks, where they are available for potential recipients. The donation of blood is regulated according to the corresponding use of the products, and the standard practice is to issue the oldest compatible blood product first to minimise waste. Red blood cells are suspended in a storage solution, containing anticoagulants and nutrients, the most common storage medium contains saline, adenine, glucose and mannitol (SAGM),<sup>51</sup> with the product being stored at 2–5°C for a maximum of 35–42 days, depending on national regulations. The mean storage time of the RBCs issued in the USA is 23 days.<sup>52</sup>

**Figure 2.** The donated whole blood after centrifuging. It is then separated into blood products. Photo by Thomas Bertelsen / the Blood Donors in Denmark. Illustration published in: Rygård et al *Blood product administration in the critical care and perioperative settings* Crit Care Clin 2018; 34(2):299-311. (Permission not required for the use in a thesis)

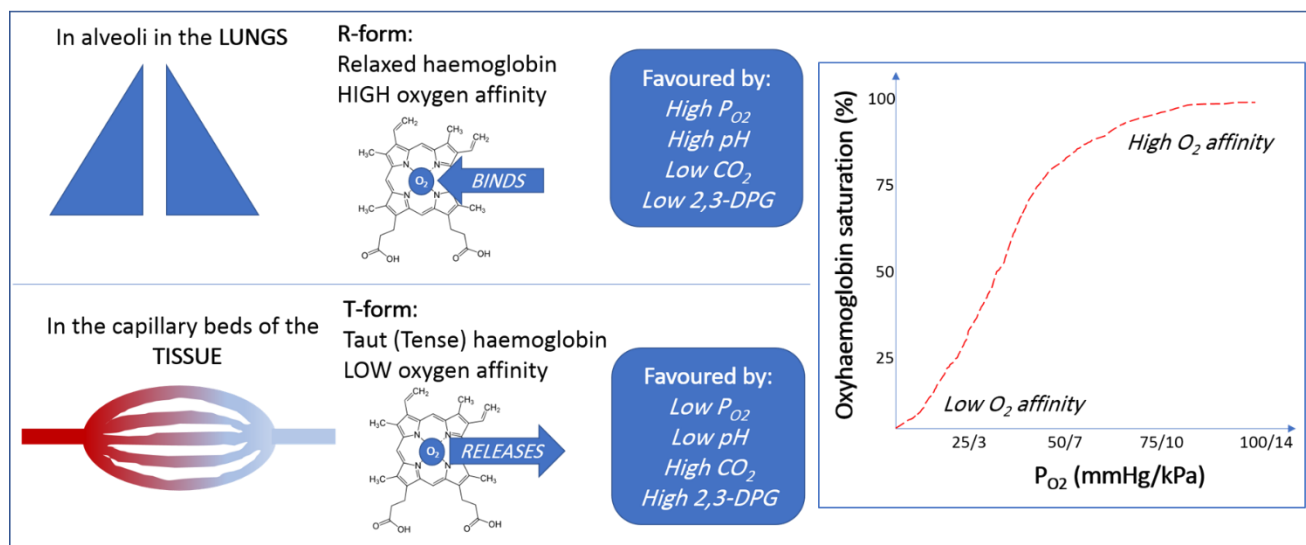


Apart from adding a storage medium, the RBC product is filtered to reduce the number of white blood cells, or leukocytes.<sup>53</sup> Leukocytes, unlike RBCs, contain a nucleus with DNA, and by transferring these immune cells with donor DNA to the recipient, adverse effects may be induced, for example transfusion-related acute lung injury (TRALI).<sup>54</sup> Therefore, universal leukoreduction by filtration was introduced and is now common practice in blood banks of high-income countries.<sup>55</sup>

The red blood cell product is in a volume of 250-350 ml, and the amount of haemoglobin is approximately 40 g, which would increase a blood transfusion recipient's blood haemoglobin level with 1 g/dl (0.5 mmol/L).<sup>56</sup> The main indications for RBC transfusion in the ICU are bleeding and anaemia.<sup>25,26,29,57</sup>

## 7.8 Oxygen delivery

The purpose of the red blood cell is transportation of oxygen from the lungs to the tissues and transportation of carbon dioxide from the tissues to the lungs. Up to four oxygen molecules bind to one haemoglobin molecule and conditions in the surroundings (lungs, bloodstream and peripheral tissues) determine the affinity for and when the oxygen is bound or released from the haemoglobin (Figure 3). When the 8  $\mu\text{m}$  RBC flows through the 2-3  $\mu\text{m}$  capillary in the peripheral tissues to release the oxygen, the cell deforms and then reshapes again in the venule. The red blood cell is also a vasoactive agent in the normal circulation. The vasodilatory molecule, nitric oxide (NO), is released from the RBC under hypoxic circumstances to cause vasorelaxation and to increase the blood flow, and the presence of free haemoglobin in the blood stream can bind to NO causing vasoconstriction.<sup>58</sup>



**Figure 3.** The binding of oxygen to haemoglobin and the oxygen-haemoglobin dissociation curve. In a normal, healthy person, the haemoglobin of the blood is saturated with oxygen (where the curve flattens) at a partial pressure of approximately 60 mmHg or 8 kPa.  $P_{O_2}$  = partial pressure. Illustration by Sofie Louise Rygård, using Microsoft PowerPoint (Microsoft Office 2016 Pro Plus).

## 7.9 Storage of blood

The red blood cell is rather robust and viable when stored, and the criteria determining the duration of storage is that less than 0.8-1.0 percent of the cells are haemolysed in addition to a survival of 75 percent of the donor RBCs in the recipient 24 hours after transfusion.<sup>59</sup> But apart from just crude survival of the RBCs, changes in the function and shape of the cell during storage is also a concern. The changes that occur during storage are collectively called the *storage lesion*.<sup>60</sup> The cell is depleted of the metabolites 2,3-DPG

and amino triphosphate (ATP) resulting in a left shift of the oxygen-haemoglobin dissociation curve (Figure 3). In other words, the haemoglobin needs a lower partial pressure of oxygen to bind oxygen, but the oxygen then binds more tightly to the haemoglobin (higher affinity) and is released more unwillingly.<sup>61</sup> Furthermore, the viability and deformability of the RBC decrease with storage, resulting in leakage from the cell and lysed cells. In the storage medium more free haemoglobin, iron, potassium and acidosis then develop.<sup>62</sup> All of the properties of the storage lesion can affect the RBCs ability to transport and deliver oxygen, because of the changed affinity, and the decreased capability to deform and flow in the capillaries to release oxygen. Furthermore, the decreased ATP and NO production of the stored RBC, together with free haemoglobin in the bloodstream binding to NO and finally RBCs adhering to the endothelium can compromise the blood flow in the recipient.<sup>63</sup> Many of the storage changes are detectable after just 24 hours of storage, but they become more pronounced with increasing storage time.<sup>64,65</sup>

Another concern of RBC transfusion and the transfusion of other blood products (plasma and platelets) is the transfusion-related immunomodulation (TRIM), which also may be increased by storage.<sup>66</sup> The transfusion of blood products can play a role in the immune-stimulatory/immunosuppressant mechanisms of critical illness or septic shock, but the impact or significance of TRIM probably depends on the patient's own underlying immune activation and the timing of the transfusion in relation to the course of the illness.<sup>67</sup>

### **7.10 Complications related to anaemia and RBC transfusion**

During critical illness, the acute consequence of anaemia is compromised oxygen delivery to tissues, leading to increased severity of the disease. But the recovery from anaemia to a normal haemoglobin level can take months,<sup>31</sup> and hence, the consequences of anaemia reach beyond the ICU. Patients discharged from hospital being anaemic have reported fatigue or depression and several studies, in both cohorts of ICU patients and other chronic diseases, have shown an association of anaemia and a lower quality of life.<sup>31,68–70</sup> Anaemia may affect oxygen delivery to the myocardium and increased myocardial damage and increase the risk of cardiovascular morbidity and mortality.<sup>71–73</sup>

Transfusion-related risks are categorised as infectious, with transmission of blood-borne pathogens, and non-infectious hazards, and such risks are generally small.<sup>74</sup> The infectious hazards of transfusion have almost been eliminated by donor selection and screening of blood products, and now the non-infectious are far more frequent areas of concern.<sup>74</sup> The most severe complications are transfusion-associated circulatory overload (TACO), haemolytic transfusion reaction (HTR), transfusion-related acute lung injury (TRALI) and acute allergic reactions, and the incidence of a serious complication is approximately one in 21,000 blood products issued and the risk of death is one in 114,000.<sup>74</sup>

There are some more subtle adverse reactions to RBC transfusion, and this is where storage lesion and TRIM might play a role. TRIM may cause an ongoing immunosuppression in the recipient and increase the risk of secondary infections.<sup>67</sup> The introduction of universal leucocyte reduction has probably minimised this risk.<sup>75–77</sup> The clinical consequences of the RBC storage time has been a concern, particularly after the publication of the large, retrospective observational study by Koch and colleagues among patients undergoing cardiac surgery.<sup>78</sup> The study suggested an association of increased RBC storage time and increased risk of post-operative complications and mortality.<sup>78</sup> Several observational studies both supported and refuted this finding,<sup>79–84</sup> and RCTs and meta-analyses have shown no connection between

storage time or the amount of blood being transfused and the increased risk of short-term morbidity or mortality.<sup>85-90</sup>

Apart from the known and unknown clinical consequences of RBC transfusion, it is important to mention the complications associated with the donation of blood by healthy donors,<sup>91</sup> the costs of blood bank services and the limited resource, which constitute the blood products. All these issues are important to calculate in the decision-making process surrounding blood administration services.

### **7.11 RBC treatment in septic shock**

The international Surviving Sepsis Campaign has strived to improve the treatment of and outcomes for patients with sepsis.<sup>92</sup> The decline in case mortality of sepsis is probably partly due to the success of the campaign in raising the awareness of the syndrome and improving the quality of treatment.<sup>15,93</sup>

After identifying patients with sepsis, initial management is focused on controlling and eliminating the infection – using antibiotics and possibly surgery – and resuscitation and maintenance of the circulation and organ perfusion by the administration of intravenous fluids and vasoactive agents.

Blood transfusion is recommended as an adjunctive therapy to increase the oxygen-carrying capacity of the circulating blood and improve tissue oxygenation. Initially it was recommended to use RBC transfusions, during the first 6 hours of resuscitation, to achieve a haematocrit of 30 percent or more – which corresponds to a haemoglobin level of 10 g/dl.<sup>92</sup> The recommendation was based on the single-centre RCT by Rivers and colleagues randomising 263 patients who had severe sepsis or septic shock in the emergency department to an early goal-directed therapy (EGDT) or standard therapy.<sup>94</sup> After the initial resuscitation, the recommendation was to use the restrictive threshold of 7 g/dl for RBC transfusion (in the absence of extenuating circumstances) and to target the treatment for a haemoglobin level between 7 and 9 g/dl.<sup>92</sup> This was based on the Transfusion Requirements in Critical Care (TRICC) trial,<sup>95</sup> but only 26 percent of the trial patients had either a severe infection or septic shock, and the patients were already resuscitated.<sup>95</sup> The primary outcome of the TRICC trial was mortality at day 30, which did not differ statistically between the treatment groups, but other outcomes showed a trend towards harm with the liberal (10 g/dl) strategy and a mortality benefit in the restrictive group among some subgroups of patients (younger than 55 years and less severely ill).<sup>95</sup> The Rivers study, on the contrary, showed a statistically significant decreased in-hospital mortality in the group receiving EGDT, where the use of RBC transfusions were more liberal than the standard care group (68 versus 45 percent of the patients received RBC transfusion in the first 72 hours of resuscitation).<sup>94</sup>

The evidence behind the first Surviving Sepsis recommendations of RBC transfusion in septic shock were somewhat conflicting, and there were no RCTs investigating the isolated treatment for patients with septic shock. The unknown tolerance of anaemia, the unknown effect of RBC transfusion and the unknown optimal haemoglobin level as threshold for transfusion in patients with septic shock was the basis, the clinical equipoise, that led to the Transfusion Requirements in Septic Shock trial.<sup>96</sup>

### **7.12 The Transfusion Requirements In Septic Shock trial**

The Transfusion Requirements in Septic Shock (TRISS) trial was an investigator-initiated randomised clinical trial which took place in 32 different ICUs in Denmark, Finland, Norway and Sweden from December 2011 to December 2013.<sup>97</sup> Patients were eligible for randomisation when they fulfilled the criteria for septic

shock<sup>9</sup> and were anaemic (with a haemoglobin level of 9 g/dl or less). 1005 patients underwent randomisation and were allocated 1:1 to either the standard, liberal practice of using a higher blood haemoglobin level of 9 g/dl (equivalent to 5.6 mmol/L) as the threshold for RBC transfusion or the intervention of using a restrictive threshold, namely a lower blood haemoglobin level of 7 g/dl (4.3 mmol/L) for RBC transfusion. The randomisation was stratified according to pre-existing haematological malignancy and trial site. The clinicians were to give a single unit of RBCs if the blood haemoglobin level fell to or below the allocated threshold, and the haemoglobin level should be re-assessed before the commencement of another transfusion or at least 3 hours after the termination of the transfusion. The remaining treatment of the patient was at the discretion of the treating clinician.

The lower threshold group received 50 percent less RBC transfusions and 36 percent of the patients did not receive any transfusions as compared to one percent in the higher threshold group. The relative risk of death in the lower threshold group versus the higher was 0.94 (95% CI 0.78 – 1.09; P=0.44) and no statistically significant differences were found in any of the secondary outcomes (time alive and out of life-support, ischemic events or severe adverse reactions).<sup>97</sup>

The evidence from the TRISS trial was rapidly implemented to support the use of a restrictive transfusion threshold for critically ill patients in national transfusion guidelines<sup>98-100</sup> and also in the updated version of the Surviving Sepsis Campaign.<sup>50</sup>

### 7.13 Long-term outcomes after septic shock

Patients who survive an episode of sepsis or septic shock, and who are discharged from ICU and eventually from the hospital have been reported to hold an increased risk of long-term (one year and beyond) mortality.<sup>101-104</sup> Additionally, sepsis survivors experience more cognitive impairment, physical disabilities, fatigue and decreased health-related quality of life (HRQOL) as compared to matched, hospitalised, non-septic patients or the general population.<sup>102,105</sup> Prescott and colleagues performed an extensive propensity score matched cohort study which showed that late mortality after sepsis was independent of health status before sepsis, and that sepsis alone carries an additional risk for late mortality.<sup>106</sup> This study underlined, that the course of sepsis and the interventions given may affect long-term survival for patients. Another recently published study from Thompson and colleagues in Australia has questioned the view, that sepsis-survivors have worse long-term outcomes than other critically ill patients.<sup>107,108</sup> In this study they also performed a large propensity score matched cohort study of ICU patients with sepsis and no sepsis comparing differences in health-care costs, HRQOL and mortality. They found a higher usage of health-care resources among patients with sepsis during their index hospitalisation, but the HRQOL and long-term mortality were similar to the matched ICU patients not having sepsis.<sup>108</sup> From the view of this thesis, the question is whether anaemia or RBC transfusion are one of the modifiers of long-term survival and health status for sepsis survivors?<sup>109</sup>

## 8 Aims of studies

The overall aims of this PhD study were to further investigate the long-term consequences and details of red blood cell transfusions for patients with septic shock. To give more qualified recommendations of RBC transfusion for patients with septic shock, we need to know more regarding the effect on different types of patients, the long-term outcomes and the effect of RBC storage time.

The present PhD thesis comprises the following four studies:

- **Study I (paper I):** Higher vs. lower haemoglobin threshold for transfusion in septic shock: subgroup analyses of the TRISS trial
- **Study II (paper II):** Long-term outcomes in patients with septic shock transfused at a lower versus a higher haemoglobin threshold: the TRISS randomised, multicentre clinical trial
- **Study III (paper IV):** Effects of shorter versus longer storage time of transfused red blood cells in adult ICU patients: a systematic review with meta-analysis and Trial Sequential Analysis (**+protocol (paper III)**): Effects of red blood cell storage time on transfused patients in the ICU - protocol for a systematic review)
- **Study IV (paper V):** Storage time of red blood cells in patients with septic shock.

## 9 Study I: Higher vs. lower haemoglobin threshold for transfusion in septic shock: subgroup analyses of the TRISS trial

### 9.1 Aim and hypothesis

The objectives were to investigate if subgroups of patients in the TRISS trial had differing degrees of effect size or differing directions of effect than the whole trial cohort. We hypothesised that some groups of patients would benefit from being transfused at a higher threshold (patients with chronic lung disease, haematological malignancy and metastatic cancer) and others would benefit from being transfused at a lower threshold (surgical patients and patients fulfilling the new criteria for septic shock).

### 9.2 Methods

#### 9.2.1 Study design

A post-hoc exploratory study of the TRISS trial investigating subgroup heterogeneity of the effect of a higher versus a lower haemoglobin threshold for RBC transfusion.

#### 9.2.2 Patients

We dichotomised the full ITT-population (n=998) of the TRISS trial in five subgroups based on comorbidity (presence of chronic lung disease (yes/no), metastatic cancer (yes/no) or haematological malignancy (yes/no)), surgery during the hospitalisation prior to randomisation (yes/no) and fulfilment of the new clinical criteria for septic shock at the time of randomisation (lactate above 2 mmol/L and vasopressor use (yes/no)).

#### 9.2.3 Outcome measures

The outcome investigated was the primary outcome from the TRISS trial – mortality at day 90.

#### 9.2.4 Statistical analyses

We performed a logistic regression analysis to test the interaction of each subgroup on the intervention effect, and we presented the relative risk with 95% CI for all dichotomised subgroups, and the P-value of the test of interaction and P-value of the test of interaction with adjustment for stratification variables (haematological cancer and trial site) for all subgroup comparisons. The investigated subgroups were described in, and the analyses were performed according to a pre-planned protocol and statistical analysis plan.

### 9.3 Results

Four patients had missing data regarding their highest plasma lactate level in the 24 hours prior to randomisation, and these patients could not be dichotomised in the subgroup of patients fulfilling/not fulfilling the new definition of septic shock. In the remaining subgroups, all patients could be dichotomised. There were 213 patients with chronic lung disease (and 785 patients in the complementary group); 75 patients with haematological malignancy (no haematological malignancy: 923 patients); 95 patients with metastatic cancer (no metastatic cancer: 903); 520 patients with surgery before randomisation (no surgery: 478); and 554 patients who fulfilled the new criteria for septic shock at the time of randomisation (patients who did not fulfil: 440). The number transfused patients and the number of transfused RBC units per patient seemed very similar in the subgroups compared to the full trial cohort, though among patients with

haematological malignancy and patients undergoing surgery it appeared that more patients were transfused with more units received (Table 1).

Regarding mortality at day 90, there were no statistically significant differences between the higher versus the lower haemoglobin threshold group in the subgroups, and there was no heterogeneity of the intervention effects in any of the subgroups (Figure 4).

## 9.4 Conclusion

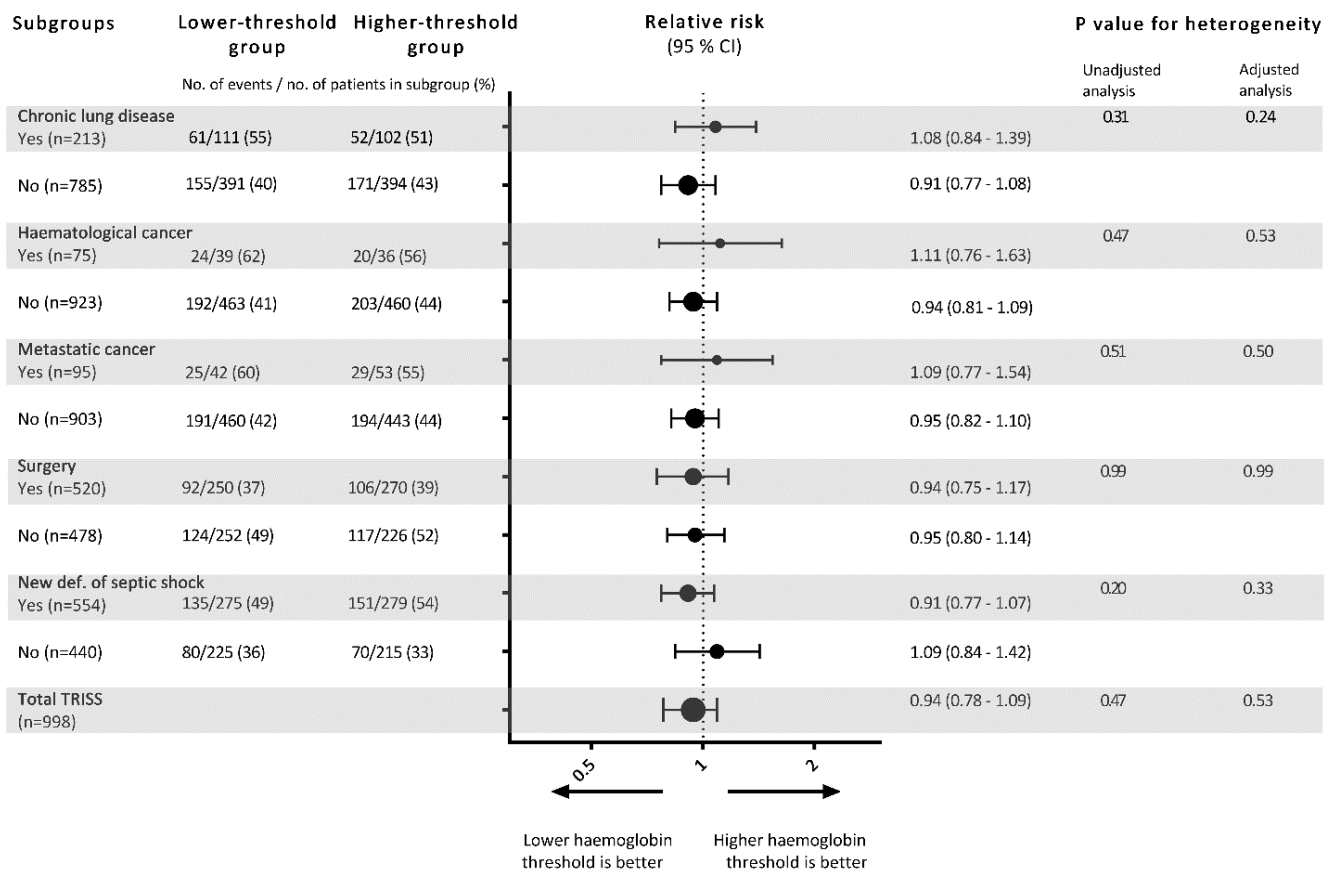
We could not detect any subgroups of patients who could benefit from either a higher or a lower haemoglobin threshold for RBC transfusion. Though, the results should be interpreted with caution because of imprecision in some subgroups, and because the study was a post-hoc analysis.

**Table 1.** Blood transfusions in subgroups after randomisation.

Subgroup	Lower haemoglobin threshold group	Higher haemoglobin threshold group
<b>Chronic lung disease</b>		
Yes, n = 213	n = 111	n = 102
Patients transfused – no. (%)	68 (61)	101 (99)
Units per patient – median (IQR)	1 (0 – 3)	3 (2 – 6)
No, n=785	n = 391	n = 394
Patients transfused – no. (%)	247 (63)	388 (98)
Units per patient – median (IQR)	1 (0 – 3)	4 (2 – 7)
<b>Haematological malignancy</b>		
Yes, n = 75	n = 39	n = 36
Patients transfused – no. (%)	27 (69)	36/36 (100)
Units per patient – median (IQR)	2 (0 – 7)	5 (2 – 10.5)
No, n = 923	n = 463	n = 460
Patients transfused – no. (%)	288 (62)	453 (98)
Units per patient – median (IQR)	1 (0 – 3)	3 (2 – 7)
<b>Metastatic cancer</b>		
Yes, n = 95	n = 42	n = 53
Patients transfused – no. (%)	24 (57)	52 (98)
Units per patient – median (IQR)	1 (0 – 3)	3 (2 – 5)
No, n = 903	n = 460	n = 443
Patients transfused – no. (%)	291 (63)	437 (99)
Units per patient – median (IQR)	1 (0 – 3)	4 (2 – 7)
<b>Surgery before randomisation</b>		
Yes, n = 520	n = 250	n = 270
Patients transfused – no. (%)	170 (68)	268 (99)
Units per patient – median (IQR)	1 (0 – 4)	4 (2 – 10)
No, n = 478	n = 252	n = 226
Patients transfused – no. (%)	145 (58)	221 (98)
Units per patient – median (IQR)	1 (0 – 3)	3 (2 – 5)
<b>New definition of septic shock</b>		
Yes, n = 554	n = 275	n = 279
Patients transfused – no. (%)	183 (67)	278 (100)
Units per patient – median (IQR)	1 (0 – 4)	4 (2 – 7)
No, n = 440	n = 225	n = 215
Patients transfused – no. (%)	130 (58)	209 (97)
Units per patient – median (IQR)	1 (0 – 3)	3 (2 – 6)
<b>Total TRISS<sup>a</sup></b>		
	n = 502	n = 496
Patients transfused – no. (%)	315 (63)	489 (99)
Units per patient – median (IQR)	1 (0 – 3)	4 (2 – 7)

IQR= Inter-quartile range. <sup>a</sup> As reported in Holst et al.<sup>97</sup>





**Figure 4.** Relative risks of death at day 90 with 95% confidence intervals (CI). The overall TRISS results were as reported by Holst et al.<sup>97</sup> The adjusted analyses were done using multiple logistic regressions with adjustment for the stratification variables (modified trial site (all sites including less than 10 patients were grouped to one) and haematological malignancy). Figure appearance has been amended from the published version, but no numbers have been changed.

## **10 Study II: Long-term outcomes in patients with septic shock transfused at a lower versus a higher haemoglobin threshold: the TRISS randomised, multicentre clinical trial**

### **10.1 Aim and hypothesis**

The objective of the study was to assess the effect of a lower versus a higher haemoglobin threshold on long-term outcomes – mortality and patient-reported HRQOL at one year after the randomisation. The hypothesis was that the intervention of a lower threshold (resulting in higher degree of anaemia during ICU stay) was as safe as a higher threshold (resulting in a higher exposure to RBC transfusion during ICU stay) regarding long-term outcomes.

### **10.2 Methods**

#### **10.2.1 Study design and patients**

This study was the report of the long-term secondary outcomes of the TRISS trial pre-defined in the primary protocol.<sup>96</sup>

#### **10.2.2 Outcome measures**

The long-term outcomes assessed were mortality and health-related quality of life (HRQOL). The mortality among all patients in the TRISS trial was assessed as the landmark mortality at 1 year after randomisation, and as survival at the time of longest follow-up. The longest follow-up time was at one year after the last patient was included in the trial. The HRQOL was assessed at one year after randomisation among the Danish patients (n=777) in the TRISS trial. We used the 36-item short form health status questionnaire (SF-36).<sup>110</sup> The questionnaire covers eight physical and mental health domains, all used to calculate a summary score for physical and mental health (physical component summary (PCS) and mental component summary (MCS)) each with a possible score from 0 to 100.

#### **10.2.3 Statistical analyses**

The analyses of mortality were performed in the intention-to-treat (ITT) population and we followed a predefined statistical analysis plan. The ITT population was all patients randomised (n=1005), excluding the patients who withdrew their consent (n=6) and one patient who was erroneously randomised, leaving 998 patients to be analysed for mortality. We performed a logistic regression analysis of the mortality at one year and the survival at longest follow-up was assessed by a Cox proportional hazards regression analysis accompanied by a Kaplan-Meier curve and log-rank test. The differences between the HRQOL scores were analysed with a general linear univariate model. In the primary analysis of HRQOL we included the patients who were dead at one year and they were given the worst possible score (zero). All primary analyses were adjusted for the stratification variables being trial site and haematological malignancy. Supplementary analyses were performed with adjustments for patient characteristics and baseline variables.

### **10.3 Results**

One patient was lost to follow-up for vital status at one year and additionally one patient at the time of longest follow-up; one was lost to follow-up in each allocation group (Figure 5). The median follow-up time was 21 months (range of 12 – 37 months). Among the Danish patients alive at one year after randomisation (n=330), 321 patients had the questionnaire mailed (Figure 5). Of these, 208 (65%) responded, and 182 (57%) of the patients had complete questionnaires to calculate the PCS and MCS scores. When the dead

patients at one year were added, a total of 629 (81%) of the Danish patients were included in the HRQOL analysis.

#### 10.3.1 Mortality

At one year after the randomisation 53% had died in the lower threshold group and 55% in the higher threshold group (relative risk of 0.97, 95% confidence interval (CI) 0.85-1.09; P-value 0.62) (Table 2). At a median of 21 months after randomisation the mortality had increased to 57% in the lower and 61% in the higher threshold group (hazard ratio (HR) of 0.88, 95% CI 0.75-1.03; P-value 0.12). The Kaplan-Meier curve and log-rank test showed no statistically significant difference in the probability of survival between the two groups (Figure 6). The supplementary analyses showed similar results.

#### 10.3.2 Health-related quality of life

In the primary analysis including the patients who were dead at one year, the mean difference of the PCS score was 0.4 (95% CI -2.4 to 3.1; P-value 0.79) and for the MCS score 0.5 (95% CI -3.1 to 4.1; P-value 0.79) (Table 2). The crude mean PCS scores were 37 in both groups and the crude mean MCS scores were 48 in the lower and 49 in the higher threshold group (Table 3) In the supplementary analysis adjusted for different risk factors and in the analysis only including the patients with complete HRQOL scores (n=182) the results were similar to the primary analysis.

### 10.4 Conclusion

There were no differences in long-term mortality or HRQOL between patients with septic shock and anaemia transfused with RBC transfusion at a blood haemoglobin threshold of 7 g/dl versus 9 g/dl.

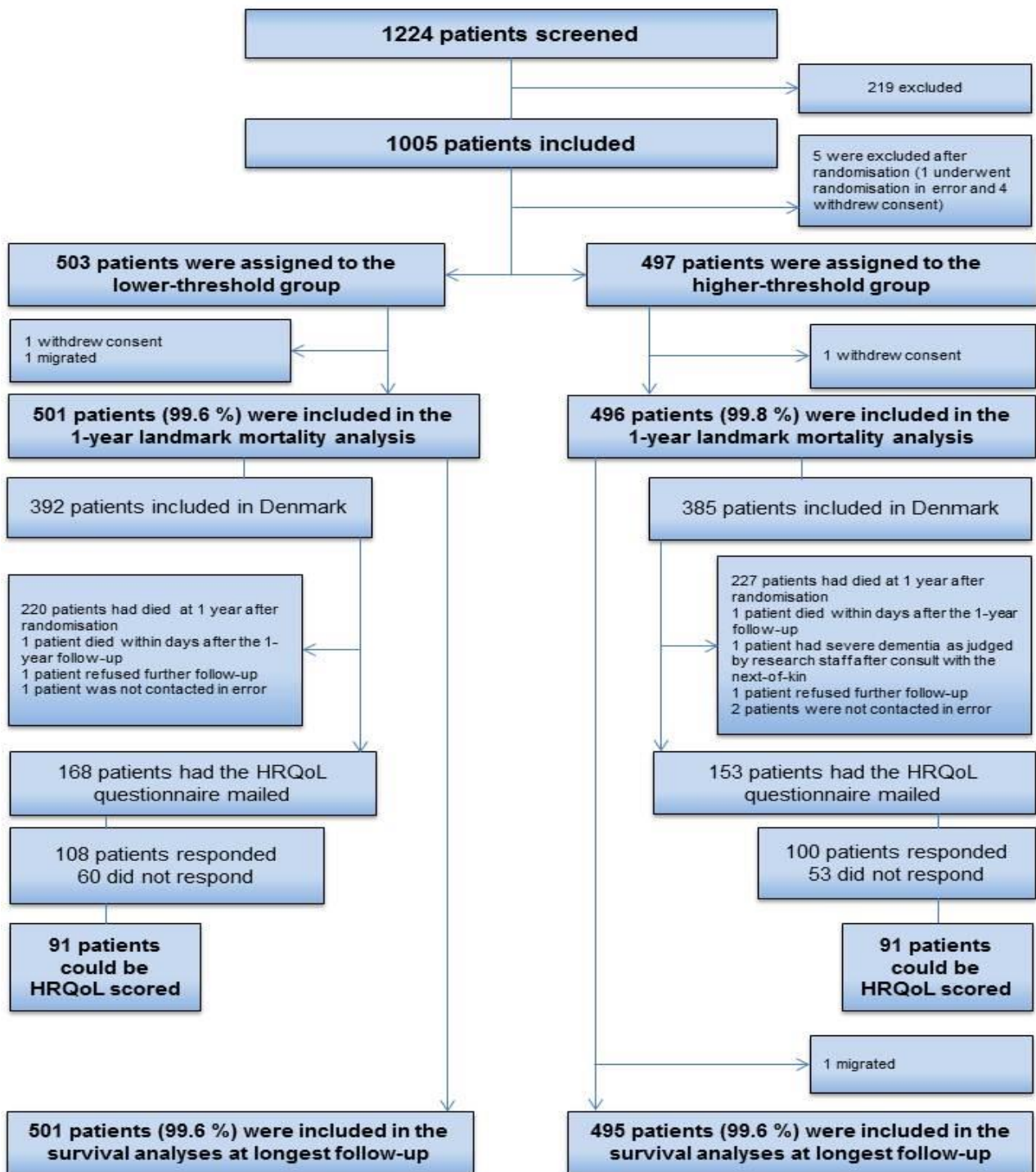


Figure 5. Trial flow of patients. HRQoL= health related quality of life.

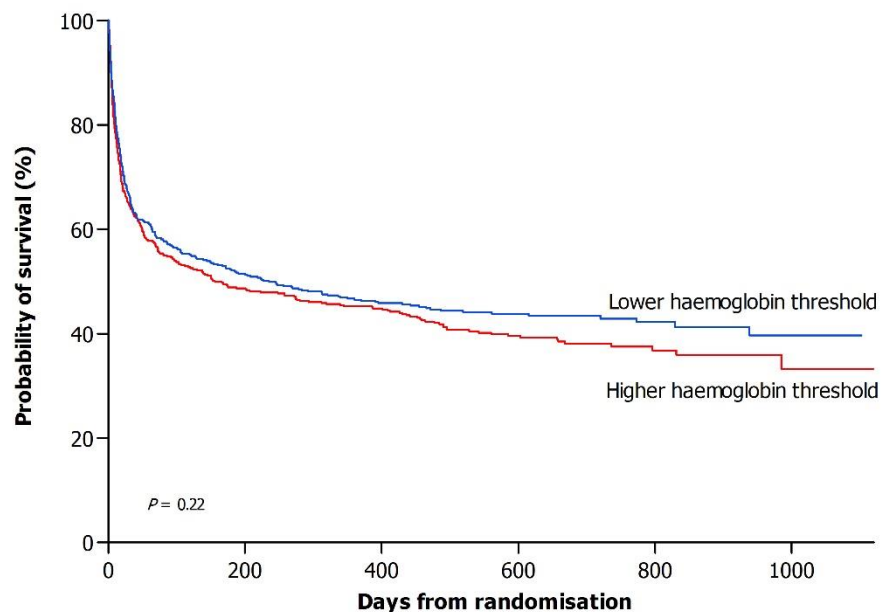
**Table 2.** Outcome measures in Study I.

	Lower haemoglobin threshold group	Higher haemoglobin threshold group	Relative Risk or Hazard Ratio or Differences between Estimated Means, all with 95% CI	P value <sup>a</sup>
<b>Mortality</b>				
Death by 1-year - no./total no. (%)	268/501 (53.5)	271/496 (54.6)	0.97 (0.85 to 1.09)	0.62
Death at the time of longest follow-up - no./total no. (%)	284/501 (56.7)	302/495 (61.0)	0.88 (0.75 to 1.03)	0.12
<b>Health-Related Quality of Life<sup>b</sup></b>				
PCS score, mean (SD)	7.6 (27.8)	7.2 (29.2)	0.4 (-2.4 to 3.1)	0.79
MCS score, mean (SD)	10.0 (36.0)	9.5 (37.7)	0.5 (-3.1 to 4.1)	0.79

CI=Confidence interval; PCS= physical component summary; MCS= mental component summary; SD=Standard Deviation.

<sup>a</sup> All analyses were adjusted for the stratification variables being modified trial site (sites including less than 10 patients were combined into 1 giving 20 sites instead of 32) and presence or absence of haematological malignancy.

<sup>b</sup> The population included all Danish patients (n=777). Patients who died within 1 year following randomisation were assigned the worst obtainable summary score value (zero). Higher summary scores indicate better quality of life. A non-parametric test was applied to test the differences between the intervention group, due to the left skewed distributions of PCS and MCS scores.



**No. at Risk**

Lower haemoglobin threshold	502	257	211	125	55	12
Higher haemoglobin threshold	496	241	207	122	49	8

**Figure 6.** Time to death or censoring. Shown are the survival curves in the two intervention groups in the intention-to-treat population, with data censored at the time of longest follow-up, which was 1-year after randomisation of the last patient. The Kaplan–Meier analysis and log-rank test showed that the survival time did not differ between the two groups at the 5% level of statistical significance.

**Table 3.** Crude HRQOL scores in Study I (un-published data).

	Lower haemoglobin threshold group (n=91)	Higher haemoglobin threshold group (n=91)
PCS score		
- mean (SD)	37 (10)	37 (12)
- median (1Q, 3Q) (range)	36 (31, 44) (18-66)	37 (26, 46) (13-61)
MCS score		
- mean (SD)	48 (12)	49 (14)
- median (1Q, 3Q) (range)	50 (40, 59) (11-74)	53 (36, 61) (17-73)

PCS= physical component summary; MCS= mental component summary; SD= standard deviation; 1Q= first quartile; 3Q= third quartile.

# 11 Study III: Effects of shorter versus longer storage time of transfused red blood cells in adult ICU patients: a systematic review with meta-analysis and Trial Sequential Analysis

## 11.1 Aim and hypothesis

The objectives were to gather evidence and to assess the effects of RBC storage time on patient-important outcomes. We hypothesised that the transfusion of fresher RBC units as compared to older would improve the outcomes for the general ICU population.

## 11.2 Methods

### 11.2.1 Study design

We conducted a systematic review with meta-analysis and Trial Sequential Analysis of randomised clinical trials. Large (> 500 participants) observational studies were included for detection of rare serious adverse events. The review was conducted according to the protocol registered in the PROSPERO database (CRD42017065366) and published.<sup>111</sup> We followed the recommendations by the Cochrane Collaboration<sup>112</sup> and prepared the manuscript according to the preferred reporting items for systematic review and meta-analysis (PRISMA) statement.<sup>113</sup>

### 11.2.2 Eligibility criteria

We included trials with adult patients in the ICU that investigated transfusion with fresher (as defined in the included trials) as compared to transfusion with older (defined in the included trials) RBC units.

### 11.2.3 Search strategy

The Cochrane library, Medline Ovid, Embase Ovid, CINAHL, BIOSIS, Science Citation Index Expanded and Conference Proceedings Citation Index-Science were searched based on a search strategy of a PICO question. A manual search was also performed. We did not restrict our search by publication status, language or date.

### 11.2.4 Outcome measures

The primary outcomes were all-cause mortality and proportion of patients with adverse events, and the secondary outcomes were HRQOL, proportion of patients with post-transfusion infections, renal failure or thrombo-embolic events and economic and blood-stock inventory outcomes, as defined in the included trials. The time-point of outcome assessment was day 90 or the time-point closest to day 90.

### 11.2.5 Data extraction and evaluation of risk of bias

Two review authors reviewed title and abstracts, and selected studies for full text screening. All studies fulfilling the eligibility criteria were included and data were extracted. The risk of bias was assessed in all the included studies by two review authors, independently.<sup>112,114</sup> The following domains were assessed: random sequence generation, allocation sequence concealment, blinding of patients and personnel, blinding of outcome assessment, incomplete outcome data, selective outcome reporting, vested financial interest and any other bias. The studies were judged as having *overall high risk of bias* if one or more domains were judged to be of *uncertain* or *high* risk of bias. Regarding the blinding of patients and personnel, we accepted that blood bank personnel and clinical personnel not involved in the treatment of

the patient was un-blinded, because of the necessary safety procedures of double-check when blood products are used.

#### 11.2.6 Grading the quality of evidence

According to the grading of recommendations assessment, development, and evaluation (GRADE) approach we graded the over-all quality of evidence for each pre-planned outcome as *high, moderate, low* or *very low*.<sup>115</sup>

#### 11.2.7 Statistical analyses

We calculated the summary relative risks (RRs) with 95% confidence intervals (CIs) for dichotomous outcomes and planned to calculate the standardized mean difference with 95% CIs for continuous outcomes. We used an adjusted P value as statistical significance level because of two primary outcomes ( $P=0.05/((2+1)/2)=0.033$ ) and 5 secondary outcomes ( $P=0.05/((5+1)/2)=0.017$ ).<sup>116</sup> We used the Review Manager (RevMan, version 5.3; Copenhagen: The Nordic Cochrane Collaboration, 2014) for data management and meta-analyses.

#### 11.2.8 Trial Sequential Analysis

In order to assess the risk of random errors due to sparse data and multiple testing we used Trial Sequential Analysis.<sup>117–120</sup> We estimated the required information size (the number of included patients and trials needed) to reject or detect an intervention effect of a relative risk reduction (RRR) or relative risk increase (RRI) of 20 percent, with a family wise error rate (FWER) of 5 percent and a power of 80 percent ( $\beta=0.20$ ).<sup>118</sup> FWER is the probability of a false positive test (type-I error) when testing a family of hypotheses. We used the TSA program version 0.9 beta ([www.ctu.dk/tsa](http://www.ctu.dk/tsa)).<sup>120,121</sup>

#### 11.2.9 Assessment of heterogeneity

We looked for heterogeneity in the description of the trial participants in each study, by inspection of the Forest plots and the estimates of statistical heterogeneity, the diversity ( $D^2$ ),<sup>122</sup> and the inconsistency ( $I^2$ ). We also planned to perform subgroup analyses (using the test of interaction) of trials with overall high versus low risk of bias and pooling the results of different sub-populations within each trial – e.g. patients with sepsis or septic shock as compared to patients not having sepsis or septic shock.

### 11.3 Results

#### 11.3.1 Trial characteristics

We identified seven RCTs including a total of 18,283 patients in the ICU (Table 4).<sup>85,87,88,123–126</sup> We also identified three publications of two post-hoc analyses<sup>127,128</sup> and one report of predefined secondary outcomes<sup>129</sup> of one included trial.<sup>85</sup> We identified six large observational studies.<sup>130–135</sup> Two trials were judged as having over-all low risk of bias.<sup>85,88</sup>

#### 11.3.2 Description of the intervention

The observed storage time in the intervention groups of fresher blood varied from 2 to 12 days of storage and in the comparator groups of older blood varied from 21 to 28 days of storage.

#### 11.3.3 Primary outcomes

Five trials reported mortality. In the primary analysis with trials of over-all low risk of bias the RR of death at day 90 was 1.04 (95% CI 0.97-1.11; 7349 patients;  $P=0.32$ ;  $I^2=0\%$ ; TSA-adjusted 95% CI 0.93-1.15) for transfusion of fresher versus older RBC units. The Z-curve in the TSA graph reached the area of futility for a



relative risk change of 10% (Figure 7). In the subgroup analysis of mortality in trials with overall low versus overall high risk of bias, there was no sign of bias effect (Figure 8). The quality was judged to be of *high* certainty and *critical* importance. The two trials judged as overall low risk of bias both reported adverse transfusion reactions with some clinical heterogeneity in the type of reactions registered. The relative risk for an adverse reaction was 1.26 (95% CI 0.76-2.09; 7332 patients; P=0.36; TSA adjusted CI 0.16-9.87). The GRADE quality was judged to be *very low*.

#### 11.3.4 Secondary outcomes

No difference was found in the proportion of infections (RR 1.08, 95% CI 0.96-1.20; two trials, 7332 patients; P=0.23; TSA adjusted CI 0.90-1.27) The cumulative Z curve reached the futility area in the TSA graph for a relative risk change of 20% and the GRADE quality was judged to be moderate.

No meta-analysis was performed on the outcome of proportion of renal failure, thrombo-embolic events, HRQOL or economic outcomes, but the results from individual trials reporting one or more of these outcomes showed no statistically significant differences between the intervention group of fresher blood versus the comparator of older blood.

#### 11.3.5 Subgroup analyses and observational studies

None of the planned subgroup analyses of the intervention effect in subpopulations were performed. Four of the six large observational studies among patients in the ICU reported an association of increased RBC storage and worsened clinical outcomes (complicated sepsis and hospital mortality) and no association to other outcomes (severe kidney failure and 90-day mortality).

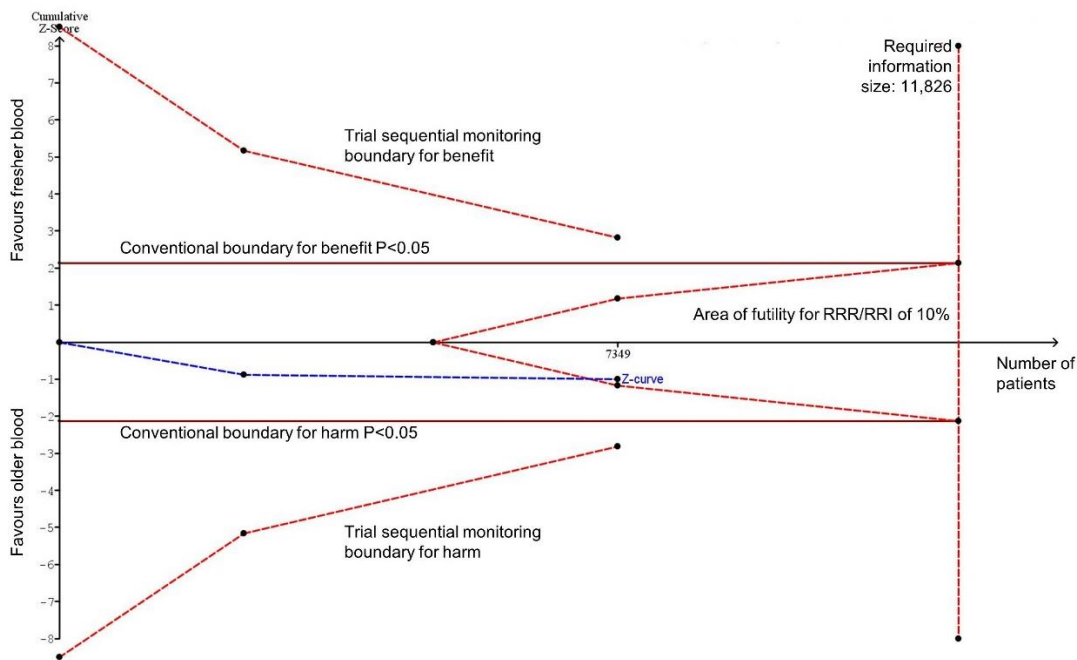
### 11.4 Conclusion

We did not find a benefit of using fresher versus older blood for patients in the ICU. The TSA showed the required information size was reached for both mortality and post-transfusion infections and we may reject a more than 10 percent relative risk change of death and a more than 20 percent relative risk change of post-transfusion infections when comparing fresher versus older blood for transfusion in adult patients in the ICU.

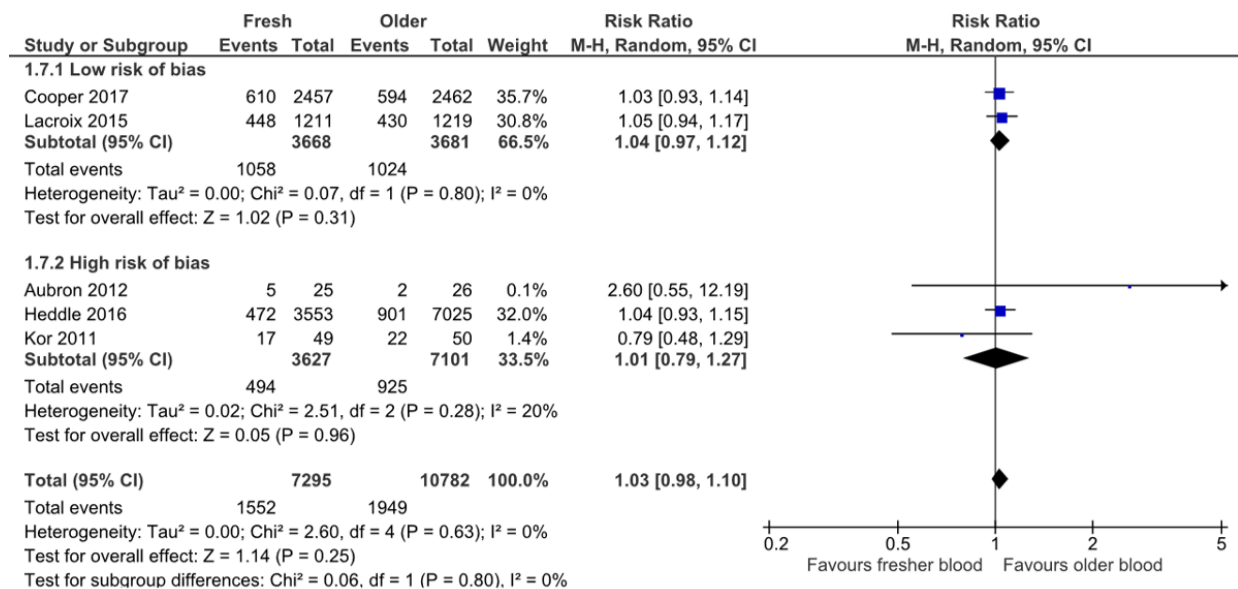
**Table 4.** Characteristics of the included trials.

Study	Country	Inclusion period	No of patients/ No of trial sites	Clinical setting	Eligibility criteria	RBCs – type/ suspension/ leukocyte reduced	Transfusion guidelines/ duration of intervention	Intervention and comparison – storage time of RBC unit
Aubron 2012	Australia	September 2010 – January 2011	52/2	General ICUs	Patients aged $\geq 18$ years; prescribed at least one RBC unit.	Allogeneic/ SAGM/ leukocyte reduced	At discretion of the clinician / until ICU discharge	Intervention: freshest RBC unit Comparator: standard issue <sup>a</sup>
Cooper 2017	Australia/ Finland/ Ireland/ New Zealand/ Saudi Arabia	October 2012 – December 2016	4994/59	General ICUs	Patients aged $\geq 18$ years hospitalised in ICU with an anticipated stay of at least 24 hours; decision to transfuse at least one RBC unit.	Allogeneic/ SAGM/ leukocyte reduced	At discretion of the clinician / during the index hospital stay.	Intervention: freshest RBC unit Comparator: oldest RBC unit
Damiani 2015	Italy	February 2011 – February 2012	20/1	General ICU	Patients aged $\geq 18$ years with sepsis, severe sepsis or septic shock as diagnosed according to standard criteria and requiring blood transfusion.	Allogeneic/ NA/ not leukocyte reduced	$\leq 8$ g/dl or as indicated by the attending physician / NA	Intervention: $<10$ days Comparator: $> 15$ days
Hedde 2016	Australia/ Canada/Israel / USA	April 2012 – October 2015	10,578 in the ICU subgroup/6	All hospitalised patients	Hospitalised patients; $\geq 18$ years; requiring at least one RBC unit.	Allogeneic/ SAGM <sup>b</sup> / leukocyte reduced <sup>b</sup>	According national guidelines / throughout the initial admission and any subsequent admissions during the study period. Minimum follow-up time of 30 days.	Intervention: freshest RBC unit Comparator: oldest RBC unit
Kor 2012	USA	June 2008 – May 2010	100/1	General ICU	Patients aged $\geq 18$ years; ET and MV; arterial catheter in situ; an order for RBC transfusion.	Allogeneic/ NA/ leukocyte reduced	At discretion of the clinician but not above 9.5 g/dl / the first RBC transfusion after randomisation	Intervention: $\leq 5$ days Comparator: standard issue <sup>a</sup>
Lacroix 2015	Belgium/ Canada/ France/ Netherlands/ UK	March 2009 – May 2014	2510/64	General ICUs	Patients aged $\geq 18$ years; first RBC transfusion prescribed within 7 days after ICU admission; MV expected for $\geq 48$ h	Allogeneic/ SAGM/ leukocyte reduced	At discretion of the clinician / until hospital discharge or 90 days after randomisation	Intervention: $<8$ days Comparator: Standard issue <sup>a</sup>
Walsh 2004	Scotland	November 1999 – December 2000	29/1	General ICU	Patients aged $> 16$ years; planning of transfusion of 2 RBC units with no sign of bleeding; haemoglobin level $\leq 9$ g/dl; no RBC transfusion 48 hours prior to inclusion.	Allogeneic/ SAGM/ leukocyte reduced	NA / 10.5 hours (one transfusion-episode of 2 RBC units)	Intervention: $\leq 5$ days Comparator: $\geq 20$ days

ET=Endotracheal tube; ICU=intensive care unit; IQR=inter-quartile range; MV=mechanical ventilation; N=not available; RBC=red blood cell; SAGM=saline, adenine, glucose and mannitol. <sup>a</sup> For all studies, standard issue was the oldest compatible blood unit available. <sup>b</sup> According to the trial protocol. Information from the Israeli site was not available.



**Figure 7.** TSA graph of the primary analysis. Two trials with overall low risk of bias reporting mortality at day 90 were included. The graph presents the TSA of the anticipated relative risk change of 10%, alpha of 3.3%, beta of 20%, a-priori planned Diversity of 20% (the actual Diversity was in fact 0%). The relative risk of death was 1.04 and the TSA adjusted confidence interval 0.93 – 1.15. The cumulative Z-curve (the blue dotted line) reached the area of futility, and we may exclude a 10% relative risk increase or reduction.



**Figure 8.** Forest plot of mortality in trials with overall low risk of bias versus trials with overall high risk of bias. Size of squares for risk ratio reflects weight of trial in pooled analysis. Horizontal bars represent 95% confidence intervals. Arrow on horizontal bar indicates that the 95% CI is outside the shown range.

## 12 Study IV: Storage time of red blood cells in patients with septic shock

### 12.1 Aim and hypothesis

The objectives were to describe the RBC transfusions given before, during and after the ICU stay among a Danish cohort of patients with septic shock; to describe the RBC transfusions given in the TRISS trial and in the two allocation groups; and to describe the crude mortality in both cohorts in groups of storage time and amount of RBC units received.

### 12.2 Methods

#### 12.2.1 Study design

The Study IV was a descriptive cohort study of two populations of patients with septic shock, named the DK cohort and the TRISS cohort.

#### 12.2.2 Patients and data

The DK cohort comprised adult patients with septic shock registered in a electronic medical record system in 8 different ICUs in Denmark between 1<sup>st</sup> of January 2008 and 31<sup>st</sup> of December 2010. The TRISS cohort comprised the patients included in the TRISS trial. Data on patient characteristics and baseline variables (for the DK cohort the baseline was admission to ICU and for the TRISS cohort the baseline was time of randomisation) were collected from the medical record system or the TRISS trial database. Transfusion data (RBC issued, storage time and blood type of recipient and RBC unit) were collected from the SCANDAT database,<sup>136</sup> or from the blood banks servicing the sites in the TRISS trial. The collection of transfusion data was described in the original TRISS trial protocol.<sup>96</sup>

#### 12.2.3 Exposure

The exposures of interest were the RBC units transfused, the number of units and their storage time, within the period of 14 days prior to ICU admission and until 90 days after (the DK cohort) or only while in the ICU (the TRISS cohort).

#### 12.2.4 Outcome measures

The outcome was mortality, described at day 90 and at 1 year.

#### 12.2.5 Descriptive statistics

A protocol describing the data-handling and the descriptive statistics was published online before any statistics were performed.<sup>137</sup> We planned to describe the RBC transfusions, the blood type of the patients and RBC units, the storage time and the number of RBC units transfused. We grouped the patients in to exposure groups: no transfusions; only fresh RBCs (<7 days of storage); intermediate RBCs (mixture of storage time, from 1 to 42 days); only old RBCs (>24 days); and the number of transfusions within the exposure groups.

## 12.3 Results

### 12.3.1 Patients and transfusions

The DK cohort included 1637 patients with septic shock. From the SCANDAT database, we identified 20,239 RBC transfusions issued to these patients within the period of 14 days before and 90 days after first ICU admission (Figure 9). During the observation period, 85 percent of the patients were transfused; 33 percent before the admission, 77 percent while in the ICU, and 36 percent after the ICU stay. They received a

median of 7 (IQR 2-15) units and the median of the mean storage time per patient was 14 (IQR 10-18) days. Of the transfused patients, 3 percent received exclusively very fresh and 4 percent received exclusively very old blood.

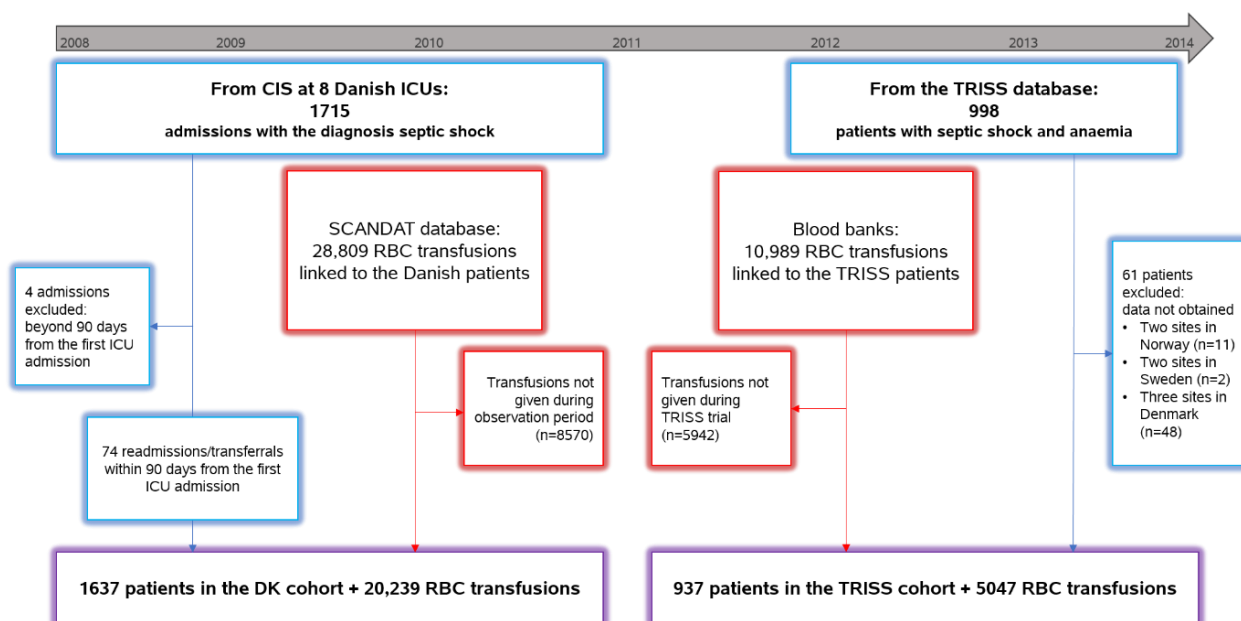
Data of transfusions were obtained for 937 patients in the TRISS cohort. 77 percent were transfused, and they received a total of 5047 RBC units while in the ICU from randomisation and 90 days forward (figure I). They received a median of 3 (IQR 2-7) with a median of the mean storage time per patient of 17 (IQR 13-23) days. There was no difference in the storage time of the blood transfused to patients in the lower threshold group as compared to the higher threshold group.

### 12.3.2 Mortality

At day 90, 959 (59%) of the patients in the DK cohort had died, which increased to 1054 (65%) deaths at one year. In the TRISS cohort 400 (43%) of the patients had died at day 90, and at one year the deaths were 493 (53%) patients. The mortality in the groups of very fresh, intermediate and very old, and the amount of blood received are presented in Figure 10.

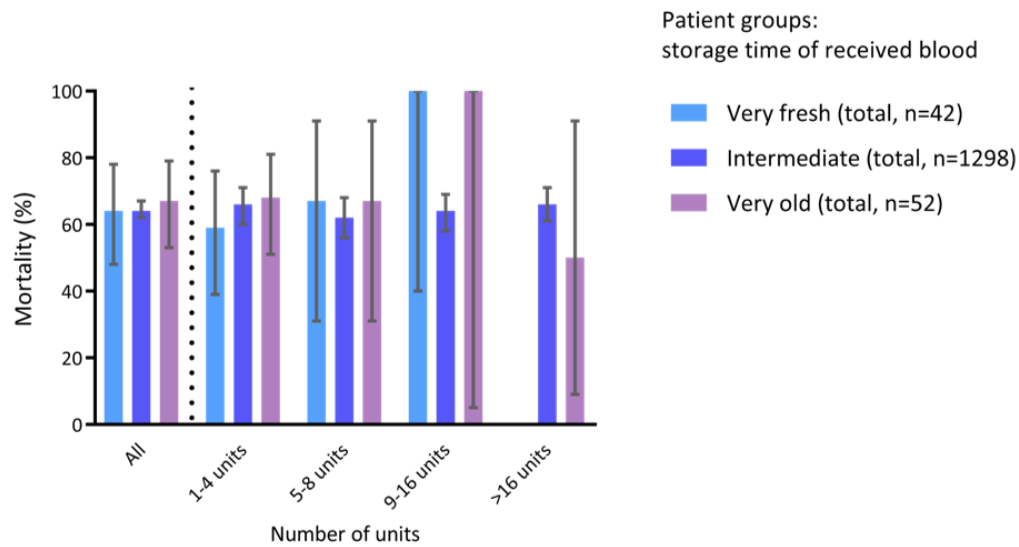
## 12.4 Conclusion

Patients with septic shock received blood both before, during and after their ICU stay, but the majority were transfused while in the ICU. The mean storage time was the same in the allocation groups of the TRISS trial, and the general exposure to blood of less than 7 days or of more than 24 days is limited, and especially an exclusive exposure to the extremes of storage time was rare and no obvious danger was detected.

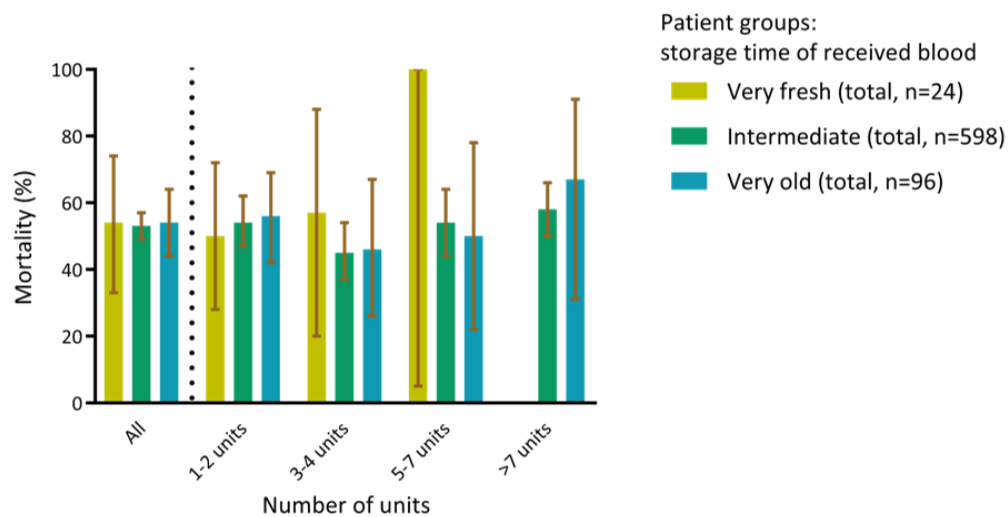


**Figure 9.** Patient selection. CIS is the ICU-specific electronic medical record system, Critical Information System, by Daintel, Copenhagen, Denmark. TRISS: Transfusion Requirements in Septic Shock. SCANDAT: Scandinavian Donations and Transfusions Database.

## A. DK



## B. TRISS



**Figure 10.** Crude 1-year mortality with 95% confidence interval in the DK (A) and the TRISS (B) cohort in groups of storage time and number of units received. On the left side of the dotted vertical line is the total cohort (with number of patients presented in the figure), and on the right side the total cohort is separated into groups based on the number of transfusions received. Bars indicating 95% confidence interval.

## 13 Discussion

### 13.1 Principal findings

The principal findings of the two first studies were; the restrictive transfusion strategy of using a haemoglobin level of 7 g/dl compared to 9 g/dl as threshold for RBC transfusions in patients with septic shock did not result in differing effects regarding 90-day mortality among subpopulations of patients with septic shock and comorbidity, patients with septic shock undergoing surgery or patients fulfilling the Sepsis-3 criteria for septic shock as compared to the complementary subpopulations; furthermore, the restrictive transfusion strategy as compared to the liberal did not result in a differing mortality at 1-year, probability of survival at the time of longest follow-up among all patients, or differing patient-reported HRQOL among the Danish patients in the TRISS trial.

The systematic review with pooled data from the recent RCTs with meta-analysis and TSA showed that among adult patients in the ICU there was no increased or reduced mortality, adverse events or post-transfusion infections when transfusing fresher versus older RBC units. The TSA supported the conventional meta-analyses and showed that the required information size was reached for a relative risk change of 20 percent and the area of futility was reached with a relative risk change of 10 percent for mortality. For the risk of post-transfusion infection, the area of futility was reached for a relative risk change of 20 percent. The subgroup-analysis of patients with sepsis or septic shock versus no sepsis or septic shock was not able to be performed due to missing separated subgroup-data.

The descriptive cohort study showed that patients with septic shock received an important part of the RBC transfusions both before their ICU admission and after their ICU stay. However, still the majority of transfusions were given in the ICU, where patients were exposed to a mixture of RBC unit storage times. Only small fractions of patients were exposed exclusively to very fresh or exclusively very old blood, to which there were no obvious danger observed.

### 13.2 Strengths and limitations

#### 13.2.1 Study I

##### 13.2.1.1 Design

The design and conduct of the TRISS trial were major strengths of Study I. The trial was pragmatic regarding patient selection with few inclusion and exclusion criteria; the participating trial sites were in different countries and in both university and non-university hospitals; the treatment, apart from the allocated transfusion threshold, was by discretion of the treating clinician and of standard practice; and blinding was used where feasible (central, computer-based randomisation and blinding of the outcome assessors).

The subgroup analyses of this study were not described in the primary protocol of the TRISS trial and the study was designed post-hoc. To prevent data-driven analysis and minimize the risk of chance finding, we prepared a protocol and statistical analysis plan before any statistics were performed, and the subgrouping was based on clinically meaningful patient sub-populations, where differing intervention effects were biologically plausible. Despite the limitations in performing post hoc subgroup analyses, it is important to investigate new hypotheses and to gain the full potential of a high-quality RCT.

### 13.2.1.2 Patient selection

When selecting subgroups post-hoc, we were at risk of selective reporting bias and imprecision due to decreased power and small sample sizes. The selective reporting bias was minimized by preparing a protocol with a description of the subgroups of interest and the planned analyses. We limited the number of subgroups investigated and chose the ones, who could be identified at baseline and the ones, who we hypothesised could respond differently to the intervention. The choice of subpopulations investigated in this study was based on the existing literature, suggesting that there may be patients with specific comorbidities or characteristics, that would tolerate a higher degree of anaemia and would perhaps be more susceptible to the risks inherent in RBC transfusion, and others who would be more susceptible to anaemia and would benefit from a more liberal transfusion strategy.

Patients with comorbidities have more often chronic anaemia and might have a delayed recovery from severe anaemia after a period of septic shock. They could benefit from being transfused at a higher haemoglobin level. Patients with chronic lung disease, including patients with chronic obstructive lung disease (COPD), have been described often as having chronic anaemia associated with increased mortality.<sup>138</sup> In one case-control study focusing on mechanically ventilated patients with COPD with and without anaemia in an ICU, anaemia was associated with an increased 90-day mortality.<sup>139</sup> The optimal transfusion threshold for patients with chronic lung disease and anaemia had not been investigated in a RCT, and the results from the subgroup analysis of this study, would contribute to the knowledge of the patient population.

Patients with cancer, both solid tumours and haematological cancers, often suffer from chronic anaemia due to the treatments of surgery and chemotherapy, nutritional problems and the underlying nature of their disease. Again, chronic anaemia has been associated with increased mortality,<sup>140</sup> and this group of patients often receives a large amount of RBC transfusions. The results of the subgroup analyses with the patients with haematological malignancy and metastatic cancer were limited by the small number of patients and showed very wide confidence intervals. We could have pooled all patients with malignancies into one subgroup analysis, but the group would still be small and result in an imprecise estimate of the intervention effect. In the existing literature and other trials assessing transfusion thresholds in patients with malignancy, there is a segregation between the patients with solid and haematological cancer,<sup>141–145</sup> and therefore we kept the two patient groups separated.

Patients undergoing surgery was another important, but also a heterogeneous subpopulation to investigate. Previous RCTs with surgical patients and the pooled data in meta-analysis have suggested a benefit of using a restrictive transfusion strategy.<sup>73,146</sup> However, there may be differing directions of the estimated effect in patients with a more vulnerable cardiovascular system (patients with cardiovascular disease undergoing surgery or patients undergoing cardiac or vascular surgery),<sup>147,148</sup> and the results from this subgroup analysis could be diluted by practice misalignment.<sup>149</sup>

We amended the original protocol of Study I after publication of the Sepsis-3 criteria.<sup>12</sup> We found it important to investigate the consistency of the results from the TRISS trial among patients who would have fulfilled the new criteria for septic shock. We were able to base the dichotomization on baseline values of the highest lactate value within 24 hours before randomisation and the use of vasopressor by the time of randomisation. The inclusion of this subgroup increased the external validity of the TRISS trial results.



### *13.2.1.3 Statistics*

We followed the recommendations for subgroup analyses,<sup>150</sup> and used the test of interaction. The test of interaction tests the null hypothesis, that the relative effectiveness of the intervention is the same, when grouping patients in subgroups, based on different baseline or patient characteristics.

The risk of a chance finding (a type I error) was increased because of multiple testing when grouping the trial population into 5 different subgroups. We did not present the P-values for the within-subgroup comparison of the intervention effect. As a result we limited the number of analyses from 10 to 5 – reducing the risk of finding at least one statistically significant difference where there were actually none (finding of a false positive result) from approximately 40 percent to 25 percent.<sup>151</sup> Additionally, we only performed the analyses on one outcome and by this means limited the number of tests and risk of chance findings.

We could have planned correction for multiple testing and adjusted the level of significance. The adjusted significance level for each test of interaction could be determined by using the Bonferroni correction, where the level of alpha or type 1 error is divided by the number of tests. If we used a FWER of 5%, this would lead to a statistical significance level for each interaction test of 1% ( $P=0.05/5=0.01$ ). However, we did not perform these corrections, but instead prepared to further investigate the clinical significance of a possible trend in the tests of interaction (if one test showed a significance level of 0.05 or less) (described in the statistical analysis plan), and we would interpret the results cautiously, as hypothesis-generating and not firm evidence of a subgroup effect.

### *13.2.1.4 Missingness*

We had missing data on highest lactate level and vasopressor use at baseline for four patients who could not be dichotomised in the group of patients fulfilling the Sepsis-3 criteria for septic shock or those not fulfilling the criteria. This is a very low number of missing values, and the exclusion of these patients from the analysis should not have changed the results notably. We used the primary outcome from the TRISS trial, 90-day mortality, and there were no missing outcome data.

## **13.2.2 Study II**

### *13.2.2.1 Design*

Again, the design of the TRISS trial is a major strength of Study II. Additionally, the secondary outcomes of the TRISS trial reported in this study were predefined in the TRISS trial protocol,<sup>152</sup> but as the power calculation was not based on these outcomes, the power to detect statistically significant differences was reduced.

### *13.2.2.2 Outcomes*

Both mortality and patient-reported functional status or quality of life are essential and emphasised patient-centred outcomes to assess in RCTs among critically ill patients.<sup>153</sup> The importance of assessing the effect of an intervention on survival beyond the first 90 days has also been emphasised.<sup>154</sup>

The SF-36 questionnaire has been validated for use in patients with sepsis,<sup>155</sup> and the shorter version, SF-12, has been used for follow-up after a RCT of restrictive versus liberal transfusion strategies.<sup>156</sup> The optimal timing for HRQOL follow-up remains to be established.<sup>157</sup> The optimal approach may be to have a baseline value and compare the decline and/or restitution to the baseline value at different time points

after the episode of septic shock and ICU stay – e.g., follow-up at 6 months, one, two and three years. But the idea of follow-up at one year together with survival, was for the outcome measures to complement each other. A small, non-statistically significant difference in mortality could be noticeable and even statistically detectable when measuring the HRQOL. And the fact that the point of interest was the difference between the two intervention groups, and not the level of the scores, further limits the need for a baseline value, and several, sequential follow-up scores of HRQOL. Other tools to assess, e.g. the cognitive impairment, could be useful and informative when investigating the effect of RBC transfusion and anaemia, but also qualitative comparisons of patient experience and preferences are becoming more common and recommended when planning clinical trials.<sup>158</sup> The idea of involving the patients in the design and in the follow-up phase of clinical trials has become more debated since the TRISS trial started, and in retrospect, a more nuanced follow-up regarding HRQOL could have been optimal also in the TRISS trial.

It would be interesting to compare the causes of death after septic shock and allocation to a lower versus a higher haemoglobin level for transfusion. It could be speculated, that the lower threshold and a lower haemoglobin level during septic shock could result in persisting anaemia and increased cardiovascular morbidity and mortality.<sup>73</sup> A higher exposure to RBC transfusions could on the other hand result in persistent inflammation and immunosuppression causing increased risk of secondary infections and hence death caused by infection.<sup>77</sup> Another disease suspected to be associated to the exposure to RBC transfusion is cancer.<sup>159</sup> The cause of death is difficult to investigate in large scale studies, where a thorough journal audit of all deaths occurring before a certain time-point is an extremely time-consuming task. The use of national cause of death registries could be an option, but even in a country like Denmark, where the register is almost complete,<sup>160</sup> the accuracy of the register is limited by low number of autopsies and erroneous coding.<sup>161–163</sup> Therefore, we did not consider cause of death as a reliable or very informative outcome to include in the study.

#### *13.2.2.3 Statistics*

The outcomes were defined in the original TRISS trial protocol, and we adhered to our predefined statistical analysis plan to prevent data-driven analysis and risk of chance findings. By the report of these secondary outcomes, all the predefined primary and secondary outcomes of the TRISS trial were published.

When comparing the HRQOL between two groups treated with different transfusion strategies, and the mortality at the time of follow-up is very high among these patients, we judged it necessary to include the people who died. The primary outcome of the TRISS trial showed a small, statistically insignificant difference in 90-day mortality in favour of the lower threshold group.<sup>97</sup> We also expected to find a similar difference at one year, and in order to let the mortality weigh in the analysis of HRQOL, we included the dead, and gave them the worst possible score of zero. This is a measure, which has been suggested and validated,<sup>164–166</sup> but may decrease the power to detect any differences, because the standard deviations of the result become very large.

#### *13.2.2.4 Missingness*

We had high missingness in the HRQOL follow-up: out of the 321 questionnaires mailed out to the patients who survived 1 year after the inclusion in the trial, only 182 questionnaires were returned and complete enough to calculate the summary scores. This is an absolute missingness of 43 percent, but because we had planned to include the non-survivors in the primary analysis, the missingness was only 19 percent – with a corresponding response rate of 81 percent.

Different approaches to reduce the missingness have shown increased response rates in other trials. An additional follow-up by phone-call after mailing the paper version, and the approach of completing the questionnaire by telephone interview resulted in a very low number of unanswered questions in the questionnaire and a response rate of 87 percent in the INSTINCT and the EAT-ICU trials.<sup>167,168</sup> Though the method of completing the questionnaire may affect the results.<sup>169</sup> We could also have used the shorter questionnaire, SF-12, particularly when we only used the summary scores for comparison.<sup>170</sup> This might have improved the response rate and the completeness of the responses.<sup>156,171</sup>

We investigated the nature of the missingness in all the patients, who could not be PCS and MCS scored, using P of Little's test (test of missing completely at random) and a search for additional variables correlated with the missingness (a search for auxiliary variables by an expectation-maximisation algorithm). The only difference detected were lower age among the patients who did not answer/incomplete questionnaires, but after adjustment for multiple testing (Bonferroni-method), this difference was not statistically significant, and together with a P of Little's test of 0.69, the conclusion was, that the missing questionnaires were missing completely at random. After the publication of this study, we performed an additional investigation of the missingness.<sup>172</sup> The aim was to describe the non-responders (those not returning the questionnaire) and to investigate if any characteristics of the patients were associated with not responding. The pre-planned covariates investigated were obtained from the TRISS database (age and number of days in hospital post randomisation), and additional data from nation-wide registries (level of education, cohabitation and employment status at time of follow-up). The multi-variable logistic regression analysis showed an association of not responding and younger age, living alone and more days in hospital, and hence, results suggested, that the non-responders were a selected group of patients, and were not missing completely at random. However, we do not know if their HRQOL scores would differ from the ones responding, and therefore we do not know if they were missing at random or not missing at random. The number of non-responders were the same in each treatment group, but we cannot determine if the results of Study II may have been affected by selection bias, and this limits our confidence in the results of the HRQOL.

### 13.2.3 Study III

#### 13.2.3.1 Design and methods

The strength of Study III was the strict, pre-planned and transparent methodology. The methods were thoroughly described in the protocol published before the commencement of the systematic review.<sup>111</sup> We followed the recommendations of the Cochrane Collaboration regarding multiple-database, structured literature search; selection of studies with no limitations of publication date, language or publication status; assessment of risk of bias and full transparency in the judgements (presented in the supplementary material); and evaluation of heterogeneity. Furthermore, we based our conclusions of the results on the trials of overall low risk of bias and assessed the overall quality of the evidence using the GRADE recommendations.

We limited the expected clinical heterogeneity between the trials by focusing on patients in the ICU. As described in the background of this thesis, the critically ill patients in the ICU could be more vulnerable to the detrimental effects of the storage lesion, because they are frequently exposed to RBC transfusion, they often suffer from tissue hypoxia, and an immunomodulation could be boosted. Since there is high mortality among patients in the ICU, an effect of RBC storage time on mortality could be possible to detect in this

patient population. The differences in the mortality of the included trials revealed, that there in fact was some clinical heterogeneity. In the three largest trials a hospital mortality of 13 percent was reported in the trial by Heddle and colleagues (the INFORM trial)<sup>87</sup> and a 90-day mortality of 36 percent in the ABLE trial and 24 percent in the TRANSFUSE trial.<sup>85,88</sup> Also, according to the baseline data, the patients in the ABLE trial received more organ-supportive therapy than in the TRANSFUSE trial, indicating a more severely ill group of ICU patients in the ABLE trial. Though when we pooled the outcome data in the meta-analysis, no statistical heterogeneity was present, and the results were extremely uniform between the trials, supporting the choice to pool the data.

#### *13.2.3.2 Use of Trial Sequential Analysis*

By using the recommendations of the Cochrane Collaboration, we were able to identify systematic errors and we limited the primary analysis and conclusion to the trials of overall low risk of bias. But when pooling trial data in meta-analysis, and as meta-analyses are updated and new ones are published, the risk of chance findings will increase due to repetitive testing and insufficient sample sizes.<sup>173,174</sup> We therefore introduced a sequential method, the Trial Sequential Analysis,<sup>118</sup> as a sensitivity analysis to account for the risk of type I errors.<sup>117,119</sup> On the basis of a pre-defined level of beta (risk of type II errors), alpha (risk of type I errors), event rate in the control group, and uncertainty due to heterogeneity between trials (diversity) we could estimate the required information size from which to draw firm conclusions on a certain pre-defined intervention effect.<sup>175</sup> We pre-planned the adjustments of the TSA (level of beta, alpha, a conservative estimate of diversity) and the estimated intervention effect.<sup>111</sup> Because the required information size was reached for a relative risk change for mortality of 20 percent, we performed an additional post-protocol TSA of a intervention effect of 10 percent relative risk change.

The use of TSA is not easily understood, and there is a risk of not being able to perform the TSA correctly and that the communication of the results may be misunderstood.<sup>176</sup> Albeit, the limitations of a meta-analysis to condense the evidence without committing either type I or II errors, call for the use of sequential methods.<sup>175</sup> Consistent use and clear communication of the reasoning, methods, and results may lead to a higher understanding in the research community and among clinicians.

### **13.2.4 Study IV**

#### *13.2.4.1 Design and statistics*

This study was an observational study with an inherent risk bias due to confounding by indication. The patients in the TRISS trial were randomised to different thresholds for transfusion, but the amount of blood received would depend on the time spent in the ICU and of the severity of the disease (or the severity of their anaemia). Hence, when grouping patients according to the amount of blood received or the storage time of the blood received, the association to the outcome would be affected by time-dependent confounding and immortal time bias.<sup>177</sup> We did not find an optimal approach or statistical model, which could control for all possible interactions, and where the results could be interpreted for clinical use. We therefore chose a descriptive cohort design to describe blood transfused to patients with septic shock, pre-planned the data-handling and descriptive statistics, and presented the crude mortality data with cautious interpretation. The investigation of two separate cohorts strengthened the external validity, and a strength of the internal validity was the use of data from high-quality and complete databases.<sup>97,136,178</sup>

#### 13.2.4.2 Patient selection

The data from the TRISS trial were limited to transfusions within the ICU.<sup>96</sup> We therefore included the cohort of patients with septic shock in Danish ICUs pre-TRISS, to get a more nuanced picture of the exposure to RBC transfusion among patients with septic shock. According to the crude 90-day and 1-year mortality in the DK cohort, which was higher than observed in previous studies among patients with septic shock in Scandinavia,<sup>29,57</sup> this was a group of severely ill patients. The positive predictive value was estimated in a validation study prior to data extraction, which showed that 99 percent of patients with the diagnosis of septic shock in the medical records fulfilled the criteria of the 1992 definition of septic shock.<sup>9,178</sup> However, we did not assess the false negative rate, and were not able to find the patients fulfilling the criteria, but lacking the diagnosis.

### 13.3 Current evidence and clinical implications

#### 13.3.1 Effect of transfusion thresholds among different populations

The knowledge from the TRISS trial including the pre-planned subgroup analyses of the primary publication (patients aged 70 or less/aged more than 70; chronic cardiovascular disease (yes/no); and SAPS II of 53 or less/SAPS II of more than 53), and the subgroup analyses included in this thesis (Study I), did not detect any subpopulations of patients with septic shock, where the intervention effect differed statistically significantly. Neither was using a lower haemoglobin level as threshold for RBC transfusions associated with a higher 90-day mortality. These results are generally in line with the evidence from other trials assessing a restrictive versus a liberal transfusion strategy in different settings. The updated Cochrane systematic review by Carson and colleagues published in 2016 included 31 RCTs; among these, 6 were in the critical care setting, 16 in surgical settings, and two in the haematological setting.<sup>90</sup> The pooled evidence of 23 trials reporting 30 day mortality showed an statistically insignificant difference in the relative risk of death (RR 0.97; 95% CI 0.81 to 0.16) between patients treated with a restrictive versus liberal transfusion strategy, but the restrictive transfusion strategy reduced the risk of being transfused by 43 percent.<sup>90</sup>

There are still subgroups of patients about whom knowledge of the optimal transfusion threshold is limited – and these include patients with cancer and haematological malignancy.<sup>90</sup> There was a limited power of detecting any true difference of the intervention effect among the patients with cancers in our study, and the evidence from other trials is somewhat conflicting. Two single-centre trials from Brazil investigated the effect of transfusion thresholds (7 g/dl versus 9 g/dl) in two groups of oncology patients in the ICU – one undergoing major surgery and one with septic shock.<sup>142,145</sup> They found in both trials the liberal transfusion strategy to be superior regarding a composite outcome including 30-day mortality among the surgical patients and the secondary outcome of 90 day mortality among the patients with septic shock.<sup>142,145</sup> One recent feasibility trial of transfusion thresholds (7 g/dl versus 8 g/dl) among patients with leukaemia showed no harm by the restrictive strategy, but reduced the use of RBC transfusion.<sup>144</sup> The decreased utility of RBC transfusions and safety with a restrictive strategy were also the conclusions from a recent systematic review of both observational studies and RCTs among patients with haematological malignancy and solid cancers,<sup>143</sup> and an observational study among patients with haematological malignancy and septic shock found a possible association between RBC transfusions and in-hospital death.<sup>179</sup>

With the limitations of Study I, including small sample sizes and the post-hoc design, we do not have firm evidence of the safety of a restrictive transfusion strategy among all subgroups (especially among patients with cancer), but on the other hand, no obvious trends were detected indicating harm of the restrictive

transfusion strategy. And taken together with the additional knowledge, the safety of the restrictive strategy among subgroup of patients with septic shock seems plausible.

### 13.3.2 Effect of transfusion thresholds on long-term outcomes

Only three other RCTs of transfusion thresholds have reported long-term outcomes (beyond day 90).<sup>156,180,181</sup> The FOCUS trial by Carson and colleagues included 2016 patients undergoing hip-fracture surgery and of 50 years or older, with risk factors for or a history of cardiovascular disease and post-operative haemoglobin level below 10 g/dl. They were randomized to a restrictive (8 g/dl) or liberal (10 g/dl) transfusion threshold group. The primary results showed no benefits of the liberal strategy.<sup>180</sup> Pre-defined secondary outcomes were the long-term mortality and comparisons of causes of death, which showed no statistically significant differences of the survival at a median on 3 years after inclusion to the trial (HR 1.09; 95% CI 0.95-1.25; P=0.21) and no differences in the causes of death.<sup>182</sup>

In a feasibility study by Walsh and colleagues, 100 mechanically ventilated ICU patients (for more than 4 days), of more than 54 years, and with a haemoglobin level of 9 g/dl or less were randomised to a restrictive transfusion strategy of 7 g/dl or a liberal transfusion strategy of 9 g/dl.<sup>156</sup> The aim of the study was to show feasibility regarding obtaining a difference in haemoglobin level during the intervention period between the groups. Secondary outcomes included mortality, HRQOL and physical function assessed by the SF-12 and Rivermead Mobility Index questionnaires at day 60 and 180. The results showed feasibility and a trend towards lower mortality in the restrictive group (RR of death at day 180: 0.68; 95% CI 0.44-1.05).<sup>156</sup> At 180-day follow-up the level of the PCS/MCS scores were similar to the crude results from our study (Table 3). Though, they found a trend of better outcomes for the survivors in the restrictive transfusion group regarding mental health at day 180 and survival. This could have been a chance finding but it could also indicate that RBC transfusions affected the mental health status because of persisting immunosuppression.

The recently completed Transfusion Requirements for Cardiac Surgery (TRICS) III trial included 5243 adult patients undergoing cardiac surgery with cardio-pulmonary bypass and a European System for Cardiac Operative Risk Evaluation I of minimum 6 (a tool for calculating predictive operative mortality for patients undergoing cardiac surgery).<sup>181</sup> Patients were randomised to a restrictive (7.5 g/dl) or a liberal (9.5 g/dl in the operating room and the ICU; 8.5 g/dl in the ward) transfusion threshold group. The restrictive transfusion threshold resulted in fewer patients being transfused and fewer units transfused per patient, and it was non-inferior to the liberal regarding the primary composite outcome (in-hospital death, myocardial infarction, stroke, or new-onset renal failure with dialysis).<sup>181</sup> They had pre-planned the same composite outcome to be followed-up at 6 months, and again, the restrictive transfusion strategy was non-inferior to the liberal (odds ratio for the composite outcome 1.02; 95% CI 0.87-1.18).<sup>183</sup>

The above evidence supports the findings from the TRISS trial and Study II, where neither the restrictive nor liberal transfusion strategy resulted in a survival benefit and where the point estimates of the short-term mortality were similar in direction and degree to the long-term mortality – and all point estimates in favour of the restrictive threshold.<sup>97,156,180-184</sup> In TRISS we excluded some of the patients who would be particularly vulnerable to anaemia (patients with ongoing ischemia or within the index hospitalisation), but some patients with chronic cardiovascular disease were included.<sup>97</sup> The subgroup analysis showed no difference in 90-day mortality and there were no differences in the number of ischemic events between the groups.<sup>97</sup> The TRICS III trial included patients who would be vulnerable to anaemia, and their results were no different.<sup>181,183</sup> Interestingly, a subgroup analysis of the long-term outcome of the TRICS III trial, showed a

trend towards difference in the effect of the intervention between patients of different age groups (P-value of the test of interaction: 0.004). It appeared that among the elderly (85 years and above) a restrictive transfusion strategy were beneficial, but the opposite (a trend towards a beneficial liberal strategy) was present for the younger patient groups.<sup>183</sup> A similar, non-statistically significant direction of the point estimates in younger versus older patient subgroups were also found in the TRISS trial and the Transfusion Indication Threshold Reduction (TITRe2) trial (post-operative transfusion threshold trial among cardiac surgery patients) subgroups,<sup>97,147</sup> but the subgroup analysis of patients of 55 years or younger in the TRICC trial showed the opposite finding of a lower mortality among the patients in the restrictive threshold group.<sup>95</sup> All these results could be chance findings, as they were secondary analyses of the trials, but there could also be a reverse age-dependent susceptibility to the risks of anaemia, where older patients with chronic anaemia are less vulnerable to subsequent or additional anaemia.

The results of the HRQOL from the TRISS trial indicated no harm by either a lower or a higher exposure to RBC transfusions, or a lower or higher level of blood haemoglobin during the ICU. But we do not know the transfusion exposure or haemoglobin level after the ICU, and if the results of long-term outcomes could have been diluted by different transfusion strategies outside the ICU. It could be that anaemia was corrected in the ward before discharge. Even though we have no transfusion data from the ward, the results of Study IV suggest the majority of transfusions take place within the ICU, which support that the findings of the long-term outcomes from TRISS trial were results of the intervention.

### 13.3.3 The storage issue among patients with septic shock

The results of Study III resemble other meta-analyses published with the same data, and support the findings and conclusions from our study.<sup>185-187</sup> The safety of standard aged blood (mean 22 days of storage) appears similar to the fresher blood (mean 6 to 12 days of storage) in ICU patients receiving a mean of 4 RBC units.<sup>185</sup> The point estimate was in favour of older blood with possibly more transfusion reactions among patients treated with fresher RBCs, but we were not able to exclude harm from very fresh blood (less than a week of storage) or very old blood (more than four weeks of storage), because of the pragmatic design of the large trials included in the systematic review.<sup>85,87,88</sup> Concerns for the very fresh blood could be that the fresh blood has a potent immunomodulatory effect and patients receiving very fresh blood have possibly increased risk of secondary infections or acute adverse reactions.<sup>88,186</sup> Concerns for the very old blood could stem from increased content of free potassium in the storage medium, possibly increasing the risk of cardiac arrhythmia and cardiac arrest.<sup>188-190</sup> But RCTs investigating the effect of using very old blood has not shown any harm of older blood,<sup>86,191</sup> and a recently published secondary exploratory study of an RCT among massively transfused burn patients showed no association of storage time with adverse outcomes after massive transfusions.<sup>192</sup>

The concerns regarding the effect of storage time on the outcome for patients with septic shock are the same as mentioned above. In the two large RCTs among patients in the ICU, the ABLE and TRANFUSE trials, 22 and 16 percent of the included patients had sepsis at baseline, respectively.<sup>88,193</sup> Unfortunately, we have no separate data from the RCTs regarding the effect of storage time on patients with sepsis and septic shock. The pre-planned subgroup analysis of the ABLE trial of patients with sepsis/septic shock or no sepsis/septic shock will be reported soon (confirmed by contact to Jacques Lacroix).<sup>194</sup> The results from Study IV suggest that the exposure of the extremes of blood storage time is limited among patients with

septic shock. This implies safety of the current strategy of *first-in-first-out* of the blood banks and that recommendations for using an imperative restrictive transfusion strategy could further limit the exposure.

#### 13.3.4 Transfusion guidelines – pros, cons and difficulties in implementation

The results from the TRISS trial became soon after the primary publication implemented in national transfusion guidelines.<sup>98–100</sup> When the surviving sepsis campaign was updated, the recommendations were changed to the following: “ *We recommend that RBC transfusion occur only when hemoglobin concentration decreases to <7.0 g/dL in adults in the absence of extenuating circumstances, such as myocardial ischemia, severe hypoxemia, or acute hemorrhage (strong recommendation, high quality of evidence)...*”, and the recommendation was not different for the early resuscitation phase of septic shock, because of the evidence against the benefits of EGDT.<sup>50,195–197</sup>

The results from Study I and II support the recommendations, but there may still be some uncertainty and caveats to our knowledge – e.g., if there are true differences in the intervention effect among patients with cancer and no-cancer, whether the HRQOL would differ if the follow-up included all patients in the trial, and the impact of very fresh or very old blood on transfused patients with septic shock. From Study IV we learned, that patients with septic shock are often transfused both before and after their ICU stay. This underlines the importance that knowledge from a study like TRISS is introduced to non-intensive care wards and that transfusion guidelines are implemented uniformly.

Given that we would like the transfusion practice to change - away from over-transfusion and over-use of a scarce resource - a national transfusion guideline is a good start.<sup>198</sup> A national transfusion guideline would be a clear message to send from the national medical societies and health authorities, and the guidelines do not just give recommendations for the haemoglobin threshold but also recommendations for the practice surrounding the administration of blood products are given. The national guidelines include a recommendation to use single-unit transfusion (in the circumstances of no active bleeding) and a subsequent re-evaluation of the haemoglobin level to assess the effect of the transfusion.<sup>98–100,199</sup> These recommendations are also possible to use for the measurement of guideline adherence. In Denmark, the National Transfusion Database publishes each year a status report regarding four quality measures: the proportion of admissions with a blood product issued (no goal or standard); the proportion of admission where the number of blood product units given were odd compared to even numbers (goal is >45%); the proportion of admissions with RBC transfusions where the haemoglobin level was measured afterwards (goal of 80%); and the proportion of haemoglobin control measurements above the recommended level (10 g/dl) with no evidence of effect of an RBC transfusion (goal of < 2%).<sup>200</sup> The results from this report show, that we in Denmark are slowly improving practice and moving away from being one of the highest consumers of blood products in the world.<sup>200</sup> If the recommendations should be implemented properly, guidelines are just one approach to start a change in practice, and more actions may be needed, including education, change of blood product ordering forms, reminders and audits.<sup>198</sup> However, we still need more knowledge of the transfusion behaviour of clinicians in order to target the most effective implementation strategies.<sup>201</sup>

Guidelines are useful for guidance, but they are often constructed to cover all patient groups in all settings despite insufficient evidence to cover everything. The Surviving Sepsis Guidelines has for example 18 recommendations based on best practice statements, with no high-quality evidence to support the practice.<sup>50</sup> To account for limitations and to encourage clinicians not blindly adhering to a guideline, the



aforementioned national transfusion guidelines all have a comment similar to the following from the American Association for Blood Banks: “*When deciding to transfuse an individual patient, it is good practice to consider not only the hemoglobin level, but the overall clinical context and alternative therapies to transfusion.*”<sup>100</sup>

## 14 Conclusions and perspectives

In conclusion, with the studies forming the basis of this thesis we further investigated the effects of transfusion strategy for patients with septic shock and the consequences of the RBC unit storage time. The restrictive transfusion strategy of using a lower haemoglobin level as threshold for RBC transfusions in patients with septic shock appears safe in subgroups of patients with septic shock, but the results are limited by sparse data on especially patients with malignancies. The restrictive transfusion strategy also seemed safe regarding long-term survival among all patients and patient-reported health status in the Danish patients in the TRISS trial, though the latter was limited by missing responses of the HRQOL-questionnaire. Among adult patients in the ICU, we may reject a clinically meaningful effect of fresher versus older blood for transfusion on mortality. Further data are needed to investigate differences in adverse events and investigate the effect of storage time on outcomes in transfused patients with septic shock, and we still do not have evidence proving the safety of either very fresh or very old blood. Patients with septic shock received an important part of the RBC transfusions both before the ICU admission and after their ICU stay, but the majority while in the ICU. They were exposed to a RBC units of varying storage times, and only small fractions of patients were exposed exclusively to very fresh or exclusively very old blood.

Based on the knowledge from this thesis and that from other studies surrounding it, a lower haemoglobin threshold seems safe for all patients with septic shock – with the exception of patients with ongoing ischemia or suffering from traumatic brain injury. The impact of RBC the storage time may not be an important or critical factor for the outcome of the patient, and continued practice of the blood banks, issuing the oldest compatible RBC units first, appears safe. A strategy ensuring a balance between minimising the exposure to RBC cells and minimising the degree of anaemia appears rational in patients with septic shock.

Stepping back from the details of storage lesion and RBC function in septic shock; zooming out from the ICU and away from my office; asking what I learned and where do we go from here? I firmly believe that doing less can be more, and that we need to seek arguments for and knowledge of the impact of the interventions to which our patients are exposed, to remain true to the oath of *primum non nocere*. The full knowledge from large, pragmatic, international RCTs, pooled data in meta-analysis and finally clinical guidelines can help us proceed in the right direction, but we must also have in mind the caveats of knowledge, and that each patient may also present with individual risks, values and preferences. So, we should use the known evidence, some common sense and always keep our eyes open when treating patients with septic shock.

On the research path ahead of us I see interesting possibilities for gaining more knowledge. Further investigations of person-level data, in order to understand better who would benefit and who would be harmed by a RBC transfusion or who could go lower in haemoglobin level; further increasing the knowledge

of RBC transfusions and septic shock by including low- and middle-income countries in research communities and multi-centre trials – perhaps the key is to be found where the options are limited; further investigations on the effect of storage time on patients with septic shock with data from the large RCTs; and further investigations of the degree of anaemia and impact of anaemia or RBC transfusions throughout the phase of recovery from septic shock; all add to the our ability to fine-tune treatments and improve the outcome.

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