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Long Tran

Erythrocyte Transfusion and Long-Term Mortality in Open Heart Surgery in Adults

Doctoral thesis

NTNU
Norwegian University of Science and Technology
Thesis for the Degree of
Philosophiae Doctor
Faculty of Medicine and Health Sciences
Department of Clinical and Molecular Medicine

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Transfusjon av røde blodceller og langtidsdødelighet ved åpen hjertekirurgi hos voksne

Årlig gjennomføres omtrent 3000 åpne hjerteoperasjoner i Norge. Det er flere årsaker til at noen dør etter operasjonen, både knyttet til risikofaktorer hos pasienten og/eller komplikasjoner til kirurgien. Tidligere studier har vist at pasienter som får blodoverføring (blodtransfusjon) har økt dødelighetsrate etter hjertekirurgi. For noen pasienter er imidlertid blodtransfusjon helt nødvendig på grunn av ulike årsaker som anemi («lav blodprosent») og blødning. Det er derfor vanskelig å skille mellom skaden forårsaket av de utløsende årsakene til at noen pasienter får blodtransfusjon, og skaden som eventuelt kan knyttes til selve blodet. I våre studier undersøkte vi sammenhengen mellom blodtransfusjon og langtidsoverlevelse 5-10 år etter åpen hjertekirurgi hos voksne.

Våre resultater viste at forskjellen i overlevelse mellom pasienter som får og pasienter som ikke får blodtransfusjon ikke skyldtes selve transfusjonen. Pasienter som gjennomgår hjertekirurgi og får blodtransfusjon er ofte eldre, har flere tilleggssykdommer, gjennomgår lengre operasjoner, og utvikler flere komplikasjoner. Disse risikofaktorene bidrar til den høyere dødelighetsraten hos dem som får blodtransfusjon. Da vi sammenlignet pasienter som fikk og ikke fikk blodtransfusjon etter å ha tatt høyde for kjente risikofaktorer og komplikasjoner i analysen, var det ingen forskjell i dødelighetsrater mellom disse to gruppene.

Studiene våre viste at sammenhengen mellom blodtransfusjon og dødelighet ved hjertekirurgi er kompleks. Flere faktorer hos pasientene selv, samt faktorer under operasjonen og komplikasjoner etter kirurgi kan bidra til den økte dødelighetsraten. Vi fant at sentrale risikofaktorer som anemi, blødning og organskade etter kirurgi ofte ikke var tatt høyde for i tidligere studier. En viktig konklusjon fra våre studier er at selv om unødvendige transfusjoner skal begrenses, er det ingen grunn til å unngå nødvendige transfusjoner fordi man feilaktig antar at dette medfører ekstra risiko for at pasienten dør.

Metode: Studiene inkluderte voksne pasienter som gjennomgikk åpen hjertekirurgi ved St. Olavs hospital, Trondheim fra 2000 til 2017, til sammen ca. 10 000 pasienter. Vi sammenlignet langtidsoverlevelse mellom pasienter som fikk transfusjon av minst 1 enhet røde blodceller under eller etter operasjonen, med pasienter som ikke fikk transfusjon av røde blodceller. Avanserte statistiske metoder (Cox regresjon og strukturell ligningsmodellering) ble brukt for overlevelsesanalyse og for å studere sammenhengen mellom risikofaktorer, blodtransfusjon og overlevelse. Informasjon om død ble innhentet fra Dødsårsaksregisteret frem til 31.12.2018.

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Trondheim, 2021

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Summary

Background

Approximately 3,000 cases of cardiac surgery with cardiopulmonary bypass are performed each year in Norway. Cardiac surgery is performed to treat various disease of the heart, which includes ischemic, valvular, and congenital heart disease. Improvements in surgical and non-surgical techniques, as well as perioperative patient management have improved patient outcomes following cardiac surgery. Nevertheless, several risk factors and complications are associated with cardiac surgery, mortality being the most serious.

Transfusion of red blood cells (RBC) is associated with postoperative morbidity, short- and long-term mortality in cardiac surgery. However, some investigators have found contradicting results regarding the association between RBC transfusion and long-term mortality. The observed difference in mortality between patients who receive RBC transfusion and patients who do not receive any transfusion, could be attributed to residual confounding by factors such as preoperative anaemia.

Aim

The aim of this thesis was to investigate the association between RBC transfusion and long-term all-cause mortality in adult patients undergoing cardiac surgery. Another aim was to compare the risk associated with RBC transfusion and preoperative anaemia on long-term all-cause mortality in adult patients undergoing cardiac surgery.

Methods

Data on adult patients undergoing cardiac surgery been consecutively collected from 2000 through 2017 in the Trondheim Heart Surgery Database at St. Olavs Hospital, Trondheim, Norway. We investigated the association between transfusion of at least one unit of RBC intra- or postoperatively and long-term mortality, in patients undergoing primary isolated coronary artery bypass grafting (CABG). We also investigated the association between RBC transfusion and 5-year mortality in adult patients with preoperative anaemia undergoing primary cardiac surgery. Cox regression adjusted for known risk factors for mortality and common postoperative complications following cardiac surgery was employed.

To compare the association of RBC transfusion with preoperative anaemia on long-term all-cause mortality, we employed Structural equation modelling (SEM). We constructed and analysed path diagrams between known risk factors in cardiac surgery and observed mortality between 30 days and 5 years postoperatively. The total effect of preoperative anaemia on mortality was compared with the direct effect of RBC transfusion.

Results

From 2000 through 2014, 4,014 patients underwent primary isolated CABG, and from 2000 through 2017, 1,859 patients with preoperative anaemia underwent cardiac surgery at St. Olavs Hospital. The unadjusted hazard ratio showed a statistically significant association between RBC transfusion and long-term mortality in both groups. However, when adjusted for known risk factors and common postoperative complications, the association was no longer significant for the patients who underwent isolated CABG (Paper 1). For the patients with preoperative anaemia (Paper 2), the association was not statistically significant when adjusted for pre- and intraoperative variables. The hazard ratio was highest in the first postoperative year in this patient group.

A total of 9,315 patients who underwent cardiac surgery from 2000 through 2017 were included in the SEM analysis (Paper 3). The standardized total effect coefficient of preoperative anaemia on mortality between 30 days and 5 years postoperatively was 0.10. The standardized direct effect of RBC transfusion on mortality was 0.03, which was smaller compared with preoperative anaemia. All other risk factors in the SEM analysis had larger total effect on mortality compared with RBC transfusion, except for female sex.

Conclusions

RBC transfusion was associated with increased long-term all-cause mortality in patients who underwent primary isolated CABG, and in cardiac surgery patients with preoperative anaemia. RBC transfusion was no longer statistically significantly associated with long-term all-cause mortality when the analyses were adjusted for known pre- and intraoperative variables, and common postoperative complications.

RBC transfusion had smaller effect on 5-year mortality compared with preoperative anaemia. Most of the observed difference in mortality between patients who received RBC transfusion and patients who did not receive any transfusion, may be attributed to patient comorbidities and operative risk factors.

List of publications

Paper 1

Transfusion of red blood cells in coronary surgery: is there an effect on long-term mortality when adjusting for risk factors and postoperative complications? Tran L, Greiff G, Pleym H, Wahba A, Stenseth R, Videm V. Eur J Cardiothorac Surg. 2018 May 1;53(5):1068-1074. doi: 10.1093/ejcts/ezx431. PMID: 29228313.

Paper 2

Limited effect of red blood cell transfusion on long-term mortality among anaemic cardiac surgery patients. Tran L, Greiff G, Wahba A, Pleym H, Videm V. Interact Cardiovasc Thorac Surg. 2020 Sep 1;31(3):375-382. doi: 10.1093/icvts/ivaa100. PMID: 32725116.

Paper 3

Relative impact of red blood cell transfusion and anaemia on 5-year mortality in cardiac surgery. Tran L, Greiff G, Wahba A, Pleym H, Videm V. Interact Cardiovasc Thorac Surg. 2020 Dec 21:ivaa266 (published online ahead of print). doi: 10.1093/icvts/ivaa266. PMID: 33346352.

List of abbreviations

AKI	Acute kidney injury
BMI	Body mass index
CABG	Coronary artery bypass grafting
CI	Confidence intervals
CPB	Cardiopulmonary bypass
DF	Degrees of freedom
EPO	Erythropoietin
HCT	Haematocrit
Hgb	Haemoglobin
HR	Hazard ratio
MI	Myocardial infarction
MiECC	Minimally invasive extracorporeal circulation circuit
PBM	Patient Blood Management
PH	Proportional hazard
RBC	Red blood cell
RCT	Randomised controlled trials
SEM	Structural equation modelling

1 Introduction

1.1 Cardiac surgery

1.1.1 Background

Cardiac surgery is one of the major surgical disciplines and is performed to treat ischemic, valvular, and congenital heart diseases. It also includes transplantation of the heart. The world's first surgery on the heart was performed on 4 September 1895 at Rikshospitalet in Kristiania, now Oslo, by Axel Hermansen Cappelen [1]. A 24-year-old male was stabbed in the heart, and it was suspected that the patient had a bleeding in one of the coronary arteries. Cappelen performed a successful ligation but the patient died three days later due to complications. The first successful heart surgery without complications was performed a year later by Ludwig Rehn in Frankfurt, Germany [2]. Dr. Rehn performed a successful suture of a stab wound on the right ventricle of a 22-year-old male.

Modern cardiac surgery is often performed using a cardiopulmonary bypass (CPB) machine which circulates the blood of the patient while the heart is stopped [3]. Other modern techniques include off-pump cardiac surgery and minimally invasive surgery, which have shown favourable outcome for some patients [4-7]. Better general health in the population, as well as advances in medical treatment and non-surgical techniques have contributed to the reduction in cardiac surgical procedures performed each year in Norway [8]. Non-surgical techniques include percutaneous coronary intervention and transcatheter aortic valve replacement [9, 10]. Nevertheless, cardiac surgery is still necessary for some patients and it is associated with a major risk of postoperative morbidity and mortality [11-14].

1.1.2 Procedures

Approximately 3,000 cases of cardiac surgery with cardiopulmonary bypass are performed each year in Norway [8]. The most common cardiovascular procedure performed is still open-heart on-pump coronary artery bypass grafting (CABG) [15]. The procedure is performed under general anaesthesia and using a CPB and sternotomy, i.e. a surgical incision through the breastbone. CABG is used to treat severe multivessel

coronary artery disease [16]. CABG improves the circulation to the heart by grafting a healthy artery or vein and attaching it beyond the stenotic part of the coronary artery. Thus, it creates an alternative route for blood flow to the heart muscle. The most common blood vessels used are the left internal thoracic artery and the greater saphenous vein. After a CABG procedure the sternum is closed by wire fixation. Since 2005, the numbers of CABG and use of a CPB machine have steadily declined each year in Norway [8].

Another major cardiac surgical procedure is heart valve surgery, which is performed to repair or replace a damaged heart valve [13]. Valvular heart disease can affect all four heart valves. However, the most common diseases are aortic stenosis and mitral regurgitation, and predominately affect elderly patients [17]. The device inserted in valve surgery can either be a mechanical prosthesis or a bioprosthesis. Mechanical prostheses have high risk of thromboembolism and require lifelong use of a vitamin K antagonist, while bioprostheses have a high risk of structural failure [18]. There has been an increase in valve surgery performed each year in Norway up to 2013. Since then, the number of open-heart valve surgeries in Norway has declined steadily due to the increase in patients who are treated with catheter-based procedures [8].

Other procedures that will not be further described here include surgery on the aorta, correction of congenital malformations, implantation of a ventricular assist device, extracorporeal membrane oxygenation, surgical ablation, and transplantation of the heart.

1.1.3 Cardiopulmonary bypass

Several essential discoveries led to the first closing of an atrial septal defect using a CPB by John Heysham Gibbon on May 6, 1953. One of these discoveries was the identification of the ABO blood group system by Karl Landsteiner in 1901 [19, 20]. The introduction of CPB has made circulatory and respiratory support through extracorporeal circulation possible and helped facilitate increasingly complex cardiac surgery in a bloodless field. CPB allows the surgeon to perform surgery on the heart and the great vessels while the heart is stopped. The CPB device maintains circulation and oxygenation to the rest of the body during surgery. Consequently, the use of CPB is not without risk to the patient and

can cause complications such as platelet dysfunction, a strong inflammatory response, bleeding and reperfusion injury [3].

Cardiac surgery provokes an immune response which has been compared to the systemic inflammatory response syndrome. This immune response could be caused by the contact of foreign surface material in the CPB circuit, surgical trauma, reperfusion injury and ischaemia [21-23]. The contact of foreign surface material in the CPB circuit can lead to activation of both the classical and alternative pathways of the complement system. Ischaemia and reperfusion injury during cardiac surgery leads to the release of the pro-inflammatory cytokines IL-6, IL-8, and TNF-alpha [23, 24]. Another proposed mechanism for complications is coagulopathy related to cardiac surgery and CPB. Activation of the intrinsic pathway through four plasma proteins in foreign surface material; high-molecular-weight kininogen, prekallikrein, and factors XII and XI, can lead to platelet aggregation, coagulation disorders and bleeding [25].

Complications associated with cardiac surgery can also be caused by haemodilution during cardiac surgery due to priming of the CPB circuit [26]. Nadir haematocrit (HCT) and haemodilution anaemia during CPB have been associated with cardiac dysfunction, renal failure, prolonged ventilation and mortality [27, 28]. Naturally, patients undergoing cardiac surgery with preoperative anaemia have a higher risk of complications due to haemodilution during CPB.

1.1.4 Risk of morbidity and mortality

According to the 2019 annual report from the Norwegian Registry for Cardiac Surgery (Norsk Hjertekirurgiregister), short-term mortality was 0.4% among CABG patients and 1.9% for all types of cardiac surgery [8]. Short-term mortality is defined as mortality within 30 days, operative or in-hospital mortality. The observed postoperative long-term mortality rate obviously depends on the observation time. A study on the Norwegian population of Middle-Norway who underwent cardiac surgery, observed that the 1-, 3-, and 5-year all-cause mortality rates were 4.4%, 8.2% and 13.8%, respectively [29].

Despite advances in cardiac surgery there is still risk and several postoperative complications associated with cardiac surgery [30, 31]. Bleeding is a common occurrence

in cardiac surgery and some patients may experience major bleeding that require reoperation [32-35]. Myocardial and respiratory dysfunction caused by cardiac surgery-related trauma may occur postoperatively [36, 37]. Hypoxia and reperfusion injury after cardiac surgery can cause myocardial infarction (MI) [38], acute kidney injury (AKI) [39, 40], and neurological complications [41]. Sternal wound infection because of sternotomy may progress to sepsis [42, 43]. Lastly, the most severe complication associated with cardiac surgery is mortality [44, 45].

Several patient risk factors are associated with mortality in cardiac surgery. Increasing age increases the risk for mortality. Critical ill patients and patients who require more complex surgical procedures compared with standard CABG, also have higher risk of long-term mortality. Chronic illnesses in the major organs such as heart, lungs, kidneys, and peripheral arteries increase the risk of mortality. Other risk factors for mortality reported in the EuroSCORE II include diabetes and sex [45]. A proposed important risk factor for both short- and long-term mortality in cardiac surgery is the transfusion of red blood cells (RBC). Evidence from the past several years suggests a hazard associated with RBC transfusion. Therefore, guidelines from the Society of Thoracic Surgeons have been published to reduce utilisation of blood products and advocate a restrictive transfusion policy [46]. European guidelines have also been published by the European Association for Cardio-Thoracic Surgery and the European Association of Cardiothoracic Anaesthesiology, to reduce blood transfusion requirements in adult cardiac surgery [47].

1.2 Blood transfusion

1.2.1 Patient Blood Management

The term Patient Blood Management (PBM) was first coined by Professor James Isbister in 2005, who highlighted the need for clinicians to shift the focus from blood components to a patient-centred approach [48]. The idea of PBM has emerged because of growing evidence of transfusion-related adverse outcomes, gaps between cost and supply, and lack of evidence for efficacy of transfusion. PBM is a multimodal, multidisciplinary term used to describe an evidence-based approach to optimise care. The three main pillars of focus of PBM are: 1) improve RBC mass and treat anaemia; 2) minimise blood loss; and 3)

optimise patient specific physiological reserve which includes an evidence-based transfusion strategy (Figure 1) [49-51].

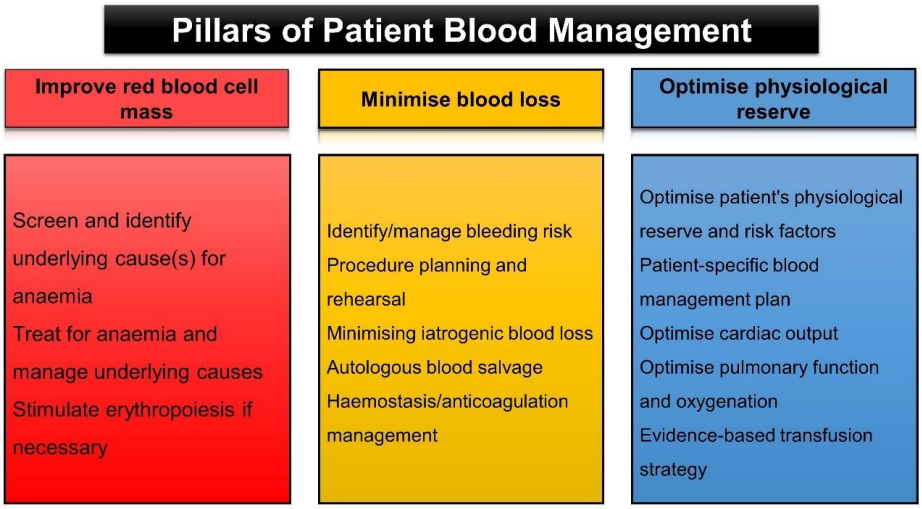

Figure 1. The three pillars of patient blood management. The list is not comprehensive and other measures such as management of blood loss and optimise tolerance of anaemia can be part of a patient blood management strategy. Adjusted from Clevenger *et al* [51].

1.2.2 Preoperative anaemia

Anaemia is the most common haematological disease and is a condition in which the absolute number of circulating RBC or haemoglobin (Hgb) concentration is reduced [52]. The reduction in Hgb, which is the oxygen carrying protein in RBC, decreases the body's capacity for oxygen delivery to organs and tissues. Erythropoiesis, the process of RBC production, occurs in the bone marrow under the stimulation of the endocrine hormone erythropoietin (EPO). A hematopoietic stem cell in the bone barrow develops to a reticulocyte through several stages before it is released to the circulation (Figure 2) [53].

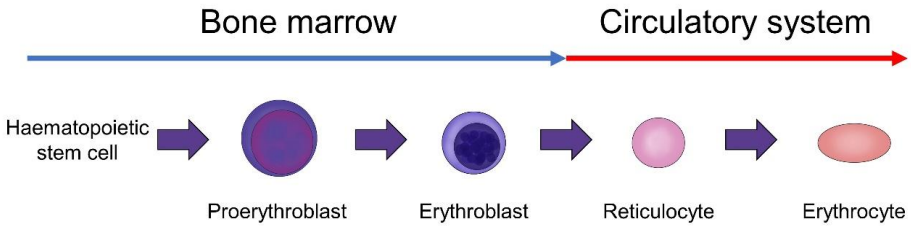

Figure 2. Erythropoiesis. Red blood cell maturation and production. Erythroblasts develop from basophilic erythroblasts to polychromatic erythroblasts, and then to orthochromatic erythroblasts, before expulsion of the nucleus and development to reticulocytes. The average life span of an erythrocyte is 120 days. Adjusted from Dzierzak *et al* [53].

After about 1 to 2 days these reticulocytes become mature erythrocytes (RBC), which have a life span from 110 to 120 days. Under normal conditions, low levels of circulating EPO produced from the interstitial fibroblasts from the kidneys stimulate a stable production of RBC, which compensates for the turnover. Secretion of EPO can be stimulated in the presence of cellular hypoxia or anaemia, which can be caused by impaired production, increased destruction, blood loss, fluid overload or intestinal inflammation [53].

Preoperative anaemia is a common finding among cardiac and non-cardiac surgery patients. In some studies, preoperative anaemia affected a third of the patients undergoing elective surgery [54]. Among cardiac surgery patients, a prospective study showed that the most common cause of preoperative anaemia is iron deficiency [55]. Iron is an essential mineral in the synthesis of haem, a component of Hgb. Anaemia may ensue if the body's iron store is depleted through blood loss, insufficient dietary intake, or malabsorption [56]. There is increasing evidence that preoperative anaemia is an important risk factor in cardiac and non-cardiac surgery. Several studies have shown that anaemia is independently associated with adverse clinical outcomes, including mortality, and it is an important determinant of RBC transfusion [57-67]. In addition, some suggest that the exposure to both RBC transfusion and anaemia is linked to worse clinical outcome than each risk factor independently [65, 68-73].

1.2.3 Major bleeding

Perioperative bleeding among cardiac surgery patients is a common clinical problem and several factors can lead to excessive bleeding. For instance, antiplatelet and anticoagulation drug use and CPB can lead to thrombocytopenia and platelet dysfunction [74]. European guidelines on PBM include several practical recommendations to minimise bleeding and reduce RBC transfusion. In most cases, perioperative bleeding is treated with RBC transfusion to maintain oxygen delivery to organ tissues [47]. Consequently, more than 30% of cardiac surgery patients receive perioperative RBC transfusion. In some cases, bleeding can be life-threatening. Even though reoperation for bleeding can be successful, the risk of long-term mortality can still be increased due to hypotension, hypoperfusion, acute anaemia, hypoxemia, and end-organ ischaemia [75].

Although major bleeding is an independent risk factor for mortality, it is also a determinant of RBC transfusion which may be unavoidable. The risk of RBC transfusion among patients who experience major bleeding is certainly higher in patients with preoperative anaemia. Some have suggested that both RBC transfusion and anaemia may exert a synergistic effect with major bleeding on mortality [68]. Because these three risk factors are intercorrelated, the mechanism of their individual effect on postoperative outcomes is difficult to determine [71]. Major bleeding has no standard definition. However, Dyke *et al* have proposed a universal definition for perioperative bleeding. This classification uses 5 classes based on the severity of bleeding during the first 12 hours postoperatively (Table 1) [76].

Table 1: Bleeding classification based on the Universal Definition of Perioperative Bleeding in adult cardiac surgery [76]

Bleeding definition	Sternal closure delayed	Postoperative chest tube blood loss within 12 hours (mL)	RBC (units)	FFP (units)	Platelets (units)
Class 0 (insignificant)	No	< 600	0	0	0
Class 1 (mild)	No	601–800	1	0	0
Class 2 (moderate)	No	801–1000	2–4	2–4	Yes
Class 3 (severe)	Yes	1001–2000	5–10	5–10	N/A
Class 4 (massive)	N/A	> 2000	> 10	> 10	N/A

Missing from the table are: use of cryoprecipitate (class 2), prothrombin complex concentrates (class 2), recombinant activated factor VII (class 4), re-exploration/tamponade (class 3).
Abbreviations: RBC: red blood cells; FFP: fresh frozen plasma; N/A: not applicable.

1.2.4 Red blood cell transfusion

The use of allogeneic blood transfusion and the development of blood banks were important medical advances in the 20th century. The first published experiment on blood transfusion was in Oxford in the 17th century, and in 1666 the English physician Richard Lower successfully transfused blood from one dog to another. In 1818, an obstetrician named James Blundell performed the first human to human blood transfusion in treatment of postpartum haemorrhage. As mentioned above, the Austrian biologist, physician and immunologist Karl Landsteiner discovered the ABO blood group system in 1901. Later several other blood groups were discovered [77].

Transfusion of whole blood is rarely practiced; instead, each unit of whole blood is filtered and separated into components; RBC, pooled platelets, and plasma. Each component can then be stored and transfused individually. In industrialised nations, over half the amount of blood products are used in the surgical setting [78]. Transfusion of blood products is, however, not without risk and transfusion is associated with several complications including mortality.

1.2.5 Transfusion reactions

Several infectious and non-infectious reactions are associated with transfusion. Allogeneic blood donation is done by voluntary donors in blood donor centres. Potential donors are evaluated and screened using rigorous quality assurance for any potential risk of infectious diseases. Therefore, the risk of transfusion transmitted infection is low and blood transfusion is considered safe [79, 80]. However, reactions from transfusion may occur. Adverse reactions to transfusion can range from mild febrile or allergic reactions to rare life-threatening events and mortality. Transfusion reactions can be categorised as either acute transfusion reactions which occur within 24 hours, or delayed transfusion reactions (Table 2) [81].

Table 2: Transfusion reactions adjusted from Suddock *et al* [81]

Acute transfusion reactions	Febrile non-haemolytic reaction
	Mild allergic reaction
	Anaphylactic reaction
	Acute haemolytic transfusion reaction
	Septic transfusion reaction
	Transfusion-associated circulatory overload
	Transfusion-related acute lung injury
	Massive transfusion-associated reaction
Delayed transfusion reactions	Post-transfusion purpura
	Delayed haemolytic transfusion reaction
	Transfusion-associated graft versus host disease

1.2.6 RBC transfusion and mortality

Observational studies have shown that patients who receive RBC transfusion have higher risk of postoperative morbidity and mortality [69, 82-96]. Similar results can be observed in non-cardiac surgery patients [97-100]. In non-urgent settings, other treatments to correct anaemia are preferred. But in the perioperative setting with blood loss, RBC transfusion is often necessary to sustain adequate tissue oxygenation. The impact of bleeding and anaemia are both associated with the need for RBC transfusion and

unavoidable at critical levels of Hgb [68, 101, 102]. The mechanism behind the negative effect of RBC transfusion is poorly understood. However, there is a consensus that minimising and avoiding unnecessary blood transfusion are better for patient outcome.

However, other studies have found contradicting results regarding RBC transfusion and mortality. Some have suggested that the risk associated with transfusion may be caused by overestimation of effects due to residual confounding, i.e. unadjusted confounders in the study design and analysis [103-108]. Patients who receive RBC transfusion are often older, have lower body mass index (BMI) and other comorbidities. The lack of proper adjustment for patient risk factors in the analysis may lead to overestimation of the effect of transfusion on mortality. Clinical factors that determine the need to transfuse, such as anaemia and blood loss, may contribute to the observed increase in mortality rates among cardiac surgery patients. A study demonstrated how inclusion of chest tube drainage was an important confounder. RBC transfusion was not statically significant associated with mortality when adjusted for in this study [107].

Several risk factors for mortality in cardiac surgery are also associated with the need to transfuse RBC; age, female sex, low BMI, renal dysfunction and the urgency of surgery [109]. Because of the complex relationship between many risk factors in cardiac surgery, it is difficult to study and elucidate the clinical efficacy of RBC transfusion in the setting of cardiac surgery. The impact of RBC transfusion on the postoperative outcome is therefore debatable, residual confounders cannot be excluded, and further research on this knowledge gap is needed to improve transfusion guidelines in cardiac surgery [46, 74, 110].

1.3 Study design

Issues caused by improper study design need to be considered with regards to the negative effect of RBC transfusion because of conflicting findings in the literature. Unmeasured confounders which could lead to false conclusions need to be considered in observational studies and survival analysis.

1.3.1 Unmeasured confounders

One of the major challenges of observational studies is the lack of proper adjustment for confounding effects and residual confounding. A confounder influences both the independent variable or exposure and the dependent variable or outcome. Thus, an unmeasured or unadjusted confounder in an analysis could lead to false association between an exposure and outcome of interest. Randomised controlled trials (RCT) avoids the issue of unmeasured confounders and in the hierarchy of study design is considered best evidence. However, ethical concerns with regards to implementing an RCT to study the association between RBC transfusion and mortality. It is unethical to expose patients who might not need it to RBC transfusion if there is any causal negative effect of transfusion.

RCTs on liberal vs. restrictive transfusion policies have shown non-inferior results of a restrictive transfusion threshold with regards to adverse clinical outcomes [110-115]. The evidence from RCTs therefore refutes the findings from observational studies, which have demonstrated that a restrictive transfusion threshold is better for patient outcome [110]. The question therefore arises whether survival analyses on the topic of transfusion were properly adjusted for confounders such as anaemia and perioperative blood loss. As a result, observational studies on the topic of RBC transfusion and mortality must address the potential consequences of improper adjustment.

1.3.2 Survival analysis

Many different outcomes may be studied using survival analysis, such as MI, AKI, and mortality. The method can be used to analyse a time-to-event outcome or event-free survival. A time-to-event outcome is distinct from event-free survival by the inclusion of the survival time function. An event-free survival investigates the outcome without regarding when the outcome occurs. It is important to consider survival time to investigate certain clinical outcomes. The difference between two groups could be distinct with regards to early vs. late mortality. For example a large portion of patients in one group may experience mortality shortly after treatment, while the other group experience mortality at the end of the follow-up time [116].

The most common methods for survival analysis in cardiac surgery are through computation of a binomial logistic regression or Cox proportional hazard (PH) model. Cox regression is widely used because of its applicability to different clinical trials, ability to compute hazard ratio (HR) and confidence intervals (CI) [117]. Cox regression is the preferred method over logistic regression models because logistic regression ignores survival time and censoring information [118].

Many clinical factors that may influence both the need to transfuse and are also independent risk factors for mortality. The validity of the results of survival analysis is therefore dependent on the quality of the data and the available adjustment variables. However, studies on transfusion and mortality can still be problematic because the need to transfuse may be unavoidable in some patients. RBC-transfused and non-transfused patients may not be comparable even after adjustments.

1.3.3 Propensity score matching

In studies where randomisation is either unfeasible or unethical, the concern regarding confounders needs to be addressed. Some illnesses or groups of patients may be well balanced, and confounders could easily be adjusted for in the analysis. However, for research where the differences in the patients are too large or quality data are difficult to acquire, the adjustment for confounding may be difficult. Thus, over- or underestimation may occur, and the results become unreliable.

An increasingly used method to deal with the bias of confounders in observational studies is to perform propensity score matching. The strength of this method is the construction and matching of two patient groups which have similar probability to the exposure of interest. After matching for the propensity score, the two matched groups in theory have the same risk due to other factors and only differ in exposure. Propensity score matching therefore reduces the confounding in observational studies, thus may be a step closer to the evidence level of an RCT [119]. An outline of the steps in propensity score matching is described in further detail in the Methods section.

1.3.4 Structural equation modelling

Another potential method to examine the relationship between RBC transfusion and long-term mortality in cardiac surgery is with structural equation modelling (SEM). Although SEM is an old statistical method, it has gained popularity in recent years because of progress made in software and model development to the SEM framework [120-123]. These techniques are well known in the field of epidemiology, but to the best of our knowledge have not been used to examine the negative effect of RBC transfusion on mortality.

SEM is a framework that uses a combination of different techniques from statistics, psychology, and epidemiology. A strength of SEM is the construction of path diagrams which graphically represent the hypothesised causal relationships between observed variables. Thus, SEM helps researchers visualise the complex theoretical models of clinical problems. In a path diagram, the observed variables are graphically represented in boxes or rectangles. A single-headed arrow in a path diagram represents the regression effect of one variable on another, and the causal effect is an assumption of the model (Figure 3).

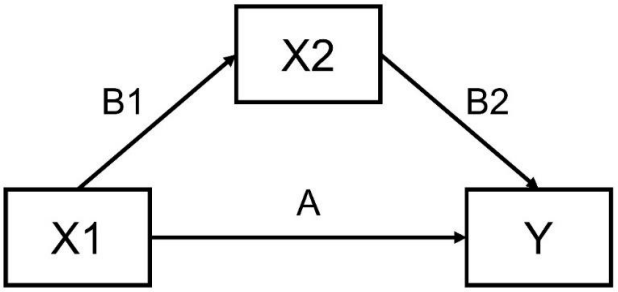

Figure 3. Example of a path diagram. Boxes represent an observed variable. Arrows represent the direct effect of one variable on another along the line. In this diagram, variable X1 has a direct effect A and B1 on variable Y and X2, respectively. Variable X2 has also a direct effect B2 on variable Y. The indirect effect of X1 on Y can be calculated as the product of B1 and B2. The total effect of X1 on Y is the sum of the direct effect A and indirect effect of the product of B1 and B2.

SEM also allows for the measurement of the indirect effects through intermediary variables or mediators. In Figure 3, the visualised example of the independent variable X1 is associated directly to outcome Y along the path A. X1 is also associated indirectly to Y through the variable X2 along the path of B1 and B2. The indirect effect of X1 on Y is the product of B1 and B2. The total effect of X1 on outcome Y can therefore be estimated as the sum of the direct effect along A and the indirect effect along B1 and B2. In the setting of cardiac surgery, SEM can compute both the direct effect and indict effect of RBC transfusion and clinical risk factors on mortality [121, 124].

A unique feature of SEM is that it also allows for the estimation of latent variables, which are variables not directly observed and represented by a circle in a path diagram. Latent variables were not used in this thesis and will not be explained in further detail.

A strength of SEM compared with Cox regression, is the ability to simultaneously compute multiple regression analysis. SEM allows for more than one dependent variable within a model. In Figure 3, both the direct effect of the independent variable X1 on the dependent variables X2 and Y can be calculated in the same model. Thus, SEM allows for more complex models than are feasible with Cox regression analysis which is limited to a single time-to-event process. A challenge when exploring the relationship between RBC transfusion and long-term mortality is that clinical factors which influence the need to transfuse are also associated with mortality. Therefore, SEM is a method that may help bridge the knowledge gaps concerning the relationship between RBC transfusion and long-term mortality [120, 124].

1.4 Knowledge gaps

As described above, the causal effect of transfusion on long-term mortality is difficult to evaluate and may be lost among other risk factors associated with mortality. Age, female sex, low BMI and anaemia are all associated with higher incidence of long-term mortality and are also risk factors for RBC transfusion [64, 71, 109, 125-128]. Associations between these risk factors and the negative outcomes could explain part of the increased long-term mortality related to RBC transfusion [64]. Thus, differences in patient

characteristics and comorbidities and not RBC transfusion itself may be an underlying reason for the higher mortality rate in transfused patients.

The focus of this thesis was to bridge the knowledge gap regarding the independent negative effect of RBC transfusion on long-term mortality. The association between RBC transfusion and long-term mortality could be due to patient and operative risk factors, and common postoperative complications associated with cardiac surgery. An important question was whether the negative effect of RBC transfusion on long-term mortality was still present with the inclusion of these variables in the analyses. Lastly, the present thesis also investigated the effect of preoperative anaemia on long-term mortality compared with RBC transfusion using SEM in patients undergoing cardiac surgery.

2 Study hypotheses

The main hypothesis of this thesis is that RBC transfusion is not associated with long-term all-cause mortality in cardiac surgery when adjusting for known perioperative risk factors.

In Paper 1 and 2, we hypothesised that there is no negative association between RBC transfusion and long-term mortality in adult patients undergoing primary isolated CABG or in adult cardiac surgery patients with preoperative anaemia.

The specific hypothesis to be tested in Paper 1 was:

1) The observed difference in long-term all-cause mortality in patients undergoing primary isolated CABG is due to underlying patient risk factors and common postoperative complications

The specific hypotheses to be tested in Paper 2 were:

2) The observed difference in 5-year all-cause mortality among patients with preoperative anaemia undergoing cardiac surgery is due to underlying patient risk factors and common postoperative complications
3) The HR of RBC transfusion in patients with preoperative anaemia undergoing cardiac surgery is largest in the first year postoperatively

In Paper 3, we hypothesised that exposure to RBC transfusion has less total effect on long-term all-cause mortality compared with preoperative anaemia among adult patients undergoing cardiac surgery.

The specific hypotheses to be tested in Paper 3 were:

4) The effect of RBC transfusion on 5-year all-cause mortality is less than the effect of preoperative anaemia among patients undergoing cardiac surgery
5) RBC transfusion has less total effect on 5-year all-cause mortality compared with patient and operative risk factors among patients undergoing cardiac surgery

3 Aims

The main aim of this thesis was to investigate associations between RBC transfusion and long-term all-cause mortality in adult patients undergoing cardiac surgery. The present thesis also investigated the difference in total effect of RBC transfusion and preoperative anaemia on long-term all-cause mortality in adult patients undergoing cardiac surgery.

3.1 Paper 1

Several previous studies have demonstrated an independent relationship between as little as 1 unit of RBC transfusion and long-term mortality in cardiac surgery. We suggested that if there was a negative association of RBC transfusion, it would be present in low-risk patients, i.e. adult patients undergoing primary isolated CABG. We also proposed the inclusion of common postoperative complication in the analysis. The specific aims of Paper 1 were:

1) To investigate the unadjusted association between RBC transfusion and long-term mortality in patients undergoing primary isolated CABG
2) To investigate this association when adjusted for known pre- and intraoperative factors that would influence the decision to transfuse RBC and other risk factors for long-term mortality
3) To investigate this association when further adjusted for common postoperative complications following cardiac surgery
4) To assess the uncertainty of the results through sensitivity analyses using propensity score matching in models without and with common postoperative complications

3.2 Paper 2

Cardiac surgery patients with preoperative anaemia often need transfusion of RBC in the perioperative setting, and the negative effect of RBC transfusion has been suggested to be larger in patients with anaemia. We therefore compared adult patients with

preoperative anaemia undergoing cardiac surgery who received RBC transfusion with patients who did not receive any transfusion. The specific aims of Paper 2 were:

5) To investigate the unadjusted association between RBC transfusion and 5-year mortality in cardiac surgery patients with preoperative anaemia, defined by the World Health Organization criteria
6) To investigate the association between RBC transfusion and 5-year mortality in cardiac surgery patients with preoperative anaemia when adjusted for known pre- and intraoperative factors, and common postoperative complications
7) To investigate the association of RBC transfusion and mortality in observation time between 30 days to 1 year postoperatively, and 1-5 years postoperatively

3.3 Paper 3

The complex relationship between risk factors associated with the need to transfuse RBC and long-term mortality in cardiac surgery is difficult to study using Cox regression or logistic regression. Therefore, we employed SEM to investigate the relationship between RBC transfusion and 5-year mortality in adult patients undergoing cardiac surgery. The specific aims of Paper 3 were:

8) To use SEM to investigate the relative total effect of RBC transfusion and preoperative anaemia on all-cause mortality between 30 days to 5 years postoperatively in adult patients undergoing cardiac surgery
9) To assess the results through sensitivity analyses with observation time between 30 days to 1 year, and 1-5 years postoperatively
10) To assess the results through sensitivity analyses in patients undergoing isolated CABG, and in patients undergoing multiple procedures

4 Patients and methods

4.1 Cardiac Surgery Outcome Study

The work in the present thesis was part of the Cardiac Surgery Outcome Study, which is a larger project investigating clinical and genetic risk factors for different complications following adult cardiac surgery. The Cardiac Surgery Outcome Study was approved by the Norwegian Data Inspectorate and by the Regional Committee for Medical and Health Research Ethics in Middle-Norway (project number 4.2007.1528), Trondheim, Norway, on 27th of June 2007.

Patient data from the Trondheim Heart Surgery Database at St. Olavs University Hospital, Trondheim, Norway, were used in this thesis. Patient and procedure-related characteristics from patients undergoing cardiac surgery at St. Olavs University Hospital have been registered consecutively into this database since 1992. This is a single-centre database which is part of local quality assurance work, and the information in the database has undergone rigorous quality controls and ascertainment by a senior anaesthesiologist. Patients were followed from admission and until discharge from the hospital.

The need for informed consent was waived up to April 2008. Thereafter, all patients have signed an informed consent. For the present thesis, data on adult patients undergoing on-pump open-heart cardiac surgery from January 2000 through December 2017 were used.

4.2 Patient selection and endpoint

4.2.1 Paper 1-3

In Paper 1, we investigated the association between RBC transfusion and long-term mortality in low-risk patients, i.e. adult patients who underwent primary isolated CABG. Other surgical procedures, multiple procedures or reoperation following surgery were excluded. Blood loss above 700 mL intraoperatively or above 800 mL postoperatively until the first postoperative morning, were found to indicate a complicated perioperative course and a higher risk for blood transfusion and were used as exclusion criteria. Other exclusion criteria were short-term mortality (operative or postoperative death within 30

days), or observation time less than 30 days. Data from January 2000 through December 2014 were available at the time of the study (Figure 4).

In Paper 2, we included all adult patients with preoperative anaemia who underwent primary cardiac surgery from January 2000 through December 2017. Exclusion criteria for this study were short-term mortality, observation time < 30 days, emergency surgery or missing data (Figure 4).

In Paper 3, we included all primary adult cardiac surgery patients from January 2000 through December 2017. The exclusion criteria for this study were short-term mortality, and salvage or emergency procedures (Figure 4).

All non-Norwegian citizens were excluded because deaths in this patient group are not registered in the Norwegian Cause of Death Registry.

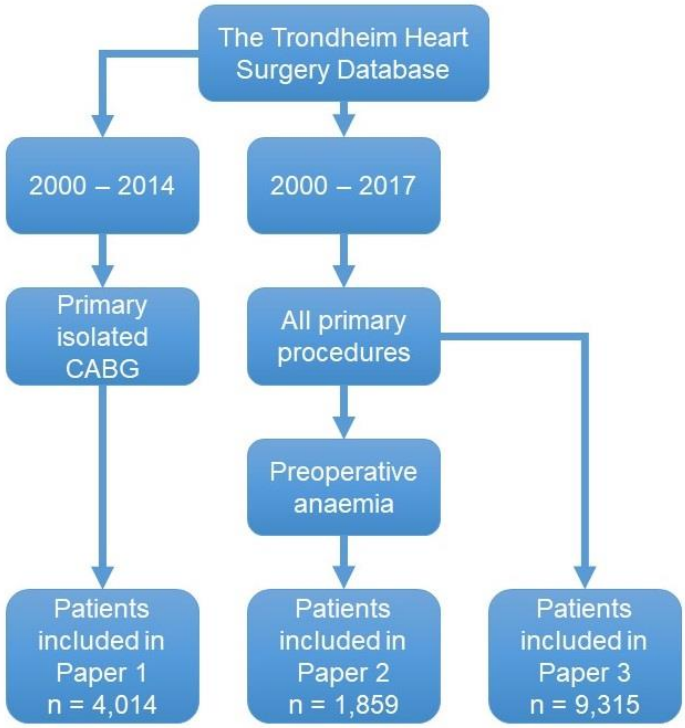

Figure 4. Patient selection and sample size for all papers. Abbreviations: CABG, coronary artery bypass grafting.

4.2.2 Exposure and endpoint

The primary exposure in all 3 papers was transfusion of 1 or more units of RBC during the intraoperative or the postoperative phase. Patients who received at least 1 unit of RBC transfusion during the hospital stay were compared with patients who did not receive any RBC transfusion. In Paper 3, we also compared the effect of RBC transfusion to the effect of preoperative anaemia on 5-year all-cause mortality.

For all papers, the main endpoint was all-cause mortality beyond 30 days postoperatively, denoted as long-term mortality. In Paper 1, the patients were followed up from the day of surgery to 31 December 2014 or death, whichever occurred first. For Paper 2 and 3, the observation time was between 30 days and 5 years postoperatively. The cut-off at 5 years was used because of low mortality rate among the controls (n = 69) beyond this point in Paper 2. Furthermore, survival analysis in Paper 2 violated the statistical assumptions beyond 5 years postoperatively. Additionally, we evaluated the difference in HR associated with RBC transfusion for the periods 30 days to 1-year postoperatively, and 1-5 years postoperatively in Paper 2 and Paper 3. Data regarding death through 31 December 2018 were obtained from the Norwegian Cause of Death Registry which has data completeness > 99%.

4.3 Operative procedures

4.3.1 Cardiopulmonary bypass protocol

All patients included in this thesis underwent an on-pump procedure. Tranexamic acid (30mg/kg) was routinely administered before the start of CPB and heparin (300 U/kg) was administrated to achieve an activated coagulation time of ≥ 480 seconds [129]. The CPB circuit was primed with 1,100 – 1,500 mL of Ringer's acetate with 7,500 – 10,000 U of heparin depending on patient size. Crystalloid cardioplegia was used for isolated CABG, whereas blood cardioplegia was used for non-CABG and multiple procedures. Blood remaining in the CPB circuit after surgery was retranfused to the patient. After termination of CPB, patients were given protamine sulfate to achieve an activated coagulation time within 10% of baseline. Data regarding the use of clopidogrel and ticagrelor were not available for the entire study periods and were not included in the

studies. The use of acetylsalicylic acid was discontinued for all patients between 1 and 3 days before surgery. Low molecular-weight heparin was continued until the night before surgery for patients on this medication.

4.3.2 Transfusion threshold

All transfusions were recorded into the Trondheim Heart Surgery Database for the duration of the hospital stay. A transfusion threshold of approximately 7.0 g/dL Hgb during CPB, and approximately 8.5 g/dL Hgb postoperatively was practised at St. Olavs University Hospital throughout the study periods for all papers. Although the postoperative transfusion threshold has ranged from 8 g/dL to 9 g/dL Hgb, we considered the transfusion policy to have remained essentially the same in the study periods. The final decision to transfuse RBC to the patient was left to the attending physician. Transfusion of platelet concentrates, or fresh frozen plasma were considered when the postoperative bleeding was persistent above 200 mL/h.

4.4 Statistical analysis and methods

4.4.1 Statistics

P-values < 0.05 were considered statistically significant. Statistical analyses were performed using Stata (version 16.0, StataCorp, College Station, TX, USA) and SPSS (version 26.0, SPSS Inc., Chicago, IL, USA). For descriptive analysis between the groups in all papers, the Chi-Square ($\chi 2$) test was used for discrete variables. Results of these variables were given as number of cases and percentage of total. All continuous study variables were graphically assessed for normality, and the t-test and Mann-Whitney U-test were used for descriptive analysis between the groups. The t-test was used for normally distributed continuous variables, and the Mann-Whitney U-test for non-normally distributed variables. Results from these variables were given as mean and standard deviation, or as median with 95% CI or 25th and 75th percentiles. Multicollinearity was assessed using variance influence factors.

4.4.2 Variable definitions

RBC transfusion was defined as intra- or postoperative transfusion of at least 1 unit of RBC. Preoperative anaemia was defined according to World Health Organization criteria (Hgb < 13.0 g/dL for men and < 12.0 g/dL for women) [130]. The preoperative Hgb concentration was based on a blood sample drawn shortly before surgery. Variable definitions used in all papers are listed in Table 3.

Table 3: Independent variable definitions for all papers

Pre- and operative variables	
Hypertension	Use of antihypertensive medication or diastolic blood pressure > 90 mmHg
Chronic pulmonary disease	Use of bronchodilator or < 75% of expected forced expiratory volume in 1 second
Cerebrovascular disease	Carotid stenosis, previous transient ischaemic attack, or stroke.
Renal dysfunction	Creatinine >140 mmol/L or dialysis
Operation category	Isolated coronary artery bypass grafting, valve surgery, 2 procedures or ≥ 3 procedures
Urgent surgery	Surgery within 2 weeks
Emergency surgery	Surgery within 24 hours
Postoperative complications	
Novel renal failure	Absolute serum creatinine increase > 26 mmol/L or relative increase > 50%
Prolonged mechanical ventilation	Primary intubation more than 24 hours or need for reintubation
Acute myocardial infarction	Elevation in Troponin T > 10 times the 99th percentile upper reference limit less < 48 hours after procedure. Development of new pathological Q-waves in ECG
Cardiac dysfunction	Use of 2 or more inotropic drugs or intra-aortic balloon pump

4.4.3 Cox regression

In Paper 1 and 2, mortality was analysed with multivariable Cox PH modelling. In Cox regression, the assumption is that the mortality rate is consistent or proportional between groups by survival time. The PH test, log-log plots and Cox-Snell residuals were used to assess model fit. If the PH assumption was violated, the robustness of the models was assessed using flexible parametric survival models. Flexible parametric survival model

allows for time-dependent effect of covariates using cubic splines, and it is therefore not dependent on PH. If the HR and 95% CI for RBC transfusion were similar between the Cox and flexible parametric survival models, the Cox models were considered acceptable.

4.4.4 Patients undergoing primary isolated CABG

In Paper 1, we explored the possibility of potential confounders in the study group by evaluating the association of RBC transfusion through a 3-Step analysis plan. These three Steps were: 1) an unadjusted Cox regression analysis with RBC transfusion as the only covariate, 2) an adjusted model with pre- and intraoperative risk factors, and 3) an adjusted model including perioperative risk factors and common postoperative complications. Independent variables associated with risk of RBC transfusion were chosen based on clinical knowledge and literature [67, 131]. The specific covariates for each Step can be found in Table 4.

Table 4: Independent variables included in the main analysis of Paper 1 [a]

	Variables
Step 2 Model	Red blood cell transfusion
	Age (years)
	Sex (female)
	Body mass index (kg/m^2)
	Hypertension
	Diabetes
	Previous or current smoker
	Preoperative haemoglobin level (g/dL)
	Preoperative creatinine level (µmol/L)
	Cardiopulmonary bypass time (min)
	Mediastinal blood loss the first 16 hours postoperatively (mL)
Step 3 Model	Cardiac dysfunction
	Acute myocardial infarction
	Novel renal failure
	Prolonged mechanical ventilation

[a] Step 3 Model also included all variables from Step 2.

4.4.5 Cardiac surgery patients with preoperative anaemia

In Paper 2, we investigated the association between RBC transfusion and long-term mortality through univariable and multivariable Cox regression analyses in a 4-Step block-wise approach. The chosen covariates were based on Paper 1 and clinical knowledge.

In Step 1, we performed a univariable Cox regression analysis with RBC transfusion as the only independent variable. In Step 2, we included RBC transfusion, patient risk factors and preoperative laboratory values as adjustment variables. In Step 3, the model further included operative variables and the New York Heart Association Functional Classification which is a classification of heart failure and measures the degree of restriction in physical activity in four categories. In the final Step 4, we added postoperative complications to the model. We evaluated the long-term mortality from 30 days to 5 years postoperatively, but we also investigated the HR of RBC transfusion in two separate observation periods, i.e. 30 days to 1 year and 1 to 5 years postoperatively. The specific variables for each Step can be found in Table 5.

Table 5: Independent variables included in the main analysis of Paper 2 [a]

Step 2 Model	Red blood cell transfusion
	Age (years)
	Sex (female)
	Body mass index (kg/m^2)
	Hypertension
	Diabetes
	Chronic pulmonary disease
	Previous or current smoker
	Preoperative haemoglobin level (g/dL)
	Preoperative creatinine level (µmol/L)
Step 3 Model	New York Heart Association Functional Classification
	Operation category
	Cardiopulmonary bypass time (min)
	Intraoperative blood loss (mL)
	Mediastinal blood loss the first 16 hours postoperatively (mL)
Step 4 Model	Cardiac dysfunction
	Acute myocardial infarction
	Novel renal failure

[a] The models also included all variables from the previous steps.

4.4.6 SEM: total effect of anaemia and RBC transfusion on mortality

In Paper 3, the main objective was to compare the direct effect of RBC transfusion and the total effect of preoperative anaemia on 5-year mortality using SEM. Pathway diagrams were constructed based on hypothesised causal relations between preoperative anaemia, RBC transfusion, patient and operative risk factors, and long-term mortality. We then employed SEM to determine whether the hypothesised relationships fit with the observed data. Finally, we compared the total effects (combined direct effect and indirect effect) of the observed variables on 5-year mortality.

We employed a stepwise approach starting with a simplified Model A. The first model included preoperative anaemia, RBC transfusion and long-term mortality, and their hypothesised relationships based on the literature (Figure 5).

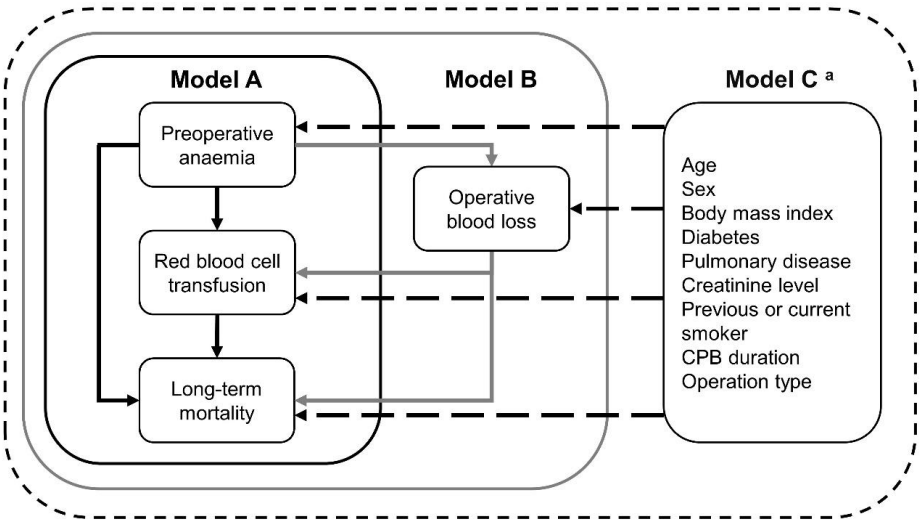

Figure 5. Simplified overview of structural equation Models A-C in Paper 3. Boxes indicate included variables. Arrows indicate causal relationship in the model. Model A confined by black solid line. Model B confined by grey solid line. Model C confined by black dashed line.

[a] Previous or current smoker was hypothesised to have direct effect on preoperative anaemia and long-term mortality. CPB duration and operation type were hypothesised to have direct effect on operative blood loss, RBC transfusion and long-term mortality. All other variables were hypothesised to have direct effect on all dependent variables.

Abbreviation: CPB, cardiopulmonary bypass.

In the first model, preoperative anaemia was hypothesised to be directly associated with RBC transfusion and 5-year mortality. RBC transfusion was hypothesised to be only directly associated with 5-year mortality. The indirect effect of preoperative anaemia on long-term mortality can be calculated by the product of the direct effect of anaemia on RBC transfusion and the direct effect of RBC transfusion on mortality. Thus, the combined total effect of preoperative anaemia was calculated for 5-year mortality.

In the second Step, we further included operative blood loss to obtain Model B (Figure 6). Operative blood loss was hypothesised to be affected by preoperative anaemia and exert a direct effect on RBC transfusion and mortality.

In the third Step, risk factors that were associated with morbidity and mortality (age, sex, BMI, diabetes, pulmonary disease, creatinine level, previous or current smoker, CPB time, and operation type) in cardiac surgery were also included to arrive at Model C (Figure 5). All risk factors that were further included in Model C had a direct effect on all the previous variables, except for previous or current smoker, CPB duration and operation type. Previous or current smoker was hypothesised to have direct effect on anaemia and mortality. CPB duration and operation type were hypothesised to have direct effect on operative blood loss, RBC transfusion and mortality.

To compare the variables measured using different scales, e.g. Hgb (g/dL) and RBC transfusion (units), the regression coefficients were standardized. This ensured that all variables were measured in standard deviations and their effects are therefore comparable.

4.4.7 Goodness of fit indices in SEM

The sample size requirement to obtain reliable estimates in SEM are considered sufficient when there is a ratio of 20 cases for each model parameter, or at least 200 cases for complex models. We assessed the overall goodness-of-fit with the $\chi 2$-test between the specified model and a model that perfectly fits the data, which denotes a model in which all the observed variables are correlated with all variables. A non-significant $\chi 2$-test indicates that the specified model fits the data, meaning that the specified parsimonious model explains the observed data equally well as a fully saturated model. However, this

test may be inadequate in large samples because small deviations may yield significant results [132].

Therefore, goodness-of-fit was also assessed with several other commonly used indices; a) the root mean square error of approximation, b) the standardized root mean squared residual, c) the Comparative Fit Index, and d) the Tucker-Lewis Index. The root mean square error of approximation is a comparison of difference between the SEM model and the observed data when considering the number of estimated parameters. The standardized root mean squared residual compares the discrepancy between the model covariance matrix and the sample covariance matrix. Lower values indicate a better fit for both tests. The Comparative Fit Index and the Tucker-Lewis Index compares the hypothesised model to a model where no variables are correlated, penalising in different ways for the number of estimated parameters. Larger values indicate better model fit for both these indexes.

Model fit is considered acceptable when the root mean square error of approximation is < 0.05, the standardized root mean squared residual is < 0.08, and the Comparative Fit Index and Tucker-Lewis Index are > 0.95 [133-135].

4.5 Sensitivity analyses

Several sensitivity analyses were performed in all papers to evaluate the robustness of the results and any potential bias.

4.5.1 Propensity score matching in Paper 1

A sensitivity analysis was performed using propensity score matching in Paper 1, because of the clinical differences between the patients who received RBC transfusion and those who did not [136]. First, we calculated the propensity score using exposure for treatment, i.e. RBC transfusion as the dependent variable in a logistic regression analysis for all patients. The covariates included in the propensity score for RBC transfusion are listed in Table 6.

Table 6: Independent variables included in the propensity score matching in Paper 1

Age (years)
Sex (female)
Body mass index (kg/m^2)
Chronic pulmonary disease
Diabetes
Hypertension
Vascular disease [a]
Previous or current smoker
Unstable angina
Preoperative haemoglobin concentration (g/dL)
Preoperative creatinine concentration (µmol/L)
Previous myocardial infarction
Previous percutaneous coronary intervention
The New York Heart Association Functional Classification III or IV
Use of acetylsalicylic acid
Use of angiotensin converting enzyme inhibitors
Use of low-molecular-weight heparin
Cardiopulmonary bypass time (min)
Cardiac dysfunction [b]
Acute myocardial infarction [b]
Novel renal failure [b]
Prolonged mechanical ventilation [b]

[a] Thoracic or abdominal aortic aneurism, history of cerebrovascular insult or intermittent claudication.

[b] Variables included only in the model with postoperative complications.

Patients were given a propensity score from 0 to 1 based on the probability of receiving RBC transfusion. Two models were analysed with propensity score matching. We calculated the propensity score without and with postoperative complications as covariates in the regression analysis (Model 5 and 6, respectively).

Next, patients were matched one-to-one among patients who received RBC transfusion with patients who did not receive any transfusion, within their respective model. The calliper width used to match an RBC-transfused patient to a non-transfused patient was one-fourth of the standard deviation of the propensity score for the model. The calliper width used was 0.0711 for Model 5 and 0.0713 for Model 6, which means that a matched pair had a propensity score within 0.0711 and 0.0713 of each other, respectively.

The purpose of propensity score matching was to analyse the association of RBC transfusion on long-term mortality in two similar groups with risk of exposure. Therefore, the differences in patient and operative variables should be well balanced between the two groups after matching. The absolute standardized difference was calculated to assess the differences in covariates between the two groups after matching. The standardized means for continuous variables or for prevalence for binomial variables were then compared between the matched groups. For categorical variables with > 2 values, the absolute standardized difference was evaluated for all dummy pairs. The absolute difference should ideally be less than 10%, which indicates a small difference of the covariate between patients in the two groups [137].

Finally, we assessed the residual confounding using a univariable random effects Cox regression model between the matched groups. The Cox regression analysis in the propensity score matching models used RBC transfusion as the only covariate and all-cause mortality after 30 days postoperatively as the outcome.

4.5.2 Observation time and operative procedures in Paper 3

In Paper 3, we evaluated the impact of RBC transfusion and preoperative anaemia on mortality from 30 days to 1 year (Model D) and 1-5 years (Model E) postoperatively. These sensitivity analyses were performed for two reasons: 1) SEM does not evaluate the time to event as in Cox regression, and 2) based on results from Paper 2, which indicated that the HR of RBC transfusion on mortality may be largest in the first year postoperatively.

The main analysis in Paper 3 included all surgical procedures. Isolated CABG, valve surgery and multiple procedures have different patient characteristics and operative risks. The risk associated with RBC transfusion on long-term mortality may be different for patients undergoing either of these procedures. We therefore performed additional sensitivity analyses in patients undergoing isolated CABG (Model F) or multiple procedures (Model G).

These sensitivity analyses used the complete Model C in Paper 3, excluding the variable operation category for analysis on isolated CABG and multiple procedures.

5 Summary of results

5.1 Patient characteristics

There were several clinical differences between the patients who received RBC transfusion and patients who did not receive any transfusion in the Trondheim Heart Surgery Database. Patients who received RBC transfusion were older, had lower BMI and Hgb-concentrations, more comorbidities, and higher incidence of postoperative complications (Table 7). Detailed patient characteristics can be found in each specific attached Paper.

Table 7: Patient characteristics and operative variables of adult patients included from the Trondheim Heart Surgery Database in the present thesis [a]

	Patients without RBC transfusion (n = 5,101)	Patients with RBC transfusion (n = 4,214)	*P*-value
Preoperative variables			
Anaemia	337 (6.6)	1,552 (36.8)	< 0.01
Sex – Men	4,449 (87.2)	2,504 (65.8)	< 0.01
Age (years)	64 ± 10.1	70 ± 10.0	< 0.01
Body mass index (kg/m^2)	27.6 ± 4.0	26.2 ± 4.1	< 0.01
Present or previous smoker	2,943 (57.7)	2,138 (50.7)	< 0.01
Chronic heart failure [b]	500 (12.8)	934 (27.0)	< 0.01
Diabetes	683 (13.4)	703 (16.7)	< 0.01
Hypertension	2,645 (51.9)	2,412 (57.2)	< 0.01
Chronic pulmonary disease	613 (12.0)	824 (19.6)	< 0.01
Renal dysfunction	82 (1.6)	275 (6.5)	< 0.01
Haemoglobin (g/dL)	14.5 ± 1.2	13.0 ± 1.5	< 0.01
Creatinine (µmol/L)	87.0 ± 24.9	96.1 ± 68.9	< 0.01
EuroSCORE II operation category			
Isolated CABG	3,948 (77.4)	2,186 (51.9)	< 0.01
Valvular procedure	543 (10.6)	686 (16.2)	< 0.01
2 or more procedures	610 (12.0)	1,342 (31.8)	< 0.01
Postoperative complications			
Renal failure	222 (4.4)	704 (16.7)	< 0.01
Myocardial infarction	150 (2.9)	258 (6.1)	< 0.01
Cardiac dysfunction	151 (3.0)	495 (11.8)	< 0.01
Prolonged mechanical ventilation	24 (0.5)	246 (5.8)	< 0.01
Long-term mortality			
Death 30 days – 1 year postoperatively	39 (0.8)	149 (3.5)	< 0.01
Death 1-5 years postoperatively	305 (6.0)	619 (14.7)	< 0.01
Death > 30 days postoperatively	1,178 (23.1)	1,772 (42.1)	< 0.01

[a] Variables given as mean with standard deviation or number of patients (%)

[b] Patients with missing data: chronic heart failure n = 1,939

Abbreviations: CABG, coronary artery bypass grafting; RBC, red blood cell.

5.2 Paper 1

5.2.1 Main results from Paper 1

From the Trondheim Heart Surgery Database from 2000 to 2014, we identified 4,014 adult patients undergoing primary CABG included in Paper 1 after inclusion and exclusion criteria.

Among the included patients in Paper 1, a total of 1,127 (28%) patients received 1 or more units of RBC transfusion during the hospital stay. There were 298 (26%) recorded deaths among those who received RBC transfusion in the observation period, compared with 429 (15%) recorded deaths in the patient group who did not receive any RBC transfusion (n = 2,887). The Kaplan-Meier survival plot showed a difference in mortality between patients who received at least 1 unit of RBC transfusion and patients who did not receive any transfusion (unadjusted Cox regression $P < 0.01$, Figure 6).

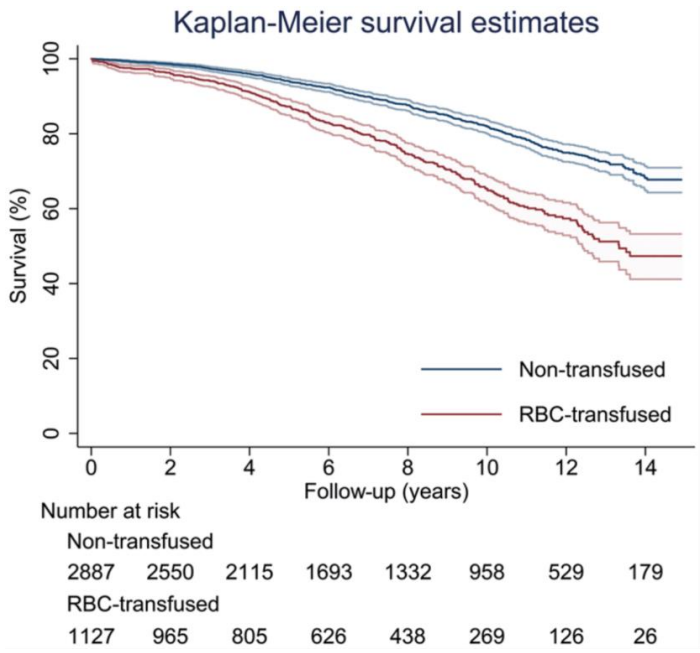

Figure 6. Survival estimates in Paper 1. Kaplan–Meier survival plot of primary isolated coronary artery bypass grafting surgery patients by RBC transfusion, corresponding to the unadjusted Cox regression analysis. Abbreviations: RBC, red blood cell

The observation time for this study ranged from 30 days to 15 years postoperatively, with a median of 7.2 years (95% CI: 7.0–7.5 years). There was a statistically significant unadjusted association between RBC transfusion and long-term mortality in patients undergoing primary isolated CABG (Table 8).

The adjusted models showed a decreasing association between RBC transfusion and long-term mortality in this patient group. When adjusted for pre- and intraoperative risk factors, RBC transfusion was still statistically significantly associated with long-term mortality (Table 8). When common postoperative complications were added to the adjustment, the association was no longer statistically significant (Figure 7, Table 8).

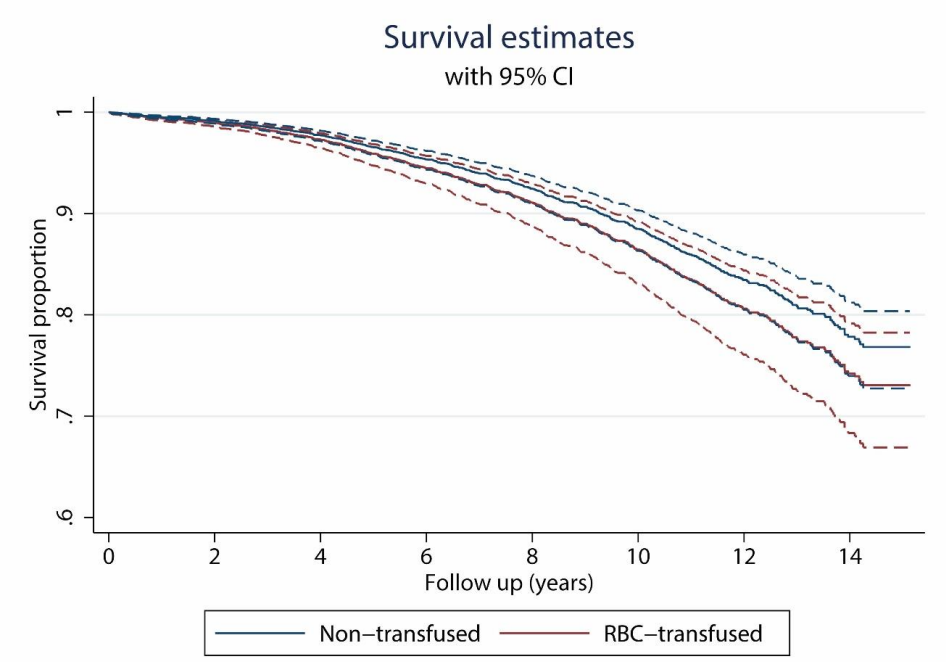

Figure 7. Multivariable survival estimates in Paper 1. Survival estimates adjusted for patient- and operative risk factors, as well as postoperative complications for primary isolated coronary artery bypass grafting patients by RBC transfusion. Shown for 100-60% cumulative survival. Abbreviations: RBC, red blood cell

Table 8: Main Cox regression models for red blood cell transfusion and long-term mortality in adult patients undergoing primary isolated CABG

	Hazard ratio	95% CI	*P*-value
Model 1 - unadjusted	2.10	1.81 – 2.43	< 0.01
Model 2[a]	1.26	1.04 – 1.53	0.02
Model 3[b]	1.19	0.98 – 1.44	0.08

[a] Adjusted for pre- and intraoperative variables.
[b] Adjusted for pre- and intraoperative variables and including postoperative complications.
Abbreviations: CABG, coronary artery bypass grafting; CI, confidence interval

The Cox regression models in Paper 1 satisfied the PH test and showed good fit. We therefore considered the models to adequately describe the observed data.

5.2.2 Sensitivity analysis: Propensity score matching

In the sensitivity analysis using propensity score matching, 596 (53%) transfused patients were matched with a corresponding non-transfused patient when adjusting for pre- and intraoperative variables corresponding to Model 2 (Table 9). In the model corresponding to Model 3 including postoperative complications, a match could be found for 590 (52%) RBC-transfused patients (Table 9). The remaining RBC-transfused patients who were not matched had a higher calculated propensity score, i.e. these patients had a higher propensity for RBC transfusion and there were no patients in the non-transfused group who had similar risk. The propensity-matched groups were balanced for all variables except for previous percutaneous coronary intervention, which had an absolute standardized difference of 13%. This was considered acceptable. The propensity score-based models did not show a significant association between RBC transfusion and long-term mortality (Table 9).

Table 9: Cox regression models for propensity-matched groups for red blood cell transfusion and long-term mortality in adult patients undergoing primary isolated CABG

	Matched pairs	Unmatched non-transfused patients	Unmatched RBC-transfused patients
Model 5	n = 596	n = 2,291 (79.4%)	n = 535 (47.5%)
Model 6	n = 590	n = 2,297 (79.6%)	n = 541 (48.0%)

	Hazard ratio	95% confidence interval	*P*-value
Model 5[a]	1.18	0.92 – 1.52	0.19
Model 6[b]	1.14	0.88 – 1.46	0.32

[a] Adjusted for pre- and intraoperative variables.

[b] Adjusted for pre- and intraoperative variables and including postoperative complications.

Abbreviations: CABG, coronary artery bypass grafting; RBC, red blood cell

5.3 Paper 2

5.3.1 Main results from Paper 2

From the Trondheim Heart Surgery Database from 2000 to 2017, we identified 1,859 adult patients with preoperative anaemia undergoing primary cardiac surgery (18%) included in Paper 2 after inclusion and exclusion criteria.

Among the patients included in Paper 2, 1,525 (82%) patients received at least 1 unit of RBC transfusion during the hospital stay. During the follow-up period, 370 (20%) deaths were registered, of which 37 (11%) patients did not receive RBC transfusion and the remaining 333 (22%) patients received at least 1 unit of RBC during the hospital stay. Between 30 days and 1 year postoperatively, 88 (24%) patient deaths were registered and 84 (96%) of those who died in this period received > 1 unit of RBC (Figure 8).

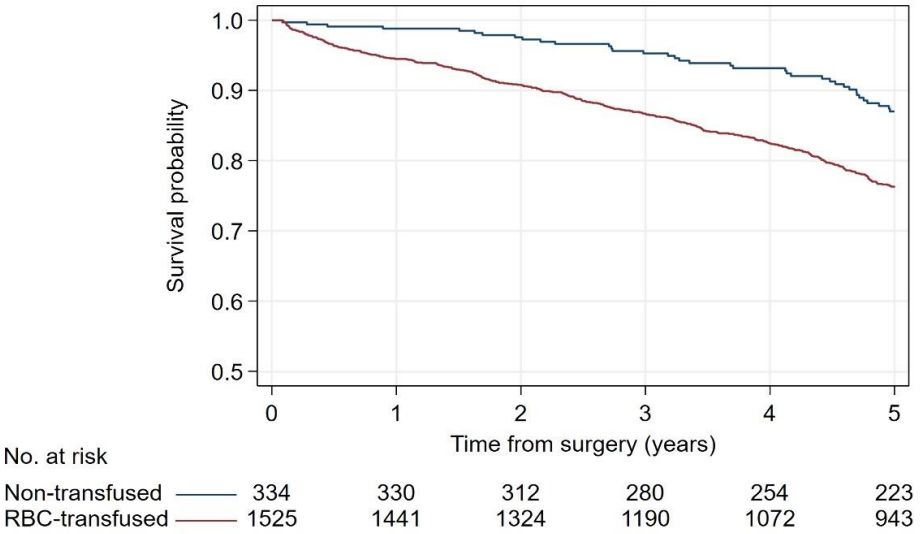

Figure 8. Survival estimates in Paper 2. Kaplan-Meier survival plot for cardiac surgery patients with preoperative anaemia by RBC transfusion, corresponding to the unadjusted Cox regression analysis. Shown for 100-50% cumulative survival. Abbreviations: RBC, red blood cell

The unadjusted Cox regression analysis in Paper 2 showed a significant association between long-term mortality and RBC transfusion in all 3 observation periods (Table 10). The HR for RBC transfusion was highest during the first year after surgery and declined thereafter. Following adjustments for patient risk factors and preoperative laboratory values in the Step 2 Model, RBC transfusion was no longer statistically significantly associated with long-term mortality in any observation period. In the Step 3 models that included operative confounding factors and the Step 4 models that also included postoperative complications, the HR for RBC transfusion were further decreased. Survivor estimates with 95% CI for the Step 4 model are shown in Figure 9.

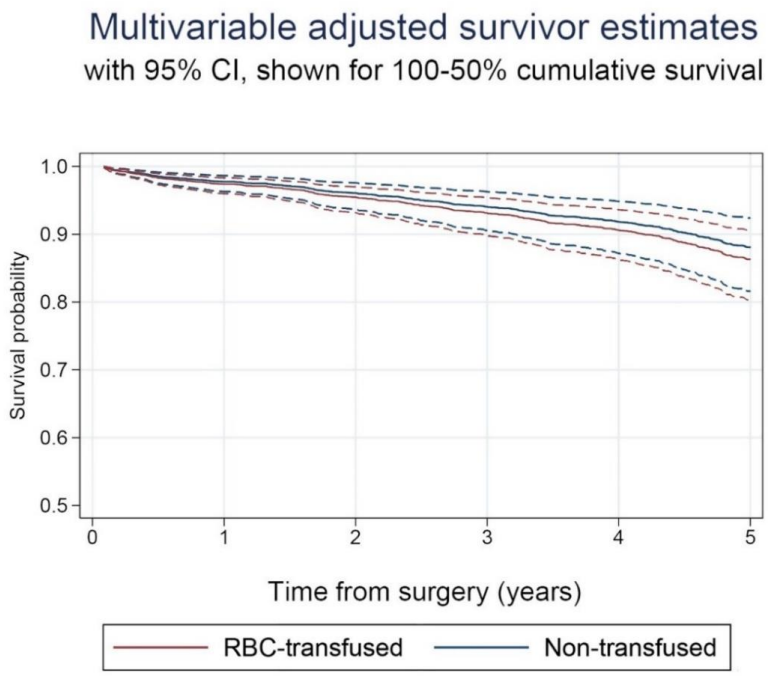

Figure 9. Multivariable survival estimates in Paper 2. Survival estimates adjusted for patient- and operative risk factors, as well as postoperative complications for cardiac surgery patients with preoperative anaemia by red blood cell (RBC) transfusion. Shown for 100-50% cumulative survival.

Table 10: Unadjusted and multivariable Cox regression analysis on long-term mortality for primary anaemic cardiac surgery patients [a]

	Hazard ratio	95% confidence interval	*P*-value
30 days – 5 year			
Model 1 - unadjusted	2.09	1.49 – 2.93	< 0.01
Model 2 [b]	1.42	0.99 – 2.03	0.06
Model 3 [c]	1.21	0.84 – 1.74	0.31
Model 4 [d]	1.16	0.80 – 1.68	0.43
30 days – 1 year			
Model 1 - unadjusted	4.70	1.72 – 12.81	< 0.01
Model 2 [b]	2.54	0.90 – 7.12	0.08
Model 3 [c]	1.92	0.67 – 5.47	0.22
Model 4 [d]	1.79	0.63 – 5.12	0.28
1 – 5 years			
Model 1 - unadjusted	1.77	1.23 – 2.55	< 0.01
Model 2 [b]	1.29	0.87 – 1.89	0.20
Model 3 [c]	1.15	0.77 – 1.70	0.50
Model 4 [d]	1.11	0.75 – 1.65	0.61

[a] Cox regression models for the association between red blood cell transfusion and long-term mortality in patients with preoperative anaemia undergoing open-heart surgery. Description of included variables can be found in Table 5.

[b] Adjusted for patient risk factors and preoperative laboratory values.

[c] Adjusted for pre- and intraoperative variables.

[d] Adjusted for pre- and intraoperative variables and including postoperative complications.

For Cox regression models in Paper 2 that did not satisfy the PH test and therefore violated the PH assumption, results were compared with similar models using flexible parametric survival models. Point estimates and *P*-values from the flexible parametric survival analysis were essentially unchanged. We therefore considered the Cox regression models to be adequate.

5.4 Paper 3

5.4.1 Main results from Paper 3

From the Trondheim Heart Surgery Database from 2000 to 2017, we identified 9,315 adult patients undergoing primary cardiac surgery in Paper 3 after considering the inclusion and exclusion criteria.

In the main analysis in Paper 3 with all included study variables (Model C), RBC transfusion (0.03) had the weakest total effects on 5-mortality of all the statistically significant variables. Preoperative anaemia (0.10) had more than three times the relative impact on mortality compared with RBC transfusion (Table 11).

Of the remaining observed variables that were significantly associated with mortality, age had the largest total effect (0.15), followed by creatinine level (0.12), pulmonary disease (0.11), diabetes (0.06) and operative blood loss (0.06). BMI was a negative predictor of 5-year mortality, meaning that a higher BMI was associated with increased survival (-0.06). Female sex was not significantly associated with mortality in the SEM model (Table 11).

Table 11: Total effects of study variables on 5-year mortality in Model C

Variables	Total effect coefficients [a]	*P*-value
Red blood cell transfusion	0.03	0.01
Preoperative anaemia	0.10	< 0.01
Operative blood loss	0.06	< 0.01
Age	0.15	< 0.01
Sex (female)	- 0.01	0.58
Body mass index	- 0.06	< 0.01
Diabetes	0.06	< 0.01
Pulmonary disease	0.11	< 0.01
Creatinine level	0.12	< 0.01
Cardiopulmonary bypass time	0.04	0.01
Operation category	0.04	< 0.01
Previous or current smoker	0.04	< 0.01

[a] All coefficients are standardized, i.e. numbers correspond to effects when all variables are measured in the same scale

5.4.2 Sensitivity analysis: Observation time

The first sensitivity analyses included observation time from 30 days to 1 year postoperatively (Model D, n = 9,315) or 1 to 5 years postoperatively (Model E, n = 9,127). The total effect of preoperative anaemia on mortality was greater than that of RBC transfusion in both models. The effect of RBC transfusion on long-term mortality was also not statically significantly associated with mortality in either observation period in the sensitivity analyses (Table 12).

Table 12. Standardized total effects in Model D (30 to 1 year postoperatively) and Model E (1-5 years postoperatively)

	Total effect on long-term mortality	*P*-value
Model D		
Red blood cell transfusion	0.02	0.10
Preoperative anaemia	0.06	< 0.01
Model E		
Red blood cell transfusion	0.02	0.06
Preoperative anaemia	0.09	< 0.01

5.4.3 Sensitivity analysis: Isolated CABG and multiple procedures

The next sensitivity analyses included patients undergoing isolated CABG (Model F, n = 6,134) and patents undergoing multiple procedures (Model G, n = 1,952). The total effect of preoperative anaemia on long-term mortality was greater than that of RBC transfusion. RBC transfusion was statically significantly associated with long-term mortality in Model F and not in Model G (Table 13).

Table 13. Standardized total effects in Model F (isolated coronary artery bypass grafting) and Model G (multiple procedures)

	Total effect on long-term mortality	*P*-value
Model F		
Red blood cell transfusion	0.03	0.03
Preoperative anaemia	0.10	< 0.01
Model G		
Red blood cell transfusion	0.02	0.44
Preoperative anaemia	0.11	< 0.01

5.4.4 Model fit indices

The sample sizes used in Paper 3 were considered sufficiently large in all models.

The third step in the study (Model C) which included all study variables and observation time from 30 days to 5 years postoperatively, the χ^2-test was significant ($P < 0.01$). Because of the relatively large sample size (n = 9,315), we accepted the model despite the result from the χ^2-test because the model showed good fit indicated by the other fit indices (Table 14).

Table 14: Goodness of fit indices for Model C

Fit statistic	Model C
Likelihood ratio	Value
Chi-square test model vs. saturated	< 0.01 (4 df)
Chi-square test baseline vs. saturated	< 0.01 (42 df)
Root mean squared error of approximation	0.03
90% CI, lower bound	0.02
90% CI, upper bound	0.04
Comparative fit index	1.00
Tucker-Lewis index	0.94
Standardized root mean squared residual	0.01
Coefficient of determination	0.40

Abbreviations: CI, confidence interval; df, degrees of freedom

In the Model D to G in Paper 3, the results regarding goodness of fit were similar to those for Model C. We considered the goodness of fit indices for all models to be acceptable.

6 Discussion

6.1 Main findings

Paper 1 included adult patients undergoing primary isolated CABG. We found that RBC transfusion was no longer statistically significantly associated with long-term all-cause mortality when adjusting for known risk factors and common postoperative complications.

Paper 2 included patients with preoperative anaemia undergoing cardiac surgery. We demonstrated that RBC transfusion was not statistically significantly associated with 5-year all-cause mortality when adjusting for patient risk factors. The same result was found between 30 days and 1 year, and 1-5 years postoperatively. The results showed a higher HR for long-term mortality associated with RBC transfusion the first year postoperatively.

Paper 3 included patients undergoing cardiac surgery and examined the relationship between preoperative anaemia, RBC transfusion, and other risk factors on mortality using SEM. The findings from Paper 3 demonstrated that all other risk factors included in the main Model C had larger effect on 5-year all-cause mortality compared with RBC transfusion, except for sex.

6.2 Methodological considerations

Several methodological uncertainties need to be considered when interpreting the results of the present thesis. Both bias and limitation of the statistical methods used in the present thesis could potentially lead to incorrect conclusions. Bias is the systematic error in research which can cause overestimation or underestimation of a true parameter. Sources of bias can occur in all phases of a study, from data collection, analysis, interpretation to publication. Selection and exclusion bias is a distortion introduced when sampling does not reflect the study population. Another form of bias is information bias, which is introduced due to error in collection, recording or handling of data.

6.2.1 Selection bias

In the present thesis, the patient exclusion and inclusion criteria used in the analyses could introduce selection bias when defining the final study samples.

In Paper 1, we excluded patients with high risk of mortality to reduce confounding. Isolated CABG is considered to have the lowest risk of mortality compared with other procedures [45]. Furthermore, we excluded patients with excessive bleeding because of the increased mortality risk in these patients [76]. Therefore, the patients in the study sample had low risk of mortality due to the selection criteria. The additional risk of RBC transfusion on long-term mortality may not have been large enough to reach a statically significant difference in patients included in Paper 1. Because of the relatively large sample size, we consider that selection bias had little impact on the results in Paper 1.

A limitation of the analyses is the low number of patients with preoperative anaemia who did not receive RBC transfusion. Therefore, all cardiac surgery patients were included in Paper 2 to preserve power. However, the patient population in Paper 2 might be incomparable because of heterogenous patient characteristics and risk factors. In Paper 2 and 3, we included operation category as an adjustment variable to mitigate this bias. In Paper 3, we included subgroup analysis of patients undergoing isolated CABG and multiple procedures as sensitivity analyses. Therefore, we do not consider the inclusion of all surgical procedures to have substantially affected the main findings.

Patients missing from the database either because of input error or having declined participation, could potentially add bias due to missing data. However, virtually all patients have provided an informed consent regarding the use of their data for research. Furthermore, compulsory inclusion of data regarding death of Norwegian citizens into the Norwegian Cause of Death Registry, guarantees little loss of follow-up of patients. We therefore consider the results in the present thesis to reflect real-world data regarding outcomes in cardiac surgery for the population in Middle-Norway.

6.2.2 Information bias

Another bias that needs to be considered is information bias, which includes misclassification, observer, recall and reporting bias. The clinical data used in this thesis

come from a single-centre university hospital, and the patient data have been collected prospectively by the treating physician. The Trondheim Heart Surgery Database was controlled several times during registration and has later undergone several quality controls by a senior anaesthesiologist. Because of these quality controls, there have been few missing data in the database. There were less than 2% missing data among the variables used in all papers. We therefore did not consider any strategy to deal with missing values and considered error during registration to be small. However, some variables in the database were not recorded during the complete study period. Therefore, not all variables available in the database were used in the analyses.

Single-centre studies often lack the sample size to infer results but avoid the problems of discrepancies of quality assurance, data management and surgical practise between centres. The Trondheim Heart Surgery Database from 2000 to 2017 included 10,288 patients and we consider the consistency and sample size as a strength of the papers. The results do, however, reflect associations to the transfusion threshold used at St. Olavs Hospital; other transfusion policies could lead to different results. The introduction of non-invasive techniques, changes in patient characteristics, and surgical procedures due to the long study period could have impacted the results. We cannot exclude that a temporal change in mortality risk in patients undergoing cardiac surgery could have influenced the association between RBC transfusion and long-term mortality.

6.2.3 Non-significant findings

The null hypothesis of this thesis was that there is no statistical difference in long-term mortality between patients who received RBC transfusion and patients who did not receive any transfusion. The finding of a non-significant result is the failure to reject the null hypothesis, meaning that there is little evidence to support that the null hypothesis is false. The non-significant results could also indicate a small or negligible effect. However, failure to reject the null hypothesis is not evidence which supports the null hypothesis. The results from observational studies can also not infer a causal relationship and the findings must therefore be interpreted cautiously.

A limitation of the present thesis is the low number of registered deaths within the first year after surgery. The lower number of deaths is reflected by the wide CI in the results which is caused by a low statistical power. The interpretation of the results from this observation period in Paper 2 must therefore be done with caution. The 95% CI gives the 95% probability that the true HR lies within the CI. The interpretation of the results must therefore consider the CI and the probability that a true value can be different from the calculated point estimates of the HR. Still, non-significant findings are important to report in the scientific literature to highlight the complexity of the present clinical dilemma. Selective reporting of only results with statistically significant findings could skew the evidence which supports a false alternative hypothesis, or falsely rejecting a true null hypothesis.

6.2.4 Inclusion of postoperative complications

The inclusion of postoperative complications could add another layer of confounding and bias to the analyses. We employed a stepwise design in all the main analyses in this thesis. The rationale behind this design was to permit evaluation of different kinds of confounders. The association of RBC transfusion with long-term mortality must therefore be evaluated with regards to the covariates used to adjust the model at each step.

The postoperative events used in the analyses could indicate a more complex operative course, which was the rationale to adjust for them. The postoperative complications included in the analyses, i.e. novel renal dysfunction, prolonged mechanical ventilation, cardiac dysfunction, and MI, could be caused by patient characteristics and operative factors [138-141]. In addition, patients who experience postoperative complications have a higher risk of mortality. Exclusion of these events among cardiac surgery patients could be an unadjusted confounder in previous studies and consequently contribute to the contradictive findings in the literature.

Data regarding time of transfusion and its relation to the occurrence of postoperative complications were not recorded in the database. The results from the use of postoperative complications in the adjusted models may therefore be biased because RBC transfusion may have preceded a complication. This was the reason why these postoperative

complications were not included in Paper 3. The finding of a statistically significant although small effect of RBC transfusion on long-term mortality in Paper 3, could be attributed to the lack of these important adjustment variables.

6.2.5 Limitation of propensity score matching

There are several strengths in the use of propensity score matching in observational studies as described in the Introduction. The objective of propensity score matching is to reduce the clinical differences and influence from potential confounders by matching patients with equal probability of the exposure. Patient differences between groups due to selection bias could potentially be eliminated. However, the regression analysis to calculate the propensity score is dependent on the adjustment variables used, and the variables available in the database. Important confounders omitted from the regression analysis could potentially be unbalanced between the groups after matching [142]. However, further inclusion of variables in the propensity score would increase the difficulty in maintaining the balance of risk factors between the groups. Therefore, other available risk factors in the database were not included in the analyses.

Another issue arises by the lack of matches between the two groups. A large portion of transfused patients with high propensity score did not have an adequate match among the controls in Paper 1. The exclusion of these high-risk patients therefore introduces a selection bias. However, the lack of matches between the groups demonstrates large clinical differences between patients who received RBC transfusion and those who did not. Propensity score matching was originally planned for Paper 2, but the analysis was not performed because of inadequate balance between the groups after matching.

6.2.6 Limitations of SEM

Because of the complex relationship between multiple risk factors in cardiac surgery, RBC transfusion and mortality, the SEM framework could potentially bridge some of the knowledge gaps. Paper 3 showed how this framework could be used to elucidate difficult clinical research questions by constructing graphical diagrams with causal pathways.

However, the construction of a measurement model in SEM should objectively be based on empirical data which. We assumed a hypothesised causal relationship between the variables in the SEM model. Therefore, the results are only valid if the relationships between these variables are true. Because the relationships between RBC transfusion and included variables are complex, other hypothesised models could fit the data just as well or better. Furthermore, omitted important variables from the model may lead to misleading estimates and effects. The effect of RBC transfusion and anaemia on mortality may be due to risk factors not included in the model. Additional inclusion of risk factors, such as those included in Paper 1 and 2, could potentially lead non-significant association between RBC transfusion and long-term mortality as seen in these papers [123].

Another consideration with SEM is the lack of adjustment of a time-to-event function. In comparison to Cox regression, SEM only estimates mortality as a binary yes or no outcome. Because mortality was considered a binary outcome variable, patients who experienced early mortality were weighted equally in the model to patients who experience late mortality. As described in the Introduction, the analysis without time-to-event with regards to long-term mortality could be problematic. Therefore, sensitivity analysis with subdivision of observation time in the SEM models was performed to reduce this early and late survival bias. These sensitivity analyses showed similar results as the main analysis.

6.3 Interpretation of results

6.3.1 Long-term mortality

In the unadjusted and multivariable models in Paper 1, which included patient and operative risk factors, RBC transfusion was statistically significantly associated with long-term mortality. The association between RBC transfusion and long-term mortality was no longer statically significant when postoperative complications were included in the final Step 3 Model. The findings of non-significant *P*-values in Paper 1 are not evidence in favour of the null hypothesis. However, the results suggest that the difference in observed mortality may be due to residual confounders. The inclusion of postoperative

complications to the analysis could be erroneous as described above, and lead to overadjustment and non-significant results in the analysis.

The sensitivity analyses with propensity score matching in Paper 1 showed similar results as the main Step 3 Model, and therefore supports the main findings. The sensitivity analyses included a model without and with postoperative complications. We consider that the inclusion of common postoperative complications to be important and with a low risk of additional bias.

In Paper 2, RBC transfusion was not significant associated with long-term mortality when adjusted for patient risk factors. The HR decreased further when operative risk factors and postoperative complications were subsequently added as adjustments in the analysis. In comparison with Paper 1, only the Step 3 Model which included all adjustments variables did not reach a statistically significant level between RBC transfusion and long-term mortality.

The difference in findings between Paper 1 and 2 could be due to the higher risk of mortality among cardiac surgery patients with preoperative anaemia. As seen in Paper 3 using SEM, the impact of preoperative anaemia on long-term mortality was at least 3-fold larger than RBC transfusion. Cardiac surgery patients with preoperative anaemia may undergo longer and more complex surgery because of comorbidities and underlying chronic diseases [127]. The additional potential negative effect of RBC transfusion among patients with preoperative anaemia may be small and not reach a statistically significant result. Furthermore, the potential small negative effect of RBC transfusion on long-term mortality could be more important among patients undergoing isolated CABG. In Paper 1, the inclusion of multiple adjustment variables was necessary to reach a statistically non-significant result between RBC transfusion and long-term mortality.

In Paper 2, the division of observation time was necessary to fulfil the PH assumption. Because of this division, the results also demonstrated the change in long-term risk associated with RBC transfusion from the first year and to the next four years postoperatively. Paper 2 indicated that any potential risk of mortality associated with RBC transfusion in anaemic patients was highest in the early postoperative period and declined after 1 year. However, the 95% CI were widest during the first year after surgery

due to low numbers of events. Other patient risk factors may be accentuated by time and the association between RBC transfusion and long-term mortality may diminish later in the follow-up period.

6.3.2 Relative effect of preoperative anaemia and RBC transfusion

Paper 3 demonstrated a large effect of patient and operative risk factors that are associated with mortality. All risk factors included in the main Model C showed a larger effect on 5-year all-cause mortality compared with RBC transfusion, except for sex. When comparing preoperative anaemia to RBC transfusion on 5-year all-cause mortality, the relative standardized effect of anaemia (10%) was three times larger compared with RBC transfusion (3%). The results from Paper 3 contribute to reducing the knowledge gap regarding RBC transfusion and mortality by employing SEM. The use of SEM allowed for the investigation of the effect of risk factors in cardiac surgery on both RBC transfusion and long-term mortality. We therefore considered the use of SEM in Paper 3 to be an important step forward in understanding the effect of RBC transfusion in cardiac surgery.

We also examined the impact of preoperative anaemia and RBC transfusion on mortality in patients undergoing isolated CABG or multiple procedures in Paper 3. Because these two patient groups have different patient and operative risks of mortality, the impact of both preoperative anaemia and RBC transfusion on mortality may therefore be dissimilar between the groups. The results from these sensitivity analyses, however, showed similar findings as the main Model C. The relative impact of preoperative anaemia was three to four times larger than RBC transfusion in all models in Paper 3. Therefore, we considered the relative effect of RBC transfusion on long-term mortality compared with preoperative to be accurate based on the consistency of the results. Sensitivity analysis of patients undergoing valve surgery did not satisfy model fit indices for SEM models and was therefore not included in Paper 3.

Taken together, our studies demonstrated that the association between RBC transfusion and the observed difference in long-term mortality in cardiac surgery patients was mainly

due to patient characteristics and risk factors. Patients who received transfusion of RBC have multiple risk factors and generally a more demanding operative course.

6.4 Comparison with previous studies

6.4.1 Residual confounders

Contradicting results from previous studies indicate a complex relationship and interaction between patient risk factors and the need to transfuse. RBC transfusion may be indicated because of patient characteristics, comorbidities, and clinical presentation during the perioperative phase. Interpretation of the results from observational studies must therefore be done with caution and covariates must be chosen with great consideration. Contradicting results from previous studies could be due to overestimation of effect when not thoroughly adjusting for confounders, which is a weakness in observational studies.

Although observational studies cannot prove causality, there seems to be a general opinion that there is a harmful effect of RBC transfusion on long-term mortality in cardiac surgery. Considering the results of the present thesis and the contradictive findings in the literature, we propose that the differences in findings could be due to residual or unmeasured confounding factors. Unmeasured variables that indicated a more complicated perioperative course not adjusted for in other studies may lead to overestimation of the effect of RBC transfusion. Covariates such as anaemia, perioperative blood loss, prolonged intubation, and postoperative end-organ damage may be unadjusted for in previous studies.

6.4.2 Preoperative anaemia

As described in the Introduction, preoperative anaemia is considered an important risk factors in cardiac surgery and increases both postoperative morbidity and mortality [57-67]. The findings from our analyses suggest that preoperative anaemia and Hgb level are important risk factors among cardiac surgery patients [65]. Previous studies lacking adjustment for preoperative Hgb concentration may therefore overestimate the negative

effect of transfusion due to residual confounding [82, 84, 87, 89, 100]. Studies with large sample sizes and multi-centre studies have difficulties attaining a broad selection of relevant adjustment variables and their results may therefore not have been properly adjusted.

Other authors have found similar result of non-significant association between RBC transfusion and long-term mortality when adjusting for preoperative Hgb or anaemia [103, 105, 106]. A study investigated the effect of RBC transfusion on long-term mortality among anaemic and non-anaemic patients undergoing isolated CABG [69]. The authors found that RBC transfusion had a higher HR for long-term mortality among anaemic patients but not among non-anaemic patients. RBC transfusion could therefore be a marker of the detrimental effect of anaemia, and a confounding factor.

Randomisation of patients to transfusion vs. non-transfusion groups is not feasible. However, patients who refuse transfusion because of religious beliefs can be compared with transfused patients. A study compared Jehovah's Witness to non-Jehovah's Witness patients with nadir Hgb < 8.0 g/dL using propensity score matching [59]. For both groups, lower Hgb was associated with an increased risk of adverse outcomes, which included postoperative MI, renal replacement therapy, stroke, or in-hospital mortality. However, the authors found no statistically significant association of RBC transfusion with the outcomes. The study also compared transfused with non-transfused patients to assess for bias and found similar results.

The studies which have found non-significant results of RBC transfusion on mortality when adjusting for anaemia therefore support the findings of the present thesis.

6.4.3 Perioperative blood loss

As described in the Introduction, perioperative blood loss is an important predictor of RBC transfusion and mortality. Patients with preoperative anaemia naturally have a higher risk of reaching the transfusion trigger in the presence of major bleeding. The results from Paper 3 suggest that both operative blood loss and preoperative anaemia have larger effect on long-term mortality than RBC transfusion. Blood loss could therefore be

a missing adjustment variable and residual confounder in previous studies on the effect of RBC transfusion on mortality.

A study analysed the independent effect of major bleeding on operative mortality adjusted for RBC transfusion and anaemia in 16,154 cardiac surgery patients [68]. The authors demonstrated an independent association of major bleeding on mortality, but also an increased hazard in the presence of RBC transfusion and anaemia. Another study demonstrated how chest tube drainage might be an unadjusted confounder and found it to be the most important predictor for mortality [107]. The authors did not find an independent association between RBC transfusion and mortality in their multivariable analysis which included chest tube drainage. The results from this study support the hypothesis that unadjusted residual confounding is the underlying cause of the observed difference in mortality between RBC transfused and non-transfused patients.

In a study on 5,342 CABG and valve surgery patients, the authors found both RBC transfusion and anaemia to be statistically significantly associated with long-term mortality [96]. The study included a wide range of adjustment variables including anaemia and prolonged intubation. Patients in this study who received massive transfusion had lower survival. However, adjustment for blood loss which could have precipitated the need for transfusion was not included. Similar results can be found in a previous study on 8,724 cardiac surgery patients [86]. The authors found RBC transfusion to be statistically significantly associated with infection and ischemic outcome, as well as increased early and late mortality. The authors stratified patients by nadir HCT to adjust for anaemia but did not include blood loss in the analysis. Therefore, the findings from the present thesis suggest that lack of adjustment for blood loss could be an important unadjusted confounder when investigating the negative effect of RBC transfusion.

6.4.4 1- and 5-year mortality

Any potential harmful effect of RBC transfusion beyond the early postoperative period is also debatable. Our analysis in Paper 2 indicated that any potential risk of mortality associated with RBC transfusion is highest in the early postoperative period and declines after 1 year. Therefore, there is potentially only an early negative effect of RBC

transfusion. The difference in mortality between RBC-transfused and non-transfused patients beyond the first year postoperatively is perhaps a result of differences in morbidity and early mortality.

In Paper 3, we evaluated the effect of preoperative anaemia and RBC transfusion on mortality separately between 30 days and 1 year, and 1-5 years after surgery. Patient and operative risk factors had larger effect than RBC transfusion on mortality in both observation periods. However, RBC transfusion was not statistically significantly associated with mortality in these sensitivity analyses. The non-significant results in the Paper 3 could be due to the low number of deaths and power issues compared with the main Model C including all patients.

The hypothesis of an early effect of RBC transfusion is supported by several other studies. A study demonstrated the independent effect of RBC transfusion on mortality only up to 6 months, and did not find any statically significant association between 6 months and 5 years postoperatively after adjusting for patient risk factors [85]. Findings from another study with a 10-year follow-up period also suggested that the association with RBC transfusion is different between short- and long-term mortality [95]. The authors described parallel survival curves between the groups after the early postoperative period. Although a different study found a statistically significant association of RBC transfusion on cardiac and late all-cause mortality, the association was mainly seen in the first 5 years postoperatively [82]. Two studies discussed above found similar results, i.e. that RBC transfusion was statistically significantly associated with mortality and the HR was largest for early mortality [86, 96]. These findings are comparable to the results of the present thesis, which suggest that if there is a negative effect of RBC transfusion on mortality it is mainly seen in the early postoperative period.

6.5 Clinical implications of the results

6.5.1 Transfusion threshold

The need to address transfusion thresholds is closely related to the topic of a harmful effect related to RBC transfusion. This topic was beyond the scope of this thesis. However, there have been studies showing similar results with respect to morbidity and

mortality when comparing liberal to restrictive transfusion policies [111-115]. As discussed in the Introduction, a restrictive transfusion threshold was non-inferior to a liberal transfusion threshold. Therefore, the observed difference in mortality in observational studies suggest a comprehensive confounding effect which was not properly accounted for [110]. Some have suggested that a liberal transfusion policy is favourable in patients over 65 years of age [143]. Not surprisingly, restrictive transfusion policies have shown a reduction in the number of RBC transfusions given [111].

The multi-centre TRICS III trial investigated a liberal vs. restrictive transfusion, which included 5,243 patients at 74 sites [113]. The study randomised patients to either a restrictive transfusion threshold of 7,5 g/dL intra- or postoperatively, or a liberal transfusion threshold of 9,5 g/dL intra- or postoperatively and less than 8,5 g/dL in a non-intensive care ward. The TRICS III trial primary outcome was death from any cause, MI, stroke, or new-onset renal failure with dialysis at 6 months after surgery. The authors found no statistically significant difference between the two groups with regards to 6-month mortality. The TITRe2 trial on non-emergency cardiac surgery patients also demonstrated that a restrictive transfusion policy was not superior to a liberal policy using transfusion thresholds of 7.5 g/dL vs 9.0 g/dL [111]. The only endpoints in the TITRe2 trial that reached statistically significant differences between the groups were 30- and 90-day mortality, which favoured a liberal threshold.

In contrast, PBM guidelines for surgical and non-surgical patients have advocated a restrictive transfusion threshold [49-51]. A Cochrane review which identified 31 trials including 12,587 patients, concluded that a restrictive transfusion threshold of Hgb value between 7 g/dL to 8 g/dL did not impact 30-day mortality or morbidity compared with a liberal threshold [115]. The difference in findings between the Cochrane review and TITRe2 trial, implies that there could be a different optimal transfusion threshold among cardiac surgery patients compared with other patient groups. Patients with ischemic heart disease or undergoing cardiac surgery may benefit from a higher transfusion threshold due to cardiac dysfunction.

The results from our papers suggest that patient and operative risk factors have larger effects on long-term mortality compared with RBC transfusion. The main findings do not

support or contradict a liberal transfusion policy. The findings, however, suggest that clinical indications for transfusion are more important risk factors for long-term mortality than RBC transfusion. European PBM guidelines for adult cardiac surgery patients favour a liberal threshold. Furthermore, the authors emphasises that the patient's clinical condition, the optimization of oxygen delivery and extraction in the tissues are more important than a fixed transfusion threshold [47]. The beneficial effect of transfusion for patient outcome is not easy to determine. Clinical judgement by the physician is therefore still important for optimal patient care.

6.5.2 Improving red blood cell mass

The findings from our papers suggest that preoperative anaemia is a more important risk factor of long-term mortality compared with RBC transfusion. The effect of preoperative anaemia on mortality could potentially be 3- to 4-fold compared with transfusion. In our SEM analysis, only age was a more important risk factor for mortality than preoperative anaemia. Treatment options to reduce the severity of anaemia could improve survival in cardiac surgery. It is therefore worthwhile to investigate some of these potential treatments for patients with preoperative anaemia undergoing cardiac surgery. Some authors have suggested to screen for preoperative anaemia at least 14 days but preferably 30 days before elective surgery [144]. The two central methods of improving RBC count are through oral or intravenous iron and/or intravenous recombinant human EPO therapy. European PBM guidelines suggests that iron or EPO supplementation could reduce the need for RBC transfusion [47].

An RCT on iron supplementation among non-anaemic cardiac surgery patients did not find any difference in transfusion rates or Hgb levels between the 3 study groups; intravenous iron, oral iron or placebo [145]. Another recent RCT compared a single 1000 mg dose of intravenous iron against placebo [146]. The authors found a statically significantly lower number of anaemic patients 1 month after surgery in the treatment group, i.e. 8.0% vs. 38.5% respectively ($P = 0.019$). However, there was no statistically significant difference in transfusion rates between the two groups, 13.3% in treatment vs. 20.0% in placebo group ($P = 0.52$).

The alternative treatment option for preoperative anaemia is EPO therapy. A study demonstrated a reduction in allogeneic RBC transfusion in patients who were given a single dose of EPO compared with placebo 2 days before cardiac surgery [147]. Among patients in the EPO group 7% received RBC transfusion compared with 39% among patients in the placebo group ($P < 0.001$). The 45-day all-cause mortality was non-significant between the two groups, but the study sample was underpowered with regards to this secondary outcome. Similar finding of a reduction in RBC transfusion in patients given EPO was found in two other studies. Results from a study found a statistically significant reduction in postoperative RBC transfusion in patients who received EPO treatment, 0.33 units of RBC compared with 0.76 units in the placebo group ($P = 0.008$) [148]. Results from a different trial demonstrated a reduction in transfusion among patients who received EPO in combination with iron sucrose compared with the placebo control group, i.e. 59.5% vs. 86.5%, respectively ($P = 0.009$) [149]. EPO therapy could therefore reduce the transfusion rates in cardiac surgery but its effect on mortality remains to be investigated.

6.5.3 Minimising blood loss

Similar to preoperative anaemia, operative blood loss had a larger effect on long-term mortality compared with transfusion in Paper 3. Furthermore, blood loss is a potential unmeasured confounder in studies that have shown a significant association between RBC transfusion and mortality. The severity of blood loss is a potential modifiable risk factor that could reduce mortality rate in cardiac surgery. Therefore, effort to minimise blood loss is an important topic of discussion to reduce both the transfusion rates and risk of mortality in cardiac surgery. The European PBM guidelines have several recommendations to minimise blood loss in cardiac surgery [47].

Routine use of tranexamic acid, ε-aminocaproic acid or aprotinin as antifibrinolytic agents in cardiac surgery is common practice and recommended in the European PBM guidelines [47]. Evidence from trials has shown that the administration of tranexamic acid has reduced bleeding and transfusion rates associated in cardiac surgery [150-152]. Although the use of tranexamic acid is recommended, it is not without risk to the patient

and is associated with increased cardiovascular events such as stroke [151]. There are limited data on the use of ε-aminocaproic acid in cardiac surgery [152].

Similarly, aprotinin reduces bleeding risk and transfusion rates, and has been extensively researched in 108 trials summarised in a Cochrane review. Results from the Cochrane review indicated that the use of aprotinin is more effective at reducing bleeding risk compared with tranexamic acid and ε-aminocaproic acid (relative risk: 0.90; 95% CI: 0.81 – 0.99) [152].

Finally, the use of minimally invasive extracorporeal circulation circuit (MiECC) could reduce blood loss and subsequently the need for RBC transfusion. Advances in perfusion technology and techniques have been made to reduce the detrimental effects of CPB. MiECC is a such technology. Common features of MiECC are small priming volume, a closed circuited and a biocompatible coated system, as well as use of a centrifugal pump [153].

Two meta-analysis showed that the use of MiECC was associated with reduced RBC transfusion when compared with CPB. In one meta-analysis including 24 studies and 2,770 patients, 17.5% of patients in the MiECC group received RBC transfusion compared with 43.1% of patients in the control group [154]. The odds ratio for RBC transfusion was 0.24 with 95% CI 0.16 – 0.37. Similarly, another meta-analysis including 29 studies and 2,335 patients showed a reduction in blood loss and number of patients transfused in the MiECC group compared with CPB [155]. The odds ratio for RBC transfusion was 0.35 with 95% CI 0.23 – 0.53 in the MiECC group. However, there were no difference in reoperation for bleeding in both meta-analyses. European PBM guidelines recommend that the use of MiECC should be considered to reduce the need for RBC transfusion [47].

6.6 Future studies

Potential negative effects of RBC transfusion have been investigated in multiple studies for many years. The consensus in the literature is that RBC transfusion is detrimental to the patients and should be avoided if possible. With the results from this thesis in mind,

we suggest that further research is necessary to fully explore the complex relationship of RBC transfusion with long-term mortality in cardiac surgery.

The findings from Paper 1 suggest that the inclusion of postoperative complications in the analysis was important when investigating the effect of RBC transfusion on long-term mortality. Similar investigations in patients undergoing valve surgery and multiple procedures could be performed to better elucidate the differences in long-term mortality in cardiac surgery. Furthermore, the inclusion or subdivision of patients with preoperative anaemia in such studies could be of interest.

The low number of patients with preoperative anaemia who did not receive RBC transfusion in Paper 2 points to the need for further investigations with larger patient groups. Because the PH assumption was difficult to obtain beyond 5 years in Paper 2, studies with larger sample sizes may also investigate the association of RBC transfusion with long-term mortality in longer follow-up time.

Finally, SEM as a novel technique to investigate cardiac surgery-related outcomes is underutilised. The implementation of SEM in cardiac surgery research could explore multiple endpoints of interest, such as postoperative morbidity. Furthermore, SEM could be used to explore the relationship between RBC transfusion and other endpoints, such as short-term mortality or postoperative complications. In addition, the inclusion of postoperative complications in a SEM model which explores the relationship between RBC transfusion and mortality, could further elucidate the relationships between these variables.

7 Conclusions

The hypothesis of this thesis was that RBC transfusion is not associated with long-term all-cause mortality in cardiac surgery when adjusting for known risk factors. The results from the papers support the main hypothesis. We therefore suggest that patient comorbidities, operative risk factors and common postoperative complications are more important risk factors for long-term mortality than RBC transfusion in cardiac surgery.

7.1 Paper 1

1) RBC transfusion either intra- or postoperatively was statistically significantly associated with an increased risk of long-term all-cause mortality in an unadjusted model for patients undergoing primary isolated CABG.
2) The association between RBC transfusion and long-term mortality was still statistically significant for patients undergoing primary isolated CABG when the analysis was adjusted for pre- and intraoperative variables.
3) The association between RBC transfusion and long-term mortality among patients undergoing isolated CABG was no longer statistically significant when adjustment also included common postoperative complications.
4) The sensitivity analyses using propensity score matching supported the results of the main analysis in Paper 1.

7.2 Paper 2

5) RBC transfusion either intra- or postoperatively was statistically significantly associated with an increased risk of 5-year all-cause mortality in an unadjusted model for patients undergoing cardiac surgery with preoperative anaemia.
6) The association between RBC transfusion and 5-year all-cause mortality among patients undergoing cardiac surgery with preoperative anaemia was not statistically significant when adjusted for patient risk factors and preoperative laboratory values. The HR for RBC transfusion on 5-year all-cause mortality was further decreased with the inclusion of intraoperative variables and common postoperative complications.

7) RBC transfusion was not statistically significantly associated with all-cause mortality between 30 days and 1-year, and 1-5 years postoperatively among patients undergoing cardiac surgery with preoperative anaemia. The sensitivity analyses were adjusted for pre- and intraoperative variables and including postoperative complications.

7.3 Paper 3

8) In the complete SEM model, the direct effect of RBC transfusion was smaller compared with the total effect of preoperative anaemia on 5-year all-cause mortality among cardiac surgery patients. The effect of preoperative anaemia was more than three times larger than RBC transfusion on mortality.
9) The sensitivity analyses which investigated mortality between 30 days to 1 year and 1-5 years postoperatively, supported the main findings of Paper 3. The effect of preoperative anaemia was three to four times larger than RBC transfusion on mortality in these analyses.
10) The sensitivity analyses which investigated patients undergoing isolated CABG and patients undergoing multiple procedures using SEM, supported the main findings of Paper 3. The effect of preoperative anaemia was three to four times larger than RBC transfusion on mortality in these analyses.

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Transfusion of red blood cells in coronary surgery: is there an effect on long-term mortality when adjusting for risk factors and postoperative complications?

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Abstract

OBJECTIVES: The aim of this study was to compare long-term mortality in patients undergoing primary isolated coronary artery bypass grafting who received ≥1 units of red blood cells (RBCs) or no RBCs. We hypothesized that a possible difference in long-term mortality was due to preoperative morbidity and/or postoperative morbidity.

METHODS: This prospective cohort study, part of the Cardiac Surgery Outcome Study (CaSOS) at St. Olavs University Hospital, Trondheim, Norway, included patients operated on from 2000 through 2014 ($n = 4014$) and excluded those with large intra- or postoperative blood loss or 30-day mortality. Observed mortality from 30 days to 15 years postoperatively was compared between patients who received RBC transfusion and those who did not. Cox regression analysis was performed with unadjusted models, adjusting for pre- and intraoperative covariates, and with further adjustment for postoperative complications. Sensitivity analyses were performed with propensity score matching or including 30-day mortality.

RESULTS: The unadjusted hazard ratio (HR) for long-term mortality was 2.10 (1.81–2.43; $P < 0.01$) for transfused patients. After adjusting for pre- and intraoperative variables, the HR was 1.26 (1.04–1.53; $P = 0.02$). With further adjustment for postoperative complications, RBC transfusion was no longer significant and the HR was 1.19 (0.98–1.44; $P = 0.08$). These results were supported by the sensitivity analyses.

CONCLUSIONS: The study indicated that most of the association between RBC transfusion and long-term mortality following primary isolated coronary artery bypass grafting was due to confounders, especially from postoperative complications.

Keywords: Red blood cell transfusion • Coronary artery bypass grafting • Long-term mortality

INTRODUCTION

The effects of transfusion of red blood cells (RBCs) on short-term and long-term morbidity and mortality in patients undergoing cardiac surgery are poorly understood. As cardiac surgery has one of the highest rates of blood transfusion among the surgeries performed, understanding the risk behind transfusion-related mortality is vital for surgical practice. Different studies have suggested that transfusion of as little as 1 unit of RBC increased mortality [1–10]. The effect of transfusion on mortality was greater in cardiac surgery patients than other patient groups [11]. However, other investigators found contradicting results when adjusting for known risk factors [12–16]. The difference in findings may be attributed to confounders.

Patients receiving RBC transfusion differed significantly clinically from those who did not receive transfusion, and had a higher risk for perioperative complications and a more complex operative course [17, 18]. Because of the presence of confounders, the effect of transfusion on long-term mortality was difficult to evaluate as it may be lost among other associated risk factors. Transfused patients often had preoperative risk factors for mortality and morbidity that were also associated with the need for RBC transfusion, such as older age, female gender, smaller body surface area or anaemia [19–23].

Associations between these risk factors and negative outcomes, for example, increased frequencies of acute kidney injury among anaemic patients also receiving RBC transfusion, could explain partly the increased long-term mortality rate among transfused patients [24]. Thus, differences in patient characteristics and comorbidities and not RBC transfusion itself may be an underlying reason for the higher mortality rate among transfused patients.

We hypothesized that there is no long-term effect of RBC transfusion on mortality in adult patients undergoing coronary artery bypass grafting (CABG) and that the increased mortality risk is due to other underlying risk factors. The aim of this study was to investigate whether there was a difference in long-term all-cause mortality associated with RBC transfusion in patients who underwent coronary artery surgery. We, therefore, compared long-term mortality adjusted for known risk factors in patients who did and who did not receive RBC transfusion in a prospective study on primary isolated CABG.

MATERIALS AND METHODS

Trondheim heart surgery database

Since 1992, adult patients undergoing cardiac surgery at St. Olavs University Hospital, Trondheim, Norway, have been registered consecutively into the Trondheim Heart Surgery Database as part of the local quality assurance work. Patient- and procedure-related preoperative characteristics, intraoperative and postoperative events and factors, as well as laboratory values have been prospectively registered and undergone rigorous quality controls. This study was part of the Cardiac Surgery Outcome Study (CaSOS), which has used the database to investigate different complications following adult cardiac surgery.

The CaSOS was approved by the Norwegian Data Inspectorate and the Regional Committee for Medical and Health Research Ethics in Middle-Norway (project number 4.2007.1528), Trondheim, Norway, on 27 June 2007. The need for informed consent was waived up to April 2008, and thereafter, all patients signed informed consent.

Patients and procedures

Data from consecutive adults undergoing isolated CABG surgery from 2000 through 2014 were included. Only the first entry into the database was used for survival analysis. Exclusion criteria were short-term mortality (postoperative death within 30 days), intraoperative blood loss ≥700 ml, postoperative blood loss ≥800 ml until the first postoperative morning or the need for reoperation following surgery (Fig. 1). Blood loss above the stated thresholds was considered to indicate a complicated intra- or early postoperative course with higher risk for RBC transfusion. Non-Norwegian citizens were excluded because of missing data regarding death, leaving 4014 eligible patients for the study.

All patients underwent an on-pump procedure. The cardiopulmonary bypass (CPB) circuit was primed with 1100–1500 ml of Ringer's acetate with 7500–10 000 U of heparin (Leo, Copenhagen, Denmark). Before CPB, patients were administered 300 U/kg of heparin to achieve a kaolin-activated coagulation time of ≥480 s. Additional heparin was administrated when needed. All patients were given tranexamic acid (Leo) 30 mg/kg routinely before the start of CPB. After CPB, blood remaining in the circuit was retransfused to the patient, and protamine sulphate (Leo) was given to achieve an activated coagulation time within 10% of baseline. Acetylsalicylic acid was discontinued 1–3 days before surgery. Data on clopidogrel and ticagrelor were not available in the database for the entire study period and were therefore not used.

In general, a transfusion threshold of 7.0 g/dl during CPB and 8.5 g/dl postoperatively was practised. Platelets or fresh frozen plasma were considered when postoperative bleeding was above 200 ml/h. The final decision to transfuse was left to the attending physician. Transfusions were recorded during the entire hospital stay. Blood loss during surgery was estimated in categories, i.e. ≤500 ml, 600 ml, 700 ml or >700 ml.

End point

The study end point was all-cause mortality with observation time starting 30 days postoperatively, denoted as long-term mortality. We compared long-term mortality between patients who received RBC transfusions during the hospital stay with patients who did not receive any RBC transfusion. Data regarding deaths were obtained from the Norwegian Cause of Death Registry (2000–14), which registers deaths of all Norwegian citizens.

Statistical analysis

Statistical analyses were performed using Stata (Stata v0.13, College Station, TX, USA). Median and 95% confidence interval (CI) are provided for continuous variables because many were non-normally distributed (mean and standard deviation are provided in the Supplementary Material Table S1). For comparison between the transfused and the non-transfused groups, the χ^2 test and the Mann–Whitney *U*-test were used for discrete and continuous variables, respectively. *P*-values < 0.05 were considered significant. Mortality was analysed with multivariate Cox proportional hazard modelling. The proportional hazard test, log–log plots and Cox–Snell residuals were used to assess model fit.

A 3-step analysis plan was employed to investigate the effects of RBC transfusion on long-term mortality, permitting separate evaluation of different types of the potential confounders. The first step was a univariate Cox regression analysis with transfusion of at least 1 unit of RBC (yes/no) as the only covariate. In the second step, the multivariate Cox regression model also included preoperative and intraoperative adjustment variables. The chosen covariates were variables that could influence RBC transfusion based on clinical knowledge and the literature [25, 26]: age, sex, preoperative haemoglobin, amount bled during the first 16 h, body mass index, hypertension, CPB time, diabetes, smoking (present or previous smoker versus never smoker) and preoperative creatinine. In the third step, the Cox regression analysis included the mentioned covariates, as well as selected postoperative complications: novel renal failure (absolute serum creatinine increase > 26 μmol/l or relative increase > 50%), prolonged mechanical ventilation (primary intubation more than 24 h or need for reintubation), acute myocardial infarction or cardiac dysfunction (use of 2 or more inotropics or intra-aortic balloon pump).

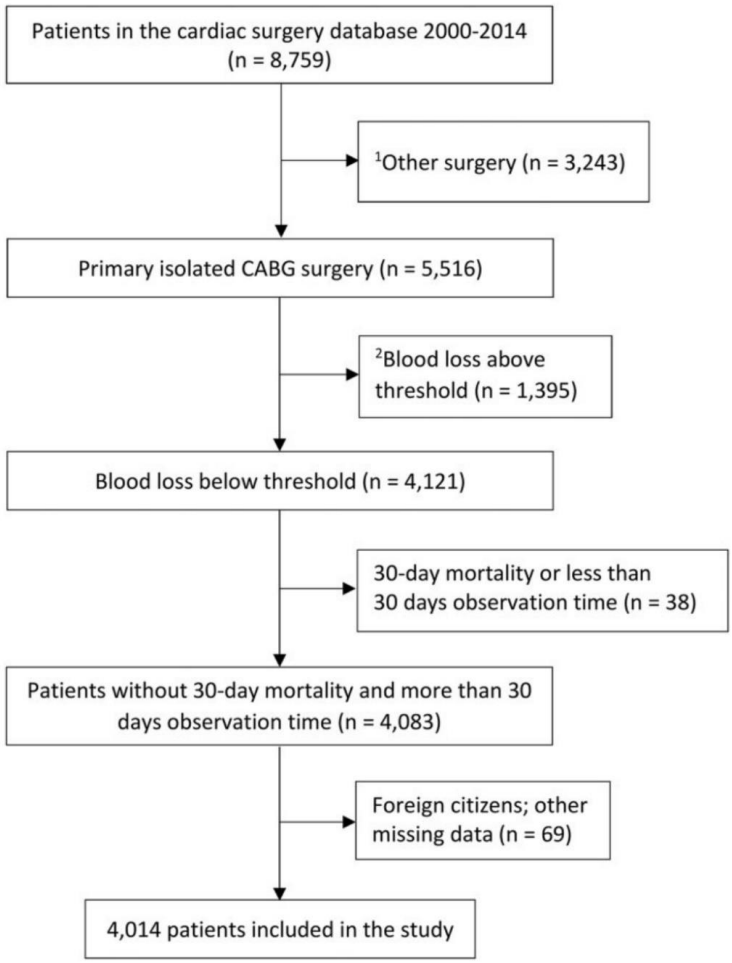

Figure 1: Flow chart of exclusion criteria and the number of patients included in the study. [1]Other surgery than isolated coronary bypass grafting, reoperation for bleeding and non-primary surgery. [2]Blood loss threshold either above 700 ml intraoperatively or above 800 ml postoperatively until the following morning after surgery. CABG: coronary artery bypass grafting.

Sensitivity analysis

Additional sensitivity analyses were performed to evaluate the robustness of the results. To test whether the findings were biased from the exclusion of the patients with early mortality or observation time <30 days, a Step 3 sensitivity analysis was performed, which also included these patients. Furthermore, because there were several preoperative differences (Table 1) between the patients who did and did not receive RBC transfusion, we performed a sensitivity analysis using greedy 1:1 propensity score matching to control for residual confounding in the Cox regression analysis.

Sequential logistic regression analysis for RBC transfusion including 18 pre- and operative variables produced a propensity score for each patient (Supplementary Material Table S2). One patient had missing New York Heart Association Functional Classification and was excluded. The caliper width was one-fourth of the standard deviation of the propensity score, i.e. 0.0711. Another propensity score included the same variables and the postoperative complications from the Step 3 main analysis, with a caliper width of 0.0713.

The balance between the groups for covariates was assessed with absolute standardized difference in covariate means or the difference in prevalence for binomial variables. For categorical variables with more than 2 values, we compared the absolute standardized difference for all dummy pairs of the covariate. Ideally, the difference should be ≤10%.

We assessed the effects of residual confounding using a univariate random effects Cox regression model for RBC transfusion between the matched groups, with all-cause mortality after 30 days following surgery as outcome.

RESULTS

Of the 4014 included patients, 1127 (28.1%) received transfusion of ≥1 units of RBC and, on average, 2 units of RBC. A total of 727

Table 1: Baseline patient characteristics and perioperative variables

	Patients without RBC transfusion ($n = 2887$)	Patients with RBC transfusion ($n = 1127$)	P-value
Preoperative variables			
Sex, male	2550 (88.3)	587 (52.1)	<0.01
Age (years)	64 (63.9–64.8)	71 (70.3–71.6)	<0.01
Body mass index (kg/m2)	27.1 (27.0–27.3)	25.8 (25.6–26.0)	<0.01
Present or previous smoker	1718 (59.5)	635 (56.3)	0.07
Haemoglobin (g/dl)	14.5 (14.5–14.6)	12.7 (12.6–12.8)	<0.01
Chronic heart failure	204 (7.1)	210 (18.6)	<0.01
Unstable angina	1053 (36.5)	644 (57.1)	<0.01
Previous myocardial infarction	1511 (52.3)	708 (62.8)	<0.01
NYHA Class III or IV	1864 (64.6)	862 (76.5)	<0.01
Previous percutaneous coronary intervention	397 (13.8)	144 (12.8)	0.42
Hypertension	1524 (52.8)	688 (61.1)	<0.01
Chronic pulmonary disease	275 (9.5)	174 (15.4)	<0.01
Renal dysfunction[a]	48 (1.7)	73 (6.5)	<0.01
Creatinine (μmol/l)	87 (86–88)	83 (82–85)	<0.01
Diabetes	402 (13.9)	209 (18.5)	<0.01
Previous cerebrovascular disease	249 (8.6)	135 (12.0)	<0.01
Intermittent claudication	173 (6.0)	109 (9.7)	<0.01
Left ventricular ejection fraction (%) ($n = 3590$)	54 (53.5–55)	52 (50.4–53.5)	<0.01
Use of medications			
Acetylsalicylic acid	2775 (96.1)	1088 (96.5)	0.53
Warfarin	103 (3.6)	41 (3.6)	0.91
Heparin/low molecular weight heparin	974 (33.7)	586 (52.0)	<0.01
Angiotensin-converting-enzyme inhibitor	964 (33.4)	503 (44.6)	<0.01
Perioperative variables			
Cardiopulmonary bypass time (min)	60 (59–60)	63 (62–64)	<0.01
Haemoglobin first postoperative day (g/dl)	10.7 (10.6–10.7)	9.4 (9.3–9.5)	<0.01
Blood loss during surgery (>500 ml)	411 (14.2)	237 (21.0)	<0.01
Postoperative blood loss until first morning (ml)	480 (475–490)	470 (460–480)	0.01
Plasma transfusion	133 (4.6)	161 (14.3)	<0.01
Platelet transfusion	16 (0.6)	43 (3.8)	<0.01
Postoperative complications			
Cerebrovascular insult	24 (0.8)	17 (1.5)	0.06
Renal failure[b]	73 (2.5)	119 (10.6)	<0.01
Myocardial infarction or cardiac dysfunction[c]	129 (4.5)	108 (9.6)	<0.01
Primary intubation more than 24 h or reintubation	9 (0.3)	29 (2.6)	<0.01
Intensive care unit stay >24 h	42 (1.5)	53 (7.3)	<0.01
Death >30 days postoperatively	429 (14.9)	298 (26.4)	<0.01

Continuous variables given as median (95% confidence interval) and categorical variables given as n (%).
[a]Creatinine >140 μmol/l or dialysis.
[b]Absolute increase of creatinine >26 μmol/l or relative increase of > 50%.
[c]Use of > 2 inotropics or intra-aortic balloon pump.
NYHA: New York Heart Association; RBC: red blood cell.

(18.1%) patients died during the follow-up period, of which 298 (26.4%) patients were in the RBC-transfused group compared with 429 (14.9%) patients in the non-transfused group ($P < 0.01$). The observation time ranged from 30 days to 15 years (median 7.2 years, 95% CI 7.0–7.5 years). There were significant differences between the groups for several variables (Table 1).

In the Step 1 univariate Cox regression model, increased long-term mortality was significantly associated with RBC transfusion (Fig. 2, Table 2). In the Step 2 model adjusting for pre- and intraoperative variables, RBC transfusion still carried a significant mortality risk but with a lower hazard ratio (Table 2). In the Step 3 model also adjusting for postoperative complications, the effect of RBC transfusion on mortality was no longer significant (Table 2). Correspondingly, survival estimates and cumulative hazards for the Step 3 model showed overlapping 95% CI for the groups with and without RBC transfusion (Fig. 3). In the Step 3 model, age, haemoglobin concentration, creatinine concentration, smoking and diabetes were significantly associated with mortality.

In the Step 3 sensitivity analysis including all patients, also those with short-term mortality, there was no significant association of RBC transfusion with mortality (hazard ratio for RBC transfusion 1.16, 95% CI 0.96–1.41; $P = 0.13$, $n = 4052$).

In the second sensitivity analysis using propensity score matching for patients who did and did not receive RBC transfusion, it was possible to match 596 transfused patients (52.9%) when adjusting for pre- and intraoperative variables (Model 4). The transfused patients without a match had high propensity scores, indicating a high probability of requiring RBC transfusion. The median propensity score for the group that did not receive RBC transfusion was 0.081 (95% CI 0.077–0.084), as compared with 0.715 (95% CI 0.679–0.737) in the group that received RBC transfusion (Supplementary Material Figure S3). When we

added postoperative complications to the variables used to develop the propensity score, 590 (52.4%) matches were found for the group with RBC transfusion (Model 5). The median propensity score for this non-transfused group was 0.078 (95% CI 0.074–0.081) compared with 0.712 (95% CI 0.680–0.736) for the RBC-transfused group (Supplementary Material Figure S3).

The propensity-matched groups were balanced for all variables as indicated by standardized differences ≤10%, except for previous percutaneous coronary intervention in Model 4 (13%, Supplementary Material Table S2). We considered this acceptable.

The sensitivity analysis using propensity score matching showed no significant effect of RBC transfusion on long-term mortality for both propensity score models (Table 2). The Cox–Snell residuals indicated a good fit for all models.

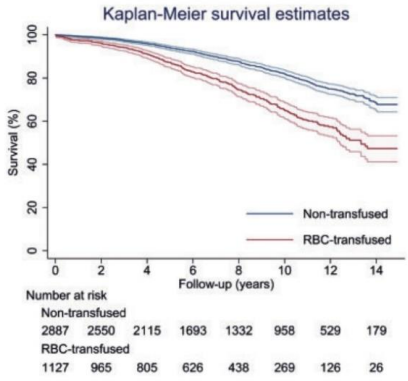

Figure 2: Survival estimates for Model 1. Kaplan–Meier survival plot of coronary artery bypass grafting surgery patients by RBC transfusion, corresponding to the unadjusted Cox regression Model 1. RBC: red blood cell.

DISCUSSION

The main finding of the study was that the association of RBC transfusion with long-term all-cause mortality (30 days to 15 years after surgery) in adults undergoing isolated primary CABG surgery was mainly due to patient characteristics and perioperative factors. The effect of RBC transfusion was diminished and no longer statistically significant when adjusted for postoperative complications as well as pre- and intraoperative variables. Thus, most of the observed difference in mortality may be attributed to well-known factors such as age, haemoglobin

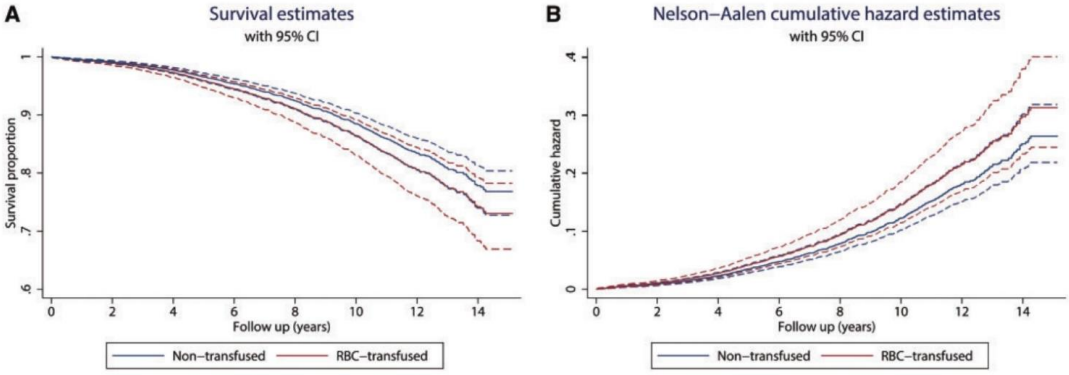

Figure 3: Survival and hazard estimates for Model 3. (**A**) Survival estimates of coronary artery bypass grafting surgery patients by RBC transfusion, including adjustments for preoperative and intraoperative variables as well as postoperative complications, corresponding to the adjusted Cox regression Model 3. (**B**) Nelson–Aalen cumulative hazard for the same model. CI: confidence interval; RBC: red blood cell.

Table 2: Cox regression models

	Hazard ratio	95% confidence interval		*P*-value
Cox regression models for RBC transfusion and long-term mortality				
Model 1	2.10	1.81	2.43	<0.01
Model 2	1.26	1.04	1.53	0.02
Model 3	1.19	0.98	1.44	0.08
Cox regression models for propensity-matched groups for RBC transfusion and long-term mortality				
Model 4	1.18	0.92	1.52	0.19
Model 5	1.14	0.88	1.46	0.32

Model 1: RBC transfusion (yes/no). Model 2: Adjusted for preoperative and intraoperative covariates. Model 3: Adjusted for preoperative and intraoperative covariates as well as postoperative complications. Model 4: Propensity score-matched group; score based on preoperative and intraoperative covariates. Model 5: Propensity score-matched group; score based on preoperative and intraoperative covariates as well as postoperative complications.
RBC: red blood cell.

concentration, smoking, diabetes and renal dysfunction. A strength of this study is that the stepwise design demonstrated that the negative effect of RBC transfusion could be due to confounding from early postoperative complications. These complications could arise because of operative factors or clinically different characteristics among the patients (Table 1) who were not adjusted for in the analysis. This study therefore suggests that the underlying comorbidities and risk factors among RBC transfused patients are an important cause of the higher rate of observed long-term mortality in transfused patients.

Another strength of this study is the inclusion of sensitivity analyses that support the main findings. With propensity score matching, which is a method to remove the influence from many confounders, the effect of RBC transfusion was far from significant ($P = 0.19$) also in the model adjusting for pre- and intraoperative variables only. This raises the question whether the association between RBC transfusion and mortality in the main Step 2 analysis, i.e. not correcting for postoperative complications, may be due to residual confounding.

Comparison with previous studies

The present findings are in accordance with several studies showing no significant effect of RBC transfusion on long-term mortality [13–16]. However, other studies have reached more ambiguous conclusions [1, 2, 4, 6–10]. Our study demonstrates the importance of cautious interpretation and consideration of how potential confounders were adjusted for. Dejam *et al.* [11] found that RBC transfusion was statistically significant in some groups, depending on patient characteristics and comorbidities, but not significant in the overall cohort. This agrees with the publication of Warwick *et al.* [13] who concluded that the increased long-term mortality may be due to underlying indications for RBC transfusion. Dardashti *et al.* showed that RBC transfusion was no longer significantly associated with long-term mortality when adjusting for preoperative haemoglobin and renal function.

In comparison, Jakobsen *et al.* [4] found an association between RBC transfusion and long-term mortality. However, their analysis was not adjusted for haemoglobin concentrations. Studies have shown lower survival among anaemic patients undergoing cardiac surgery [21, 26] and higher postoperative morbidity among patients with lower haematocrit without RBC transfusion [27]. Engoren *et al.* [2] found that RBC transfusion had a higher hazard for long-term mortality among anaemic patients but not among non-anaemic patients. Preoperative haemoglobin concentrations and RBC transfusion are highly correlated [13]. Consequently, the association of RBC transfusion and long-term mortality seen in observational studies may be due to residual confounding. As expected, patient characteristics in this study differed between the transfused and the non-transfused groups (Table 1). On the basis of similar observations, Jakobsen *et al.* discussed whether confounding may lead to overestimation of the effect of RBC transfusion in observational studies. This view was supported by the Step 3 model in this study. Murphy *et al.* [28] found more deaths within 3 months after cardiac surgery in patients randomized to a restrictive transfusion threshold. Their data suggest that in patients with moderate postoperative anaemia, the risks associated with not being transfused are higher than the risks associated with RBC transfusion. Their findings support our view that increased mortality in transfused patients found in some observational studies may rather be due to confounding than a true negative effect of transfusion.

In a recent study, Schwann *et al.* [1] stratified patients into groups with and without postoperative complications. They showed that RBC transfusion was associated with an increase in long-term mortality for the whole study population, as well as for both stratified groups. The effect was smallest in patients with the longest observation time. Surgenor *et al.* [6] found that RBC-transfused patients were at higher mortality risk up to 6 months postoperatively but not thereafter. van Straten *et al.* [5] suggested that the association of RBC transfusion with short-term mortality and long-term mortality may be different. On the basis of these findings, we excluded patients with short-term mortality from our main analysis. The sensitivity analysis supports that this exclusion did not bias our results.

We did not investigate whether the number of units transfused had an influence on mortality. Therefore, the results reflect the independent effect of RBC transfusion on long-term mortality. This strategy was supported by a previous study showing that the number of RBC units transfused was not associated with long-term mortality when adjusting for a wide range of covariates [16].

The results from this study support our hypothesis that RBC transfusion in isolated CABG surgery has no statistically significant effect on long-term mortality. However, we cannot exclude that this may be a false-negative conclusion due to the moderate size of the study. Even so, the findings suggest that confounders for long-term mortality seen in the RBC transfused patients, such as differences in comorbidities, operative course and postoperative complications, may be the major cause for differences in the observed mortality. The effect of RBC transfusion on long-term mortality may therefore be less than previously suggested.

Limitations

The database was limited to patients undergoing cardiac surgery at 1 centre, St. Olavs University Hospital. The database is considered representative of the general population in Middle Norway. Women were under-represented because they less often undergo cardiac surgery. Therefore, separate gender effects could not be evaluated.

The transfusion policy did not change significantly during the study period, although there have been some minor changes. The postoperative transfusion threshold of haemoglobin has ranged from 8–9 g/dl, but the intraoperative transfusion policy remained essentially the same during the study period. However, this probably had little impact on the results because we did not evaluate the transfusion threshold on the outcome, the dose effect of RBC transfusion or the intra- or postoperative timing of transfusion. We cannot exclude that the results could have been different using other transfusion thresholds. The storage time of RBC units was not taken into consideration, but a systematic review has not found a definite effect of storage time on mortality or morbidity [29].

This study demonstrates associations, not causation, and our models are not appropriate for mortality prediction in individual patients. There is a possibility that inclusion of postoperative complications may have introduced a bias to the Step 3 analysis, in that they could be triggered or influenced by transfusion.

The patients without a match in the sensitivity analysis had higher propensity scores, i.e. a higher risk for RBC transfusion and higher mortality by the end of the observation time. We cannot exclude that this may have biased the propensity score-matched analysis.

CONCLUSIONS

We found no statistically significant association between RBC transfusion and long-term mortality in patients undergoing isolated CABG surgery when adjusting for pre- and intraoperative risk factors and postoperative complications. These findings were strengthened by the results from sensitivity analyses. Most of the effect of RBC transfusion on long-term mortality was probably due to confounding. Concerns on increased long-term mortality should not influence the decision of whether to transfuse RBC or not in this patient group. Future studies should be performed in patients at higher risk for RBC transfusion such as anaemic patients, including adjustment for preoperative haemoglobin concentrations and postoperative complications.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *EJCTS* online.

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Conflict of interest: none declared.

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SUPPLEMENTARY MATERIAL

Table S1: Baseline patient characteristics and perioperative variables[1]

	Patients without RBC transfusion n = 2,887	Patients with RBC transfusion n = 1,127	P-value
Preoperative variables			
Age (years)	64 (9.4)	69 (8.8)	< 0.01
Body mass index (kg/m^2)	27.5 (3.8)	26.0 (3.9)	< 0.01
Haemoglobin (g/dL)	14.5 (1.1)	12.7 (1.3)	< 0.01
Creatinine (µmol/L)	88 (21)	95 (65)	< 0.01
Left ventricular ejection fraction (%) (n = 3,590)	52.3 (8.3)	50.5 (10.1)	< 0.01
Perioperative variables			
Cardiopulmonary bypass time (min)	61 (18.8)	65 (19.9)	< 0.01
Haemoglobin first postoperative day (g/dL)	10.7 (1.1)	9.5 (0.9)	< 0.01
Postoperative blood loss until first morning (mL)	492 (142)	479 (154)	0.01

[1]Continuous variables given as mean (standard deviation)

Table S2: Absolute standardized difference for propensity score matching

Variable	Standardized difference for all patients (n = 2,886 + 1,127)	Standardized difference for matched group adjusted for pre- and intraoperative variables (n = 596 + 596)	Standardized difference for matched group including perioperative variables and postoperative complications (n = 590 + 590)
Sex – Men	0.86	0.02	0.004
Age (years)	0.59	0.008	0.03
Haemoglobin (g/dL)	1.44	0.05	0.04
Body mass index	0.38	0.001	0.03
Preoperative creatinine	0.14	0.03	0.08
Cardiopulmonary bypass time	0.19	0.03	0.02
Unstable angina	0.42	0.04	0.01
Previous myocardial infarction	0.21	0.03	0.02
Previous percutaneous coronary intervention	0.03	0.13	0.02
NYHA class III or IV[1]	0.26	0.08	0.07
Chronic pulmonary disease	0.18	0.06	0.05
Diabetes	0.13	0.03	0.01
Hypertension	0.17	0.10	0.05
Vascular disease[2]	0.18	0.10	0.06
Previous or current smoker	0.06	0.10	0.10
Use of acetylsalicylic acid	0.02	0.03	0.03
Use of angiotensin converting enzyme inhibitors	0.23	0.07	0.03
Use of heparin	0.37	0.04	0.01
Postoperative heart failure or myocardial infarction	0.20	*	0.07
Renal failure[3]	0.33	*	0.10
Primary intubation > 24 hours or need for reintubation	0.19	*	0.01

[1]The New York Heart Association Functional Classification

[2]Thoracic or abdominal aortic aneurism, history of cerebrovascular insult or intermittent claudication

[3]Absolute increase of creatinine > 26 µmol/L or relative increase of > 50 %

*Omitted. Not part of the analysis and model

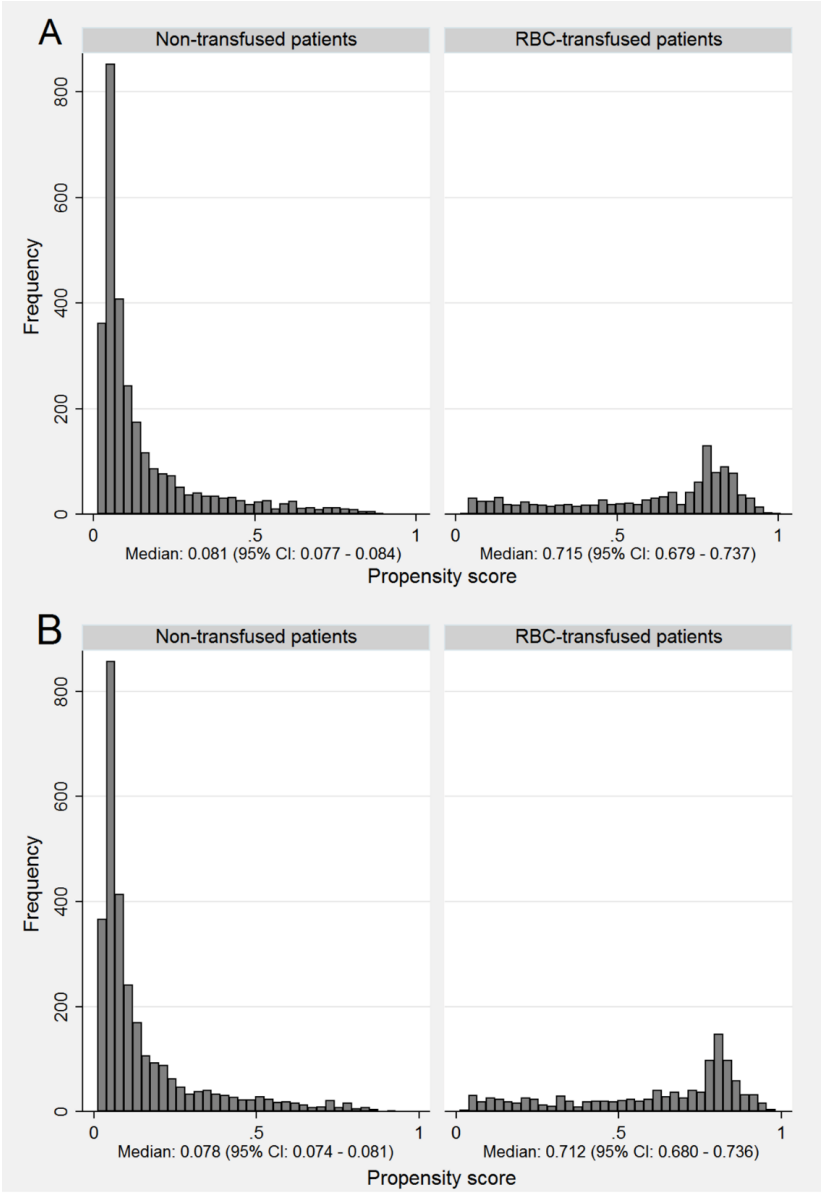

Figure S3: Propensity scores.

Histograms showing the frequencies of propensity scores for patients who received red blood cell (RBC) transfusion or did not receive RBC transfusion, used in the sensitivity analysis for RBC transfusion and long-term mortality. The first propensity score (A) was based on pre- and intraoperative variables, the second model (B) added postoperative complications. 596 RBC transfused patients of 1,127 possible were matched in the first model and 590 in the second model.

Paper 2

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Limited effect of red blood cell transfusion on long-term mortality among anaemic cardiac surgery patients

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Key question

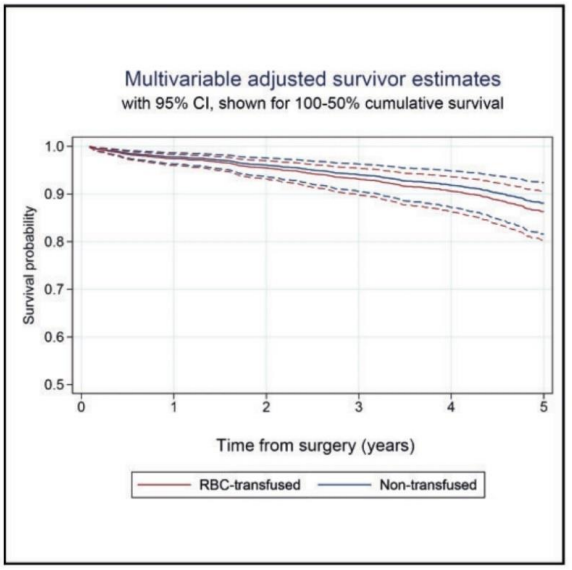
Does red blood cell (RBC) transfusion increase long-term mortality among anaemic cardiac surgery patients?

Key finding(s)

The hazard ratio for RBC transfusion was not statistically significant when adjusted for known risk factors.

Take-home message

Increased long-term mortality in anaemic patients who receive RBC transfusion is largely due to patient and operative risk factors.



Abstract

OBJECTIVES: Our goal was to investigate long-term mortality associated with red blood cell (RBC) transfusion among patients with anaemia undergoing cardiac surgery when adjusting for known risk factors.

METHODS: Adults with preoperative anaemia as defined by World Health Organization criteria undergoing open-heart surgery from 2000 through 2017 were included. Cox regression was performed for long-term mortality (30 days–5 years), comparing patients who

received ≥1 unit of RBC with those who did not. Unadjusted and multivariable analyses adjusted for risk factors were performed.

RESULTS: The study included 1859 patients, 1525 (82%) of whom received RBC transfusion. A total of 370 (19.9%) deaths were registered between 30 days and 5 years; 88 patients (23.8%) died between 30 days and 1 year. The unadjusted hazard ratio (HR) associated with RBC transfusion was 2.09 (1.49–2.93, $P < 0.001$) from 30 days to 5 years postoperatively. The HR for RBC transfusion were 4.70 (1.72–12.81, $P = 0.002$) and 1.77 (1.23–2.55, $P = 0.002$) for 30 days–1 year and 1–5 years, respectively. Adjusting for perioperative risk factors, which included postoperative complications, the HR decreased to 1.16 (0.80–1.68, $P = 0.43$), 1.79 (0.63–5.12, $P = 0.28$) and 1.11 (0.75–1.65, $P = 0.61$) for observation time from 30 days to 5 years, 30 days to 1 year and 1 to 5 years, respectively. Results were similar when postoperative complications were excluded from the adjustment variables.

CONCLUSIONS: No statistically significant association between RBC transfusion and long-term mortality was found when we adjusted for known risk factors. This study suggests that the observed difference in mortality in this patient group is largely due to patient-related risk factors.

Keywords: Anaemia • Red blood cell transfusion • Cardiac surgery • Long-term mortality • Survival analysis • Risk factors

ABBREVIATIONS

CABG	Coronary artery bypass grafting
CaSOS	Cardiac Surgery Outcome Study
CI	Confidence interval
CPB	Cardiopulmonary bypass
Hgb	Haemoglobin
HR	Hazard ratio
PH	Proportional hazards
RBC	Red blood cell

INTRODUCTION

Previous work in the Cardiac Surgery Outcome Study (CaSOS) found no significant association between red blood cell (RBC) transfusion and long-term mortality following isolated coronary artery bypass grafting (CABG) [1]. This finding suggested that future research should be directed at patients at higher risk for transfusion, such as patients with anaemia. Several previous studies have shown that both transfusion of blood products and anaemia are risk factors for morbidity and mortality following cardiac surgery [2–11]. These 2 risk factors are closely correlated because the transfusion of blood products is associated with correction of anaemia and compensating for blood loss during the perioperative course. It is difficult to ascertain which exposure is more harmful to the patient and if there are synergistic effects of anaemia and RBC transfusion even after adjustment for known risk factors, as suggested by some researchers [11–14].

Patients who receive RBC transfusion often have several risk factors and more complicated operative courses than non-transfused patients. Anaemic patients naturally have a higher risk of RBC transfusion during surgery [15]. Some investigators have suggested that the effect of RBC transfusion on mortality and organ failure in cardiac surgery seen in observational studies is due to confounding from common risk factors, such as age, sex and preoperative morbidity [1, 15–19]. A meta-analysis regarding the effects of different transfusion policies on mortality showed that results from randomized controlled trials refuted findings from observational studies [20]. The evidence is therefore contradictory. In addition, the overall effect of RBC transfusion on long-term mortality is poorly understood. Further investigation regarding the effect of RBC transfusion in cardiac surgery patients with anaemia is therefore warranted.

We hypothesized that the increased observed long-term mortality among anaemic patients undergoing cardiac surgery is due to underlying risk factors and operative complications and not to RBC transfusion. Our aim was to investigate the association between long-term mortality and RBC transfusion among anaemic patients undergoing on-pump open-heart surgery. Therefore, we compared long-term mortality between anaemic patients who received RBC transfusion and anaemic patients who did not in a *post hoc* analysis of a prospectively collected database.

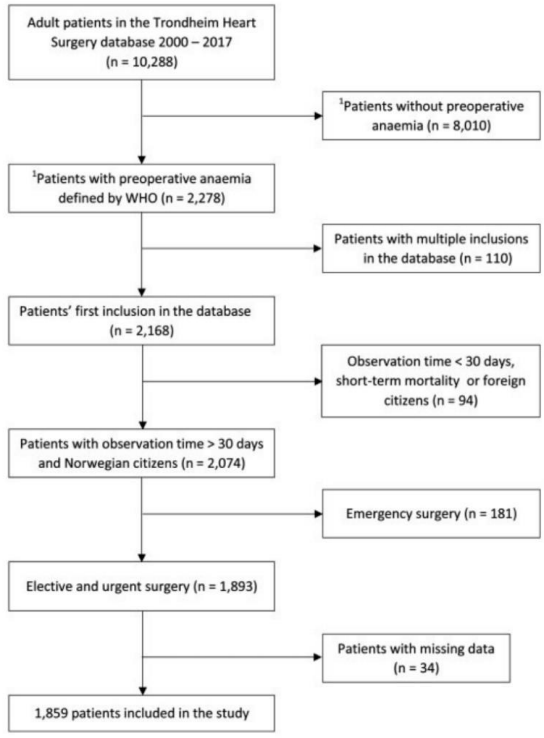


Figure 1: Patient inclusion and exclusion criteria. Anaemia is defined by the World Health Organization as <13 g/dl for men and <12 g/dl for women.

PATIENTS AND METHODS

Data source and patient population

Cardiac surgery patients at St. Olavs Hospital, Trondheim, Norway, have been registered consecutively into the Trondheim Heart Surgery Database since 1992. The database has prospectively registered patient- and procedure-related preoperative characteristics, perioperative events and factors and laboratory results as described earlier [1]. The present study was part of the CaSOS. The Norwegian Data Inspectorate and the Regional Committee for Medical and Health Research Ethics in Middle Norway approved the CaSOS (project number 4.2007.1528). Patients included after April 2008 gave written informed consent. For patients included earlier, informed consent was waived. Data regarding death and cause of death were obtained from the national Norwegian Cause of Death Registry, which has data completeness >99%.

Adult patients with preoperative anaemia undergoing cardiac surgery from 1 January 2000 through 31 December 2017 at St. Olavs Hospital were included. Anaemia was defined according to the World Health Organization's definition, i.e. haemoglobin (Hgb) concentration below 13.0 g/dl for men and 12.0 g/dl for women. For patients with multiple entries, only the first entry into the database was used. Exclusion criteria were short-term mortality (postoperative death <30 days), observation time <30 days, emergency procedures, non-Norwegian citizens or missing data (Fig. 1).

Operative procedures

All included patients underwent cardiac surgery with cardiopulmonary bypass (CPB). The CPB circuit was primed with 1100–1500 ml of Ringer's acetate with heparin (Leo, Copenhagen, Denmark) 7500–10 000 U depending on patient size. Before start of CPB, patients were routinely given tranexamic acid (Leo) 30 mg/kg and heparin 300 U/kg to achieve a kaolin-activated coagulation time ≥480 s. Crystalloid cardioplegia was used for isolated CABG, whereas blood cardioplegia was used for non-CABG and multiple procedures. Blood remaining in the CPB circuit was retransfused to the patient without centrifugation. Protamine sulphate (Leo) was administered to achieve an activated coagulation time within 10% of baseline. Acetylsalicylic acid was discontinued 1–3 days before surgery in ∼83% of the users and earlier in the remaining ones. Ticagrelor or clopidogrel was discontinued ≥7 days before surgery in ∼82% of the users of these drugs. Low-molecular-weight heparin was continued until the night before surgery for patients on this medication. A transfusion threshold of Hgb 7.0 g/dl intraoperatively and Hgb 8.5 g/dl postoperatively was practised in general at St. Olav Hospital during the observation period. The attending physician made the final decision whether to transfuse RBC, and the number of transfusions was recorded until hospital discharge.

Endpoint

The study endpoint was all-cause long-term mortality, defined by an observation time from 30 days to 5 years postoperatively. The observation period was divided into 2 parts: 30 days–1 year and 1–5 years postoperatively to compare findings between these 2 periods. The main analysis compared long-term mortality between anaemic patients who received ≥1 unit of RBC and anaemic patients who did not receive any RBC transfusion during the entire hospital stay.

Table 1: Variables included in the main analysis[a]

	Variables
Step 2 model	Red blood cell transfusion
	Age
	Sex
	Body mass index
	Hypertension[b]
	Diabetes
	Chronic pulmonary disease[c]
	Smoking (present or previous smoker vs never smoker)
	Preoperative haemoglobin concentration
	Preoperative creatinine concentration
Step 3 model	New York Heart Association functional classification
	Operation category defined by EuroSCORE II[d]
	Cardiopulmonary bypass time
	Intraoperative blood loss in ml
	Mediastinal blood loss the first 16 h postoperatively
Step 4 model	Cardiac dysfunction[e]
	Acute myocardial infarction
	Novel renal failure[f]

[a] All models include variables from previous steps.
[b] Use of antihypertensive medication or diastolic blood pressure >90 mmHg.
[c] Use of bronchodilator or <75% of expected forced expiratory volume in 1 s.
[d] Isolated CABG, single non-CABG, 2 procedures or ≥3 procedures.
[e] Use of >2 inotropics or intra-aortic balloon pump.
[f] Absolute serum creatinine increase >26 μmol/l, relative increase >50% or postoperative renal dialysis.
CABG: coronary artery bypass grafting.

Statistical analyses

All statistical analyses were performed using Stata (version 16.0, StataCorp, College Station, TX, USA). *P*-values <0.05 were considered significant. The χ^2 test was used for discrete variables. Normality of continuous variables was graphically assessed. Because several variables were non-normally distributed, the Mann–Whitney *U*-test was used for comparisons. The median and 25th and 75th percentiles are given for continuous variables. Long-term mortality was analysed with univariable and multivariable Cox proportional hazards (PH) modelling. Extreme outliers and overinfluential observations were assessed visually on scatterplots for continuous variables and using Cook's distance. The PH test, log-log plots and Cox-Snell residuals were used to assess model fit. Robustness of the Cox models was evaluated using flexible parametric survival models if the PH assumption was violated. If the hazard ratios (HRs, given as mean with 95% confidence interval [CI]) were similar, the Cox models were considered acceptable.

We evaluated the risk associated with RBC transfusion using a four-step blockwise approach. In the first step, we performed unadjusted Cox regression with transfusion of ≥1 unit of RBC using long-term mortality as the outcome. In the second step, a multivariable Cox regression model also including patient risk factors and preoperative laboratory values was analysed (Table 1). The chosen covariates were based on clinical

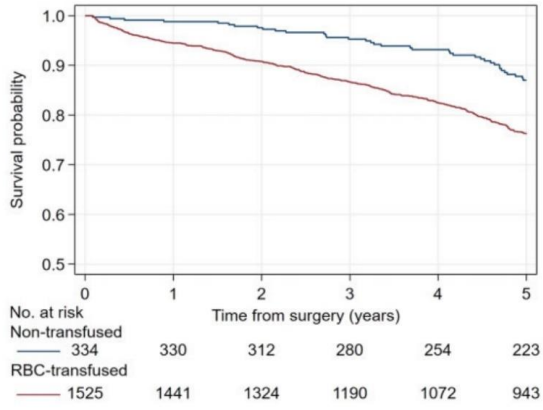


Figure 2: Kaplan–Meier survival estimates. Unadjusted Cox regression model by RBC transfusion, shown for 100–50% cumulative survival. RBC: red blood cell.

knowledge and on a previous study in the CaSOS [1]. In the third step, we further included perioperative variables associated with the need for RBC transfusion; in the fourth step, postoperative complications were also included.

Sensitivity analyses

To evaluate the potential bias introduced by exclusions of short-term mortality and emergency procedures ($n = 233$), we performed the first sensitivity analysis without excluding these patients, using an observation time from the day of surgery to 5 years postoperatively. Another potential bias caused by correlations among the use of anticoagulation (warfarin) or platelet inhibitors (ticagrelor or clopidogrel), perioperative blood loss and RBC transfusion in these patients, could affect the results from the main model. Therefore, a second sensitivity analysis excluded patients with a medical history of using these drugs ($n = 521$) from the main model. Because mortality associated with RBC transfusion may be related to the transfusion dose, a third sensitivity analysis was performed where transfusion was categorized as no transfusion, 1–2 units or ≥3 units of RBC. The sensitivity analyses included the same variables as those used in step 4 of the multivariable Cox regression of the main analysis.

RESULTS

Altogether, 1859 patients with anaemia were included in the study (Fig. 1). Of the included patients, 1525 (82%) received ≥1 unit of RBC transfusion. A total of 545 patients (29.3%) were women; 522 (95.8%) of these patients received ≥1 unit of RBC. In comparison, only 1003 (65.8%) male patients received RBC transfusion during their hospital stays. Preoperative Hgb concentrations were <11.0 g/dl in 398 patients (21.4%); 388 (97.5%) of them received RBC transfusion. All 181 female patients in this group (45.5%) received RBC transfusion. Preoperative Hgb concentrations were <10.0 g/dl in 107 patients (5.8%); 106 (99.1%) of them received RBC transfusion and 43 (40.6%) of these patients were women.

There were several differences between patients who received RBC transfusion and patients who did not, including age, sex and body mass index (Table 2). Mean observation time for all patients was 4.1 (95% CI 4.1–4.2) years. During the follow-up period, 370 (19.9%) deaths were registered, of which 37 (11.1%) were in the group that did not receive RBC transfusion and 333 (21.8%) were in the RBC-transfused group. Between 30 days and 1 year postoperatively, 88 (23.8%) patient deaths were registered and 84 (95.5%) of those who died in this period received ≥1 unit of RBC (Fig. 2).

Results from the unadjusted Cox regression step 1 model showed a significant association between long-term mortality and RBC transfusion in all 3 observation periods (Table 3). Following adjustments for patient risk factors and preoperative laboratory values in the step 2 model, RBC transfusion was no longer statistically significantly associated with long-term mortality in any observation period. In the step 3 models that included operative confounding factors and the step 4 models that also included postoperative complications, the HR for RBC transfusion were further decreased (Table 3 and Fig. 3). The survivor estimates with the 95% CI for the step 4 model are shown in the central image.

Sensitivity analyses

In the first sensitivity analysis including short-term mortality and patients undergoing emergency procedures ($n = 2092$), the HR for mortality was 1.19 (95% CI 0.82–1.71, $P = 0.36$). In the second sensitivity analysis, which excluded patients using anticoagulants or platelet inhibitors ($n = 1338$), the HR for long-term mortality was 1.00 (95% CI 0.65–1.53, $P = 0.99$). In the third sensitivity analysis, RBC transfusion was not significantly associated with long-term mortality for 1–2 units ($n = 461$, HR 1.14, 95% CI 0.75–1.75, $P = 0.45$) or ≥3 units transfused ($n = 1064$, HR 1.08, 95% CI 0.70–1.65, $P = 0.47$). The HR for RBC transfusion was not significant for any model in these sensitivity analyses (Supplementary Material).

All models showed good fit, i.e. the model adequately described the observed data. For the Cox regression models that violated the PH assumption, point estimates and *P*-values from the flexible parametric survival analysis were essentially unchanged. We therefore considered the Cox regression models to be adequate.

DISCUSSION

The results of this study showed that, when adjusting for known preoperative risk factors among anaemic patients undergoing cardiac surgery, RBC transfusion was no longer statistically significantly associated with long-term mortality. When further adjusting for intraoperative and postoperative risk factors, the point estimates for the HR of RBC transfusion became smaller compared to the unadjusted model. These results indicated that much of the mortality risk in anaemic patients was associated with factors other than the transfusion itself. Our findings are similar to those from a previous study, which demonstrated that the HR of RBC transfusion among propensity score matched patients who had CABG was not significant in several observation periods between surgery and 4 years postoperatively [16]. The sensitivity analyses supported the main findings. The results also

Table 2: Baseline patient characteristics and operative variables

	Patients without RBC transfusion ($n = 334$)	Patients with RBC transfusion ($n = 1525$)	*P*-value
Preoperative characteristics			
Sex, men	311 (93.1)	1003 (65.8)	<0.001
Age (years)	69 (61.6–74.7)	73 (66.0–78.0)	<0.001
Body mass index (kg/m^2)	26.2 (24.1–29.3)	25.2 (22.9–28.0)	<0.001
Present or previous smoker	189 (56.6)	801 (52.5)	0.18
Unstable angina	145 (43.4)	610 (40.0)	0.25
Chronic heart failure	55 (16.5)	441 (28.9)	<0.001
New York Heart Association functional classification			
I	12 (3.6)	81 (5.3)	0.19
II	100 (29.9)	331 (21.7)	0.001
III	177 (53.0)	867 (56.9)	0.20
IV	45 (13.5)	246 (16.1)	0.23
Previous myocardial infarction	193 (57.8)	782 (51.3)	0.031
Previous percutaneous coronary intervention	44 (13.2)	136 (8.9)	0.017
Diabetes	72 (21.6)	344 (22.6)	0.69
Hypertension	179 (53.6)	874 (57.3)	0.21
Cerebrovascular disease[a]	39 (12.2)	221 (15.1)	0.18
Chronic pulmonary disease	54 (16.2)	337 (22.1)	0.016
Renal dysfunction[b]	24 (7.5)	185 (12.7)	0.009
Preoperative laboratory values			
Haemoglobin (g/dl)	12.4 (12.0–12.7)	11.6 (10.9–12.2)	<0.001
Creatinine (μmol/l)	87 (74–101)	90 (74–112)	0.057
Preoperative medications[c]			
Acetylsalicylic acid	289 (86.5)	1237 (81.1)	0.019
Ticagrelor	22 (6.6)	49 (3.2)	0.004
Clopidogrel	61 (18.3)	243 (15.9)	0.30
Warfarin	26 (7.8)	132 (8.6)	0.61
Low-molecular-weight heparin	141 (42.2)	613 (40.2)	0.50
Operative and postoperative variables			
EuroSCORE II operation category			
Isolated coronary artery bypass grafting	269 (80.5)	795 (52.1)	<0.001
Single non-coronary artery bypass grafting	36 (10.8)	259 (17.0)	0.005
2 procedures	27 (8.1)	422 (27.7)	<0.001
3 or more procedures	2 (0.6)	49 (3.2)	0.008
Urgent surgery[d]	178 (53.3)	853 (55.93)	0.38
Cardiopulmonary bypass time (min)	67 (62–69)	83 (81–85)	<0.001
Blood loss ≥500 ml during surgery	76 (22.8)	650 (42.6)	<0.001
Postoperative mediastinal blood loss first 16 h (ml)	480 (380–620)	545 (390–820)	<0.001
Postoperative complications			
Renal failure[e]	23 (6.9)	304 (19.9)	<0.001
Myocardial infarction	10 (3.0)	74 (4.9)	0.14
Cardiac dysfunction[f]	20 (6.0)	206 (13.5)	<0.001
Intensive care unit stay >24	12 (3.6)	270 (17.7)	<0.001
Death >30 days postoperatively	37 (11.1)	333 (21.8)	<0.001

Continuous variables given as median (25th–75th percentiles) and categorical variables given as number of patients (%).

[a] Carotid stenosis, previous transient ischaemic attack or stroke.

[b] Creatinine >140 μmol/l or dialysis.

[c] Drug history prior to surgery. Anticoagulation and platelet inhibitor medications were discontinued up to the day before surgery.

[d] Surgery within 2 weeks.

[e] Absolute increase of creatinine >26 μmol/l, relative increase of >50% or postoperative renal dialysis.

[f] Use of >2 inotropics or intra-aortic balloon pump.

RBC: red blood cell.

correspond with a previous investigation in CaSOS, which included anaemic and non-anaemic patients undergoing primary isolated CABG [1].

Although RBC transfusion was not significantly associated with mortality in the present study, the point estimates and wide CIs revealed a potential effect during the first year postoperatively. This result is consistent with previous findings in studies not limited to anaemic patients, indicating that the negative effect of RBC transfusion, if any, is most profound during the early period after cardiac surgery [2, 6, 7, 9]. However, findings regarding the negative effects of RBC transfusion on long-term mortality among patients having cardiac surgery have been contradictory. Our study indicated that overestimation of the negative effects due to lack of proper adjustment may be a reason for contradictory results regarding mortality associated with RBC transfusion.

One of the pitfalls of observational studies of the association between RBC transfusion and long-term mortality is the lack of correction for other risk factors in the analysis. Among patients

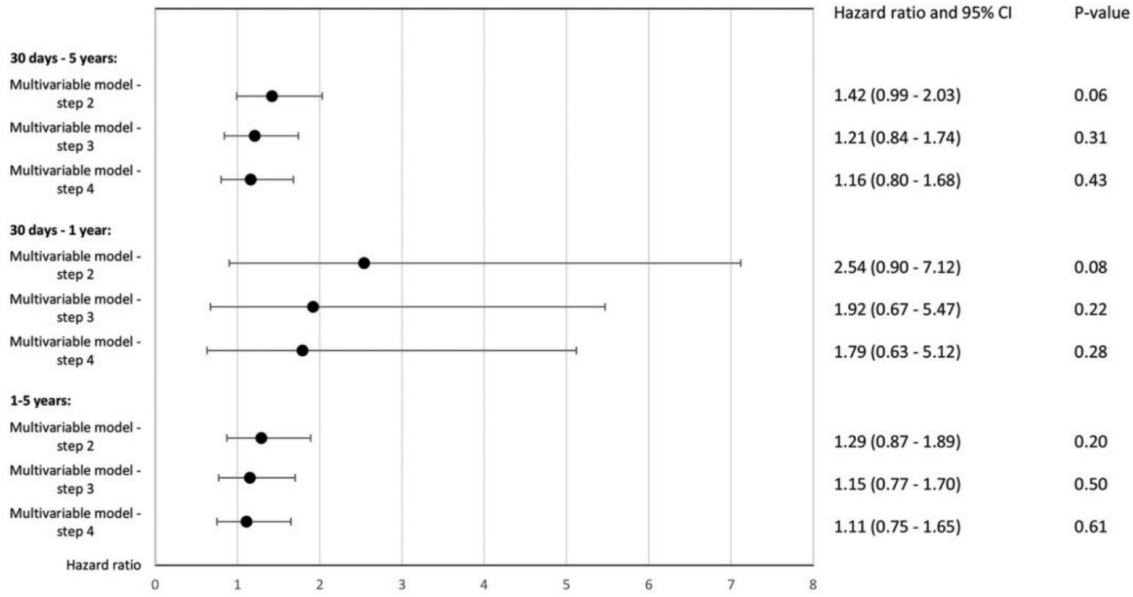


Figure 3: Multivariable Cox regression models. Hazard ratio with 95% CI for multivariable Cox regression models for patients with preoperative anaemia undergoing open-heart surgery. CI: confidence interval.

Table 3: Unadjusted and multivariable Cox regression models for long-term mortality[a]

	Hazard ratio (95% CI)	*P*-value
30 Days-5 years		
Unadjusted model—step 1	2.09 (1.49–2.93)	<0.001
Multivariable model—step 2	1.42 (0.99–2.03)	0.056
Multivariable model—step 3	1.21 (0.84–1.74)	0.31
Multivariable model—step 4	1.16 (0.80–1.68)	0.43
30 Days–1 year		
Unadjusted model—step 1	4.70 (1.72–12.81)	0.002
Multivariable model—step 2	2.54 (0.90–7.12)	0.077
Multivariable model—step 3	1.92 (0.67–5.47)	0.22
Multivariable model—step 4	1.79 (0.63–5.12)	0.28
1–5 Years		
Unadjusted model—step 1	1.77 (1.23–2.55)	0.002
Multivariable model—step 2	1.29 (0.87–1.89)	0.20
Multivariable model—step 3	1.15 (0.77–1.70)	0.50
Multivariable model—step 4	1.11 (0.75–1.65)	0.61

[a]Cox regression models for the association between red blood cell transfusion and long-term mortality among patients with preoperative anaemia undergoing open-heart surgery. Included variables detailed in Table 1.
CI: confidence interval.

having cardiac surgery, several risk factors are associated both with RBC transfusion and mortality, such as age, gender, body mass index and nadir haematocrit [14, 21, 22]. The inclusion of Hgb values in the analysis is therefore necessary when evaluating the potential risk of RBC transfusion but may cause underestimation of the effect. Although the Hgb level for anaemia is defined differently for men and women, the transfusion threshold remains the same for both genders. In the present study almost all the included female patients received RBC transfusion. Due to the inclusion criteria and haemodilution during CPB, women in the present study were at greater risk of RBC transfusion. Therefore, any potential negative effect of RBC transfusion is difficult to evaluate because there were few women in the non-transfused group. This finding could potentially lead to overestimation of the effect of RBC transfusion in the results.

A previous study in the CaSOS showed that long-term mortality was no longer significantly associated with transfusion among patients having CABG when preoperative, intraoperative and postoperative factors were considered [1]. This finding suggests that the perceived effect of mortality due to transfusion was caused by residual confounding and not by the transfusion. A previous study investigated the effect of postoperative mediastinal bleeding as a potential confounder for mortality in patients having cardiac surgery [23]. The authors found no association between RBC transfusion and death when adjusting for 24-h chest-tube drainage. They therefore suggested that the increased number of deaths seen among these patients is mediated through mechanisms other than RBC transfusion. This result and hypothesis are in conjunction with the findings of the present study.

The observed difference in long-term mortality in patients who receive RBC transfusion could be caused by increased postoperative morbidity [3, 5, 8, 10]. However, it is difficult to determine whether organ failure in these patients was the result of RBC transfusion or of other clinical factors associated with the need to transfuse. Postoperative complications may therefore add another layer of confounding in the multivariable analysis, which was why we employed a stepwise analytic approach. The association between RBC transfusion and long-term mortality was no longer significant in the second step when preoperative patient risk factors were included in the analysis. The HR decreased further when intraoperative and postoperative factors were included. The sensitivity analyses demonstrated that inclusion of high-risk patients increased the HR associated with RBC transfusion, whereas exclusion of patients with higher risk of

bleeding decreased it. These findings further strengthen the hypothesis that patient risk factors are an important underlying reason for the observed difference in mortality between the transfused and non-transfused groups.

The present study indicates that transfusion is not associated with increased long-term mortality in anaemic patients when the analysis is appropriately adjusted for potential confounders. Our results underscore the need to use statistical methods that permit adequate statistical adjustment and not the simple factorial designs that have been used in some previous studies.

Strengths and limitations

The CaSOS database is prospectively registered, has undergone quality assurance and has been supervised by a senior anaesthesiologist; therefore, there were few missing data. Because of the relatively large database, we were able to evaluate the effect of RBC transfusion in patients with anaemia having cardiac surgery separately from the rest of the patient group. Compared to non-anaemic cardiac surgery patients, patients with anaemia have additional risk factors and morbidities as well as higher mortality rates [13, 14], which may influence the outcome and therefore result in different confounding than in non-anaemic patients.

This is a single-centre study, and the local transfusion policy may have influenced the results. Studies with other transfusion thresholds and policies may therefore have different findings. The final decision to transfuse was left to the attending physician, which may bias the results. However, a meta-analysis from 2015 suggests that observational studies overestimate the effect of transfusion compared to results from randomized controlled trials [20]. Due to a relatively small sample size, we cannot exclude the possibility that transfusion volume influences the association between RBC transfusions and long-term mortality even if the sensitivity analysis argues against a dose-dependent effect. The potential effects of cardioplegia and haemodilution could not be investigated. Even though RBC transfusion was not significantly associated with long-term mortality in the present study, we cannot exclude the possibility that unmeasured confounders may bias the results.

This study was limited by the small number of patients with anaemia who did not receive RBC transfusion, especially among the women. Because most patients with anaemia received transfusion, the non-transfused patients constituted a group with fewer known risk factors. The sample size was too small to allow for subgroup analysis of different categories of operation. The influence of the aetiology of anaemia was not investigated because of lack of data. Another limitation is that almost all the registered deaths during the first postoperative year occurred in patients who received RBC transfusion, which is the reason for the wide CIs seen in the models for observation time between 30 days and 1 year. Despite adjustments in the multivariable analysis, there may therefore be residual confounding. Thus, a false-negative result cannot be excluded, and the lack of significance should be interpreted cautiously.

CONCLUSIONS

We found no statistically significant association of RBC transfusion with long-term mortality in patients with anaemia undergoing open-heart surgery when adjusting for known risk factors. This was the case for the periods from 30 days to 5 years and from 1 to 5 years after surgery. The results from the sensitivity analyses further strengthen the result. Because of the sample size, we cannot exclude an association between RBC transfusion and long-term mortality, especially between 30 days and 1 year postoperatively.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *ICVTS* online.

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Author contributions

Long Tran: Conceptualization; Data curation; Formal analysis; Investigation; Validation; Visualization; Writing—original draft. **Guri Greiff:** Conceptualization; Data curation; Writing—review & editing. **Alexander Wahba:** Conceptualization; Data curation; Writing—review & editing. **Hilde Pleym:** Conceptualization; Data curation; Writing—review & editing. **Vibeke Videm:** Conceptualization; Formal analysis; Funding acquisition; Investigation; Project administration; Supervision; Validation; Writing—review & editing.

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SUPPLEMENTARY MATERIAL

Supplementary table 1: Sensitivity analyses 1 and 2 – multivariable Cox regression models for long-term mortality[a]

30 days-5 year	Hazard ratio (95% CI)	P-value
Sensitivity analysis 1[b]	1.19 (0.82-1.71)	0.36
Sensitivity analysis 2[c]	1.00 (0.65-1.53)	0.99
30 days-1 year		
Sensitivity analysis 1[b]	1.92 (0.67-5.45)	0.22
Sensitivity analysis 2[c]	1.73 (0.40-7.58)	0.46
1-5 years		
Sensitivity analysis 1[b]	1.13 (0.76-1.67)	0.56
Sensitivity analysis 2[c]	0.99 (0.63-1.57)	0.98

a Cox regression models for the association between red blood cell transfusion and long-term mortality among patients with preoperative anaemia undergoing open-heart surgery. Included variables detailed in Table 1 of the main paper.

b Sensitivity analysis for step 4 model which included short-term mortality (death <30 days postoperatively), patients with observation time <30 days, and emergency surgery (n = 2,092). Observation time was from the day of surgery to 5 year postoperatively. Patient numbers: RBC-transfused n = 1,748 (83.6%), non-transfused n = 344 (16.4%). Observed mortality between day of surgery and 5 years: RBC-transfused n = 418 (24.9%), non-transfused n = 38 (11.0%). Observed mortality between day of surgery and 1 year: RBC-transfused n = 159 (9.1%), non-transfused n = 5 (1.5%). Observed mortality between 1 and 5 years postoperatively: RBC-transfused n = 259 (16.3%), non-transfused n = 33 (9.7%).

c Sensitivity analysis for step 4 model which excluded use of oral anticoagulation (warfarin) and platelet inhibitors (ticagrelor or clopidogrel) (n = 1,338). Patient numbers: RBC-transfused n = 1,112 (83.1%), non-transfused n = 226 (16.9%). Observed mortality between 30 days and 5 years: RBC-transfused n = 238 (21.4%), non-transfused n = 28 (12.4%). Observed mortality between 30 days of surgery and 1 year: RBC-transfused n = 53 (4.8%), non-transfused n = 2 (0.9%). Observed mortality between 1 and 5 years postoperatively: RBC-transfused n = 185 (17.5%), non-transfused n = 26 (11.6%).

CI: Confidence interval

Supplementary table 2: Sensitivity analysis 3 comparing RBC transfusion volumes - multivariable Cox regression models for long-term mortality[a]

30 days-5 years[b]	**Hazard ratio (95% CI)**	**P-value**
Multivariable model – step 4		
1-2 units of RBC	1.17 (0.78-1.73)	0.45
≥ 3 units of RBC	1.16 (0.78-1.71)	0.47
30 days-1 year[c]		
Multivariable model – step 4		
1-2 units of RBC	1.47 (0.47-4.61)	0.51
≥ 3 units of RBC	2.02 (0.69-5.93)	0.20
1-5 years[d]		
Multivariable model – step 4		
1-2 units of RBC	1.14 (0.75-1.75)	0.54
≥ 3 units of RBC	1.08 (0.70-1.65)	0.74

a Cox regression models for the association between different volumes of red blood cell (RBC) transfusion (0 units: reference, 1-2 units or ≥ 3 units) and long-term mortality among patients with preoperative anaemia undergoing open-heart surgery. Included variables detailed in Table 1 of the main paper. Patient numbers: 0 units n = 334, 1-2 units n = 461, ≥ 3 units n = 1,064.

b Observed mortality according to number of transfusions: 0 units n = 37 (11.1%), 1-2 units n = 80 (17.4%), ≥ 3 units n = 253 (23.8%).

c Observed mortality according to number of transfusions: 0 units n = 4 (1.2%), 1-2 units n = 12 (2.6%), ≥ 3 units n = 72 (6.8%).

d Observed mortality according to number of transfusions: 0 units n = 33 (10.0%), 1-2 units n = 68 (15.1%), ≥ 3 units n = 181 (20.5%).

CI: Confidence interval; RBC: Red blood cell

Paper 3

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ORIGINAL ARTICLE

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Relative impact of red blood cell transfusion and anaemia on 5-year mortality in cardiac surgery

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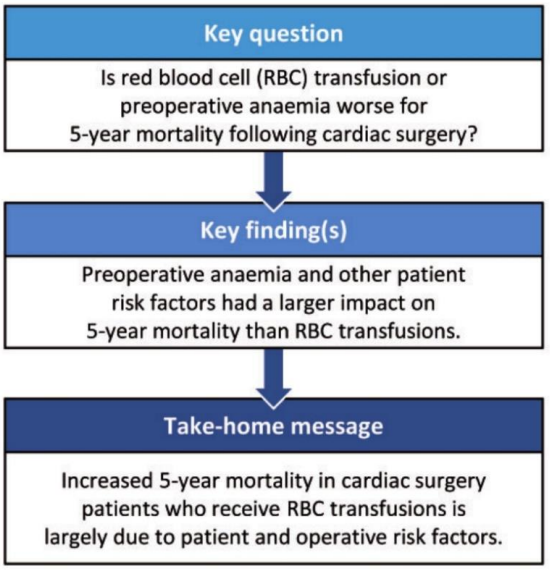

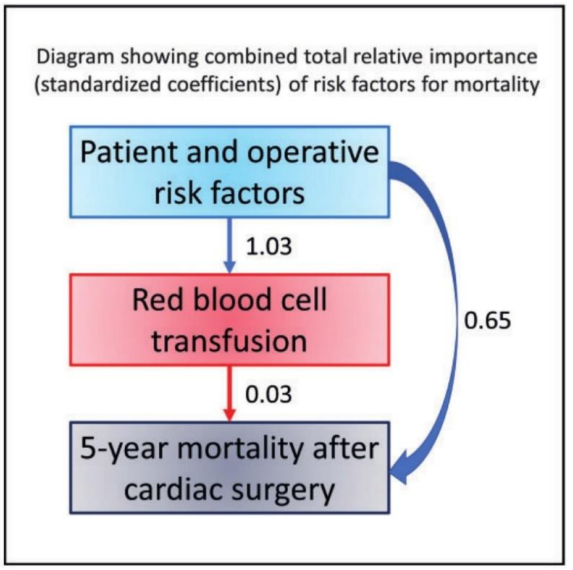

Abstract

OBJECTIVES: The aim was to compare the relative effects of red blood cell (RBC) transfusion and preoperative anaemia on 5-year mortality following open-heart cardiac surgery using structural equation modelling. We hypothesized that patient risk factors associated with RBC transfusion are of larger importance than transfusion itself.

METHODS: This prospective cohort study, part of the Cardiac Surgery Outcome Study at St. Olavs University Hospital, Trondheim, Norway, included open-heart on-pump cardiac surgery patients operated on from 2000 through 2017 ($n = 9315$). Structural equation modelling, which allows for intervariable correlations, was used to analyse pathway diagrams between known risk factors and observed

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mortality between 30 days and 5 years postoperatively. Observation times between 30 days and 1 year, and 1–5 years postoperatively were also compared with the main analysis.

RESULTS: In a simplified model, preoperative anaemia had a larger effect on 5-year mortality than RBC transfusion (standardized coefficients: 0.17 vs 0.09). The complete model including multiple risk factors showed that patient risk factors such as age (0.15), anaemia (0.10), pulmonary disease (0.11) and higher creatinine level (0.12) had larger effects than transfusion (0.03). Results from several sensitivity analyses supported the main findings. The models showed good fit.

CONCLUSIONS: Preoperative anaemia had a larger impact on 5-year mortality than RBC transfusion. Differences in 5-year mortality were mainly associated with patient risk factors.

Keywords: Red blood cell transfusion • Cardiac surgery • All-cause mortality • Anaemia • Structural equation modelling • Risk factors

ABBREVIATIONS

BMI	Body mass index
CABG	Coronary artery bypass grafting
CaSOS	Cardiac Surgery Outcome Study
CPB	Cardiopulmonary bypass
Hgb	Haemoglobin
RBC	Red blood cell
SD	Standard deviation
SEM	Structural equation modelling

INTRODUCTION

The relationship between red blood cell (RBC) transfusion and long-term mortality among cardiac surgery patients has been explored in several previous studies. Some studies found significant associations [1–4], whereas other studies suggest that there is no such significant effect [5–9]. Although the results are conflicting, there is a consensus that RBC transfusion is independently associated with a negative outcome in these patients. Furthermore, patients with low haematocrit values or preoperative anaemia have a higher mortality risk when given RBC transfusion [10–12]. The conflicting findings from observational studies may be attributed to the lack of proper adjustments and overestimation of the effect of RBC transfusion. The increased risk associated with RBC transfusion seen in earlier studies may therefore be due to residual confounding. Based on previous results from our group, we hypothesized that patient and operative risk factors are more important for mortality than transfusion itself [8, 9].

Traditionally, studies on this subject have employed a factorial design in their survival analysis. A limitation of this method is not permitting adequate analysis of intervariable correlations and more complex models where effects are modified by several variables. Risk factors such as sex, body mass index (BMI) and haemoglobin (Hgb) concentrations are highly correlated with the need to transfuse RBC, as well as with mortality [13]. Therefore, structural equation modelling (SEM) which can analyse pathways between multiple observed variables and the outcome, could address a gap in knowledge regarding transfusion and mortality [14, 15].

SEM has commonly been used in social sciences and psychology where unobserved latent variables can be investigated. Although SEM is an old statistical method, it has gained popularity in recent years because of progress made in software and modelling development [15–17]. To the best of our knowledge, the SEM framework has not previously been employed to investigate cardiac surgery-related mortality, perhaps due to the considerable familiarity with Cox proportional hazards modelling for survival analysis.

The aim of the present study was to investigate the association of RBC transfusion and preoperative anaemia with 5-year mortality among patients who undergo cardiac surgery, while allowing for intervariable correlation. We compared the associated risk of RBC transfusion and preoperative anaemia on 5-year mortality in patients who underwent on-pump open-heart surgery, in SEM models depicting potential paths among known risk factors.

MATERIALS AND METHODS

The Trondheim Heart Surgery database has consecutively registered cardiac surgery patients at St. Olavs University Hospital, Trondheim, Norway, since 1992 as part of local quality assurance work. The database has prospectively registered laboratory results, patient- and procedure-related preoperative characteristics, perioperative events and variables as earlier described [8]. Data regarding mortality and cause of death until 31 December 2018 were obtained from the national Norwegian Cause of Death Registry, which has >99% coverage regarding all Norwegian citizens.

The study was part of the Cardiac Surgery Outcome Study (CaSOS), which has used the database to investigate different complications following adult cardiac surgery and was approved by the Norwegian Data Inspectorate and the Regional Committee for Medical and Health Research Ethics in Middle-Norway (27 June 2007, reference 4.2007.1528). Patients included in the database from April 2008 have provided informed consent, and the need for informed consent was waived up to this date.

Patient population and endpoint

The operative procedure and transfusion thresholds are detailed in the Supplementary Material. All adult on-pump open-heart surgery patients operated on from 2000 through 2017 ($n = 10\ 288$) were prospectively included in the database. For patients with multiple entries, only the primary entry was used. Exclusion criteria were short-term mortality, i.e. operative mortality, in-hospital mortality or mortality within 30 postoperative days. Non-Norwegian citizens were excluded from the study because death of foreign citizens is not recorded in the Norwegian Cause of Death Registry. Salvage and emergency procedures have a higher risk of mortality and RBC transfusion, so these patients were excluded. Data were incomplete or missing in 13 patients, leaving data from 9315 patients for analysis (Fig. 1).

Preoperative anaemia was defined according to the World Health Organization criteria, i.e. Hgb concentration below 12.0 g/dl for women and 13.0 g/dl for men, based on a blood sample

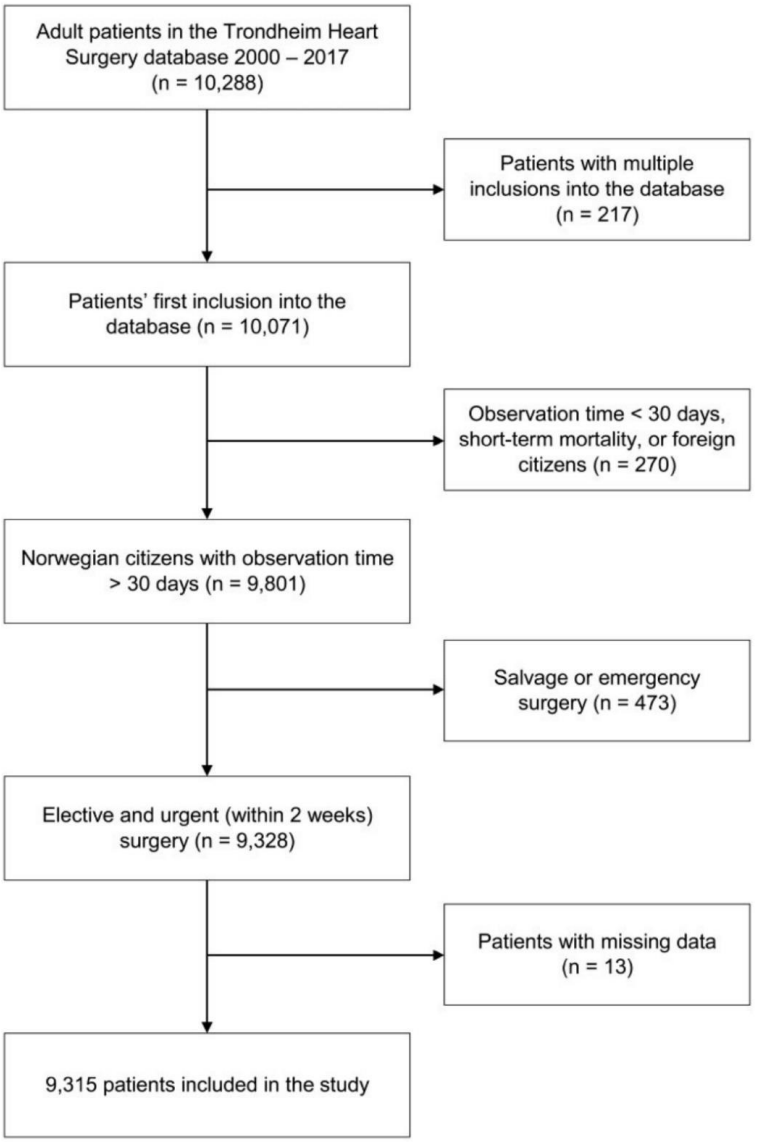

Figure 1: Inclusion and exclusion of patients to the study.

drawn shortly before surgery. The study endpoint was 5-year mortality defined as all-cause mortality between 30 days and 5 years postoperatively. The exposure was transfusion of at least 1 unit of RBC intraoperatively or postoperatively. Additionally, the study evaluated the potential effect of RBC transfusion on mortality in shorter intervals, i.e. between 30 days and 1 year postoperatively, and between 1 and 5 years postoperatively.

Study approach

We constructed pathway diagrams in the SEM framework hypothesizing causal relations between risk factors, preoperative anaemia, RBC transfusion and 5-year mortality based on literature, clinical knowledge and available data [15]. We then determined whether the hypothesized relationships fit with the observed data using a series of generally accepted fit indices as detailed in the Supplementary Material [14].

SEM allows for simultaneous estimation of multiple linear regression terms describing the relationships among observed variables, and allows for estimation of complex models [14, 15]. Compared with Cox regression analysis, which only estimates a single time-to-event process, SEM can explore intervariable relationships among multiple patient and operative risk factors both with the need to transfuse RBC and mortality. Another advantage

of SEM is the measurement of the indirect effects through intermediary variables, thus estimating both the direct and indirect effect of an independent variable on the dependent variable [16]. However, the model needs to be specified a priori based on empirical data and more than 1 hypothesized model may fit the observed data [14, 17].

The main objective of the study was to compare the direct effect of RBC transfusion and the total effect of preoperative anaemia on 5-year mortality, also with inclusion of other variables that may influence the results. To compare the variables measured using different scales, e.g. Hgb (g/dl) and RBC transfusion (units), the regression coefficients were standardized. This ensured that all variables were measured in standard deviations (SD) and their effects are therefore comparable.

Main structural equation models

We employed a stepwise model-building approach, starting with a simplified model A which included preoperative anaemia, RBC transfusion and 5-year mortality (Fig. 2, model A). In the second step, we further included operative blood loss (100 ml increments) (Fig. 2, model B).

In the third step, we included risk factors associated with morbidity and mortality in cardiac surgery (model C, Fig. 3). The additional variables were based on clinical knowledge and previous models in CaSOS [8,9]: age, sex, BMI, diabetes, chronic pulmonary disease (use of bronchodilators or forced expiratory volume <75%) and preoperative creatinine level. We hypothesized direct effects of each risk factor on all variables in model B. The model also included history of smoking, cardiopulmonary bypass (CPB) duration (min) and operation category as defined in EuroSCORE II [isolated coronary artery bypass grafting (CABG), non-CABG, 2 surgeries or ≥3 surgeries]. Smoking was only hypothesized to be associated with anaemia and 5-year mortality. CPB duration and EuroSCORE II operation category were assumed to be associated with operative blood loss, RBC transfusion and 5-year mortality (Fig. 3). Left ventricular ejection fraction was measured using different methods over the years and was therefore not included.

Sensitivity analysis

In the fourth and fifth steps, we analysed the risk associated with RBC transfusion and preoperative anaemia on mortality from 30 days to 1 year postoperatively (model D) and from 1 to 5 years postoperatively (model E), including the same pathways as in model C.

In the sixth step, we analysed the risk associated with RBC transfusion and preoperative anaemia on 5-year mortality in isolated CABG (model F) or multiple procedures (model G). Pathways were as in model C but excluding the variable EuroSCORE II operation category. Finally, in the seventh step, we analysed the risk associated with transfusion of at least 2 units of RBC (model H, RBC-transfused $n = 3507$) using the same pathways as in model C.

Statistical analysis

All statistical analyses were performed using Stata (Release 16, StataCorp, College Station, TX, USA). Comparisons between transfused and non-transfused patients were performed with the χ^2 or

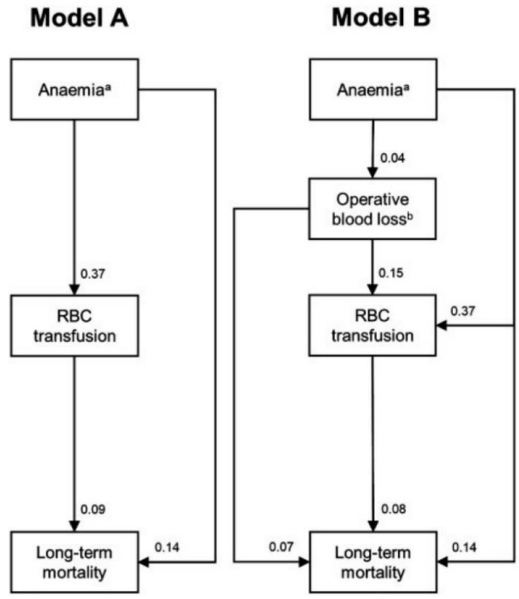

Figure 2: Pathway diagrams for model A and B. Path diagram notation: boxes indicate observed variables; arrows indicate paths, i.e. the influence of variables on each other; numbers indicate regression coefficients, i.e. the level of correlation along the path. All coefficients are standardized, i.e. numbers corresponded to effects when all variables are measured on the same scale. [a]Anaemia defined as preoperative haemoglobin concentration <12.0 g/dl for women and <13.0 g/dl for men

the *t*-test. Normality of continuous variables was graphically assessed. Multicollinearity was assessed using variance influence factors. Data are presented as mean and SD or number of patients with percentage. *P*-values <0.05 were considered significant. We calculated the unstandardized and standardized direct, indirect and total (combined direct and indirect effect) of study variables in all models. Sample size considerations and goodness-of-fit indices are explained in the Supplementary Material.

RESULTS

Patient characteristics

Nine thousand three hundred and fifteen patients were eligible for the study (Fig. 1). Among them, 4214 (45.2%) received at least 1 unit of RBC transfusion. There were 1889 patients (20.3%) who had a Hgb concentration below the threshold for anaemia and RBC transfusion was given to 1552 (82.2%) of these patients. Baseline characteristics, operative variables and postoperative complications are shown in Table 1. Several clinical variables and postoperative events differed between the groups, postoperative renal failure being the most frequent and occurring in 704 (16.7%) of RBC-transfused patients compared to 222 (4.4%) of the non-transfused patients.

In the follow-up period, 619 patients (14.7%) who received RBC transfusion died, compared to 305 patients (6.0%) in the non-transfused group. The mean observation time until death was 2.5 years (SD 1.6 years) for patients who received RBC

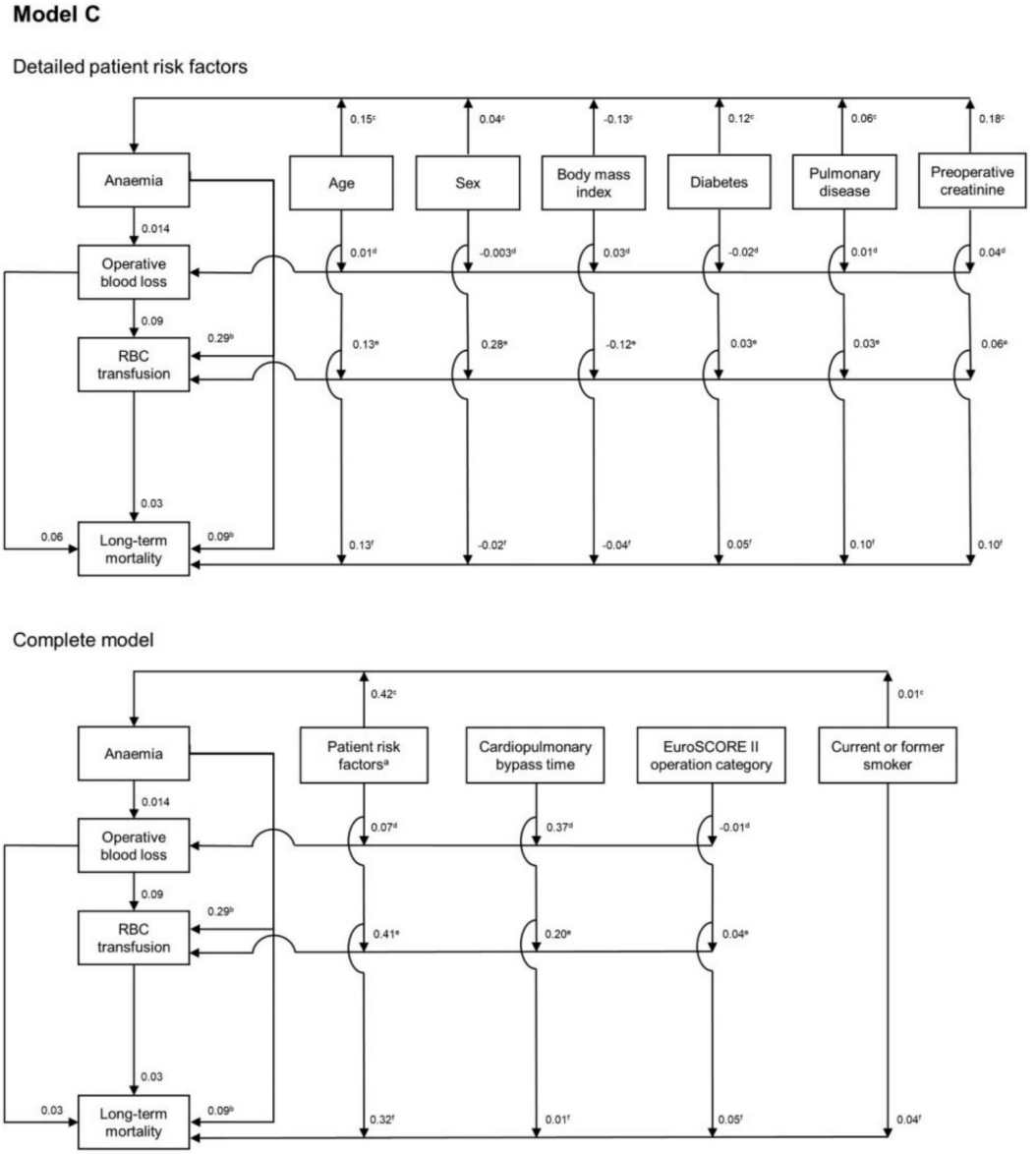

Figure 3: Pathway diagrams for model C. Upper panel: detailed part of model C, focusing on associations of preoperative patient risk factors. Excluded model variables from this panel are CPB time, EuroSCORE II operation category and history of smoking. Lower panel: the pathway diagram represents the full model C. Patient risk factors detailed in the upper panel are visualized as a composite variable. Path diagram notation: boxes indicate observed variables; arrows indicate paths, i.e. the influence of variables on each other; numbers indicate regression coefficients, i.e. the level of correlation along the path. All coefficients are standardized, i.e. numbers corresponded to effects when all variables are measured on the same scale. [a]Age, sex, body mass index, diabetes, pulmonary disease and preoperative creatinine level. [b]Direct effect of preoperative anaemia on RBC transfusion and 5-year mortality. [c]Direct effect of risk factors on preoperative anaemia. [d]Direct effect of risk factors on operative blood loss. [e]Direct effect of risk factors on RBC transfusion. [f]Direct effect of risk factors on 5-year mortality. RBC: red blood cell.

transfusion and 2.9 years (SD 1.4 years) for patients who did not receive any transfusion ($P < 0.001$). Between 30 days and 1 year postoperatively, 149 deaths (3.5%) were registered in the transfused group and 39 deaths (0.8%) in the non-transfused group ($P < 0.001$).

Main structural equation models

All main models had acceptable fit, as described in the Supplementary Material. There were no indications of

Table 1: Baseline patient characteristics, operative variables and postoperative complications

	Patients without RBC transfusion ($n = 5101$)	Patients with RBC transfusion ($n = 4214$)	*P*-value
Preoperative characteristics			
Anaemia	337 (6.6)	1552 (36.8)	<0.001
Male sex	4449 (87.2)	2504 (65.8)	<0.001
Age (years)	64 ± 10.1	70 ± 10.0	<0.001
Body mass index (kg/m^2)	27.6 ± 4.0	26.2 ± 4.1	<0.001
Present or previous smoker	2943 (57.7)	2138 (50.7)	<0.001
Congestive heart failure[a]	500 (12.8)	934 (27.0)	<0.001
NYHA functional classification[a]			
I	314 (6.2)	238 (5.7)	0.30
II	1721 (33.7)	1033 (24.5)	<0.001
III	2676 (52.5)	2401 (57.0)	<0.001
IV	389 (7.6)	541 (12.8)	<0.001
Previous myocardial infarction	2223 (43.6)	1843 (43.7)	0.88
Previous percutaneous coronary intervention	631 (12.4)	417 (9.9)	<0.001
Diabetes	683 (13.4)	703 (16.7)	<0.001
Hypertension	2645 (51.9)	2412 (57.2)	<0.001
Chronic pulmonary disease	613 (12.0)	824 (19.6)	<0.001
Renal dysfunction[b]	82 (1.6)	275 (6.5)	<0.001
Preoperative lab values			
Haemoglobin (g/dl)	14.5 ± 1.2	13.0 ± 1.5	<0.001
Creatinine (μmol/l)	87.0 ± 24.9	96.1 ± 68.9	<0.001
Operative and postoperative variables			
EuroSCORE II operation category			
Isolated coronary artery bypass grafting	3948 (77.4)	2186 (51.9)	<0.001
Single non-coronary artery bypass grafting	543 (10.6)	686 (16.2)	<0.001
2 procedures	556 (10.9)	1163 (27.6)	<0.001
3 or more procedures	54 (1.1)	179 (4.3)	<0.001
Urgent surgery[c]	1958 (38.4)	2058 (48.8)	<0.001
Cardiopulmonary bypass time (min)	74 ± 29.7	96 ± 42.7	<0.001
Haemoglobin first postoperative day (g/dl)	10.6 ± 1.1	9.5 ± 0.9	<0.001
Blood loss during surgery (ml)	560 ± 180	730 ± 720	<0.001
Postoperative mediastinal blood loss first 16 h (ml)	537 ± 231	799 ± 693	<0.001
Postoperative complications			
Renal failure[d]	222 (4.4)	704 (16.7)	<0.001
Myocardial infarction	150 (2.9)	258 (6.1)	<0.001
Cardiac dysfunction[e]	151 (3.0)	495 (11.8)	<0.001
Primary intubation more than 24 h or reintubation	24 (0.5)	246 (5.8)	<0.001
Intensive care unit stay >24 h	140 (2.7)	665 (15.8)	<0.001
Death >30 days postoperatively	305 (6.0)	619 (14.7)	<0.001

Variables given as mean with standard deviation or *n* (%).

[a]Patients with missing data: congestive heart failure $n = 1939$; NYHA functional classification $n = 2$.

[b]Creatinine >140 μmol/l or dialysis.

[c]Surgery within 2 weeks.

[d]Absolute increase of creatinine >26 μmol/l, relative increase of >50% or postoperative renal dialysis.

[e]Use of >2 inotropics or intra-aortic balloon pump.

NYHA: New York Heart Association; RBC: red blood cell.

multicollinearity. Results for all models, as well as the correlation matrix are given in the Supplementary Material.

In the first analysis step (model A, Fig. 2), the direct effects of preoperative anaemia and RBC transfusion on 5-year mortality were 0.14 and 0.09, respectively. These numbers represent the amount of variance in mortality explained by these independent variables, i.e. 14% and 9%. The total effect of anaemia on 5-year mortality when combining the direct and indirect effects was 0.17. Model A indicated that preoperative anaemia was a greater determinant of 5-year mortality than RBC transfusion (i.e. total combined direct and indirect effect: 0.17 vs 0.09).

The results from the step 2 model also including operative blood loss (model B, Fig. 2) were comparable to those of the first step (model A). Total effects for model B were: anaemia 0.17, RBC transfusion 0.08 and operative blood loss 0.08. All path coefficients in model A and B were significant.

In the third step, all study variables were included (model C). Details regarding patient risk factors with direct effects are shown in the upper panel of Fig. 3, and the complete model is shown in the lower panel. Age had the largest total effect on 5-year mortality among cardiac surgery patients (0.15), followed by creatinine level (0.12), pulmonary disease (0.11), anaemia (0.10), diabetes (0.06) and operative blood loss (0.06). BMI was a negative predictor of 5-year mortality, meaning that a higher BMI was associated with increased survival (-0.06). RBC transfusion (0.03) had the weakest total effects on mortality. The coefficients for direct and total effects of all study variables on anaemia, RBC transfusion and 5-year mortality in model C are summarized in Table 2.

Table 2: Direct and total effects of study variables on anaemia, RBC transfusion and 5-year mortality in model C

Variables	Direct effect coefficients[a]	Total effect coefficients[a]
Effects on anaemia		
Age	0.15*	0.15*
Female sex	0.04*	0.04*
Body mass index	-0.13*	-0.13*
Diabetes	0.12*	0.12*
Pulmonary disease	0.06*	0.06*
Creatinine level	0.18*	0.18*
Smoker	0.01	0.01
Effects on RBC transfusion		
Preoperative anaemia	0.29*	0.29*
Operative blood loss	0.09*	0.09*
Age	0.13*	0.17*
Female sex	0.28*	0.30*
Body mass index	-0.12*	-0.15*
Diabetes	0.03*	0.06*
Pulmonary disease	0.03*	0.04*
Creatinine level	0.06*	0.11*
CPB time	0.20*	0.23*
EuroSCORE II operation category	0.04*	0.03*
Smoker	Not applicable	0.003
Effects on 5-year mortality		
RBC transfusion	0.03*	0.03*
Preoperative anaemia	0.09*	0.10*
Operative blood loss	0.06*	0.06*
Age	0.13*	0.15*
Female sex	-0.02	-0.01
Body mass index	-0.04*	-0.06*
Diabetes	0.05*	0.06*
Pulmonary disease	0.10*	0.11*
Creatinine level	0.10*	0.12*
CPB time	0.01	0.04*
EuroSCORE II operation category	0.05*	0.04*
Smoker	0.04*	0.04*

[a]All coefficients are standardized, i.e. numbers correspond to effects when all variables are measured in the same scale.

CPB: cardiopulmonary bypass; RBC: red blood cell.

*P-value <0.05.

Sensitivity analysis

The results from the sensitivity analyses, i.e. fourth to seventh steps of the analysis, were similar for anaemia and RBC transfusion as in the main analysis (model C). Details are given in the Supplementary Material. In model D, model E and model G, RBC transfusion was not significantly associated with mortality ($P = 0.10$, $P = 0.061$ and $P = 0.44$, respectively).

The results for age, BMI, diabetes, pulmonary disease and preoperative creatinine level were similar in model D–H as in model C. Female sex was not significantly associated with mortality in any model.

DISCUSSION

The main finding of the study suggests that when appropriately adjusted for correlations among variables in a SEM model, the effect on 5-year mortality of RBC transfusion became weaker than that of other patient risk factors. In the follow-up period, age was the most important risk factor for 5-year mortality. The results from our models showed that preoperative anaemia, pulmonary disease and increased creatinine levels had equivalent effects in all observation periods. Of the significant variables, RBC transfusion had the smallest effect. Several variables were highly correlated to RBC transfusion, of which preoperative anaemia had the strongest correlation. The results from the present study expand on previously published studies because of the alternative analytical approach.

SEM is a statistical method which has not commonly been used to investigate mortality in this patient group. Cox proportional hazards modelling, which is widely used, allows for adjustment of multiple variables in a regression analysis of a time-related outcome such as mortality. But an inherent problem when using Cox regression to investigate the hazard associated with RBC transfusion is the strong correlation of patient risk factors such as age, sex, BMI and preoperative anaemia with the need to transfuse and with mortality [10, 13]. Cox regression assumes that the independent variables are uncorrelated but tolerates low-to-moderate correlation. If correlation is high as in the present setting, the coefficients and P-values may become imprecise. Therefore, a major advantage of using SEM is that it allows for more complex models based on hypothesized causal relationships and the measurement of direct, indirect and total effects on dependent variables [14, 17].

The findings of an association between RBC transfusion and 5-year mortality in cardiac surgery patients point in the same direction as previous studies [1–4], but the effect was smaller in the present study. Some studies have not found an association and attributed the difference in observed long-term mortality to patient risk factors [5–9]. Preoperative anaemia had 3 times the total effect on long-term mortality compared to RBC transfusion in the present study, both being significant. Although preoperative anaemia is sometimes unavoidable, it may also be a modifiable risk factor. The present study shows that preoperative anaemia may be considered a more harmful risk factor compared to transfusion and efforts should be directed at finding better ways to manage preoperative anaemia. The impact of preoperative anaemia is consistent with previous studies indicating that it is an independent risk factor among cardiac surgery patients [11, 12]. The results suggest that patient risk factors that are both associated with risk of transfusion and mortality, such as anaemia, are the main cause of the difference in long-term mortality seen between transfused and non-transfused patients in cardiac surgery. It is crucial that physicians continue striving to minimize unnecessary transfusion. But an important clinical implication of the present study is that RBC transfusion should not be withheld from patients who could benefit from it, because of a perceived strong harmful effect with respect to mortality.

In previous studies from CaSOS, adjustments were included for more risk factors and no significant association between RBC transfusion and long-term mortality was found [8, 9]. They were omitted in the present study because SEM models are specified a priori based on hypothesized causality and too complex models render acceptable model fit difficult to attain. Randomized controlled trials comparing adverse effects of liberal versus restrictive transfusion thresholds have not shown differences in outcomes between the groups, except for total number of RBC units transfused [18, 19]. These findings indirectly support the notions that there are no long-term effects of RBC transfusion itself and that differences in mortality may be due to patient risk factors. Therefore, the significant association of RBC transfusion with 5-year mortality in the present study may be due to residual confounding.

In our models, higher BMI had a positive effect on survival in cardiac surgery, although it is commonly associated with cardiovascular disease and mortality [20]. The term 'obesity paradox' is commonly used to denote this phenomenon, which has been observed previously [21]. Possible explanations include selection bias (obese patients with fewer risk factors are admitted to surgery), an inverse age effect (obesity is more common in younger patients) or less haemodilution among males and those with higher BMI and consequently less transfusion [13, 22].

Female sex is considered a risk factor for mortality following cardiac surgery [13] but was not significantly associated with 5-year mortality in our models. The sex effect could be related to different risk profiles in men and women undergoing cardiac surgery [23]. A previous study in CaSOS explored sex as a risk factor for survival following cardiac surgery and did not find a significant association with long-term mortality [24]. The roles of sex and BMI on RBC transfusion and 5-year mortality were not investigated in the present study.

Several postoperative complications were significantly more common in the RBC-transfused group. Whether these differences could be entirely attributed to transfusion or were also associated with patient-related risk factors, and the impact they have on long-term mortality, was not fully explored. A previous study in CaSOS suggested that postoperative complications and not RBC transfusion, are significantly associated with long-term mortality in isolated CABG patients [8]. SEM could be employed to investigate this relationship between transfusion, postoperative complications and long-term mortality.

Limitations

Important challenges in SEM are correct model specification, omitted or missing variables and small sample sizes. A strength of the present study is the large number of patients in the CaSOS database which allowed for estimation with multiple independent and dependent variables. The results differ from previous studies because SEM allows for analysis of associations among correlated variables, such as risk factors and transfusion. The findings of the present study are only accurate if the causal relationships presented are true. Other hypothesized models between variables may yield different results, and we cannot exclude that inclusion of further variables could have improved the models [17].

A limitation of SEM is that mortality is analysed as a categorical variable (yes/no) without considering the time to death as in Cox regression. Previous studies in CaSOS found no significant association of RBC transfusion with long-term mortality using Cox regression [8, 9]. Results from the sensitivity analysis showed that RBC transfusion was not significant when investigated in the separate periods from 30 days to 1 year or 1 to 5 years postoperatively, even if it was significant in the main analysis without such subdivision. This may be a statistical power issue arising because the total number of deaths was lower in each separate period. Despite the adjustments of risk factors in the SEM models and the relatively long follow-up time, residual confounding due to missing adjustment variables cannot be excluded.

The patient data in the CaSOS database were prospectively collected and have undergone quality assurance controls. There were few missing data, and therefore the database is considered representative of the general population in Middle Norway. The study was limited to a single centre and local transfusion policy may differ from other institutions.

The transfusion policy has undergone minor changes during the study period. The intraoperative transfusion threshold has remained the same, but the postoperative transfusion threshold has varied between 8 and 9 g/dl. The final decision to transfuse was left to the attending physician and this introduces a potential bias. The present study did not evaluate the dose effect, intraoperative or postoperative timing of RBC transfusion or storage time of RBC units.

CONCLUSIONS

Preoperative anaemia was statistically significant and had a greater impact on 5-year mortality compared to RBC transfusion for all observation periods. The results from the SEM models indicated that the risk associated with RBC transfusion is mainly due to patient risk factors.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *ICVTS* online.

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Author contributions

Long Tran: Conceptualization; Data curation; Formal analysis; Investigation; Validation; Visualization; Writing—original draft. **Guri Greiff:** Conceptualization; Data curation; Writing—review & editing. **Alexander Wahba:** Conceptualization; Writing—review & editing. **Hilde Pleym:** Conceptualization; Data curation; Writing—review & editing. **Vibeke Videm:** Conceptualization; Formal analysis; Funding acquisition; Investigation; Project administration; Supervision; Validation; Writing—review & editing.

Reviewer information

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SUPPLEMENTARY MATERIAL

Data sharing statement

Restrictions apply to the availability of the individual participation data for the present paper, which were used under license for the current study and are not publicly available in accordance with Norwegian law.

Operative procedure

All included patients underwent on-pump open-heart surgery. They were given 300 U/kg of heparin (Leo, Copenhagen, Denmark) to achieve a kaolin-activated coagulation time of ≥480 s and tranexamic acid (Leo) 30 mg/kg was given routinely before cardiopulmonary bypass (CPB). The cardiopulmonary (CPB) circuit was primed with 1100–1500 mL of Ringer's acetate with 7500–10 000 U of heparin. Blood remaining in the circuit after CPB was re-transfused to the patient without centrifugation, and protamine sulphate (Leo) was administered to attain an activated coagulation time within 10% of baseline. In general, the transfusion threshold at St. Olavs University Hospital was a concentration of 7.0 g/dL haemoglobin during CPB and 8.5 g/dL haemoglobin postoperatively during the observation period. Transfusion of platelets and plasma were considered when postoperative bleeding exceeded 200 mL/h. The final decision to transfuse was left to the attending physician and transfusions were recorded throughout the hospital stay.

Fit indices and model fit

The sample size requirement to obtain reliable estimates in SEM are considered sufficient when there is a ratio of 20 cases for each model parameter, or at least 200 cases for complex models. We assessed the overall goodness-of-fit with the χ^2-test between the specified model and a model that perfectly fits the data. A non-significant χ^2-test indicates that the model fits the data adequately. However, this test may be inadequate in large samples because small deviations may yield significant results. Therefore, goodness-of-fit was also assessed with the root mean square error of approximation (RMSEA, a comparison of difference between the SEM model and the observed data when considering the number of estimated parameters), the standardized root mean squared residual (SRMR, a comparison of the difference between predicted and observed correlations), and the comparative fit index (CFI) and Tucker-Lewis index (TLI). The CFI and TLI compare the hypothesised model to a model where no variables are correlated, penalising in different ways for the number of estimated parameters. The model fit was considered acceptable when RMSEA < 0.05, SRMR < 0.08, and the CFI and TLI > 0.95.

RMSEA is given with 90% confidence interval (CI). We also calculated the coefficient of determination (R^2) for all models.

In the first step of the analysis, the simplified Model A with only preoperative anaemia, RBC transfusion and long-term mortality was fully saturated, as was Model B. The χ^2-test was therefore not applicable for these models. The other fit indices showed good fit for both model A and Model B: RMSEA = 0.00 (90% CI: 0.00-0.00), SRMR < 0.01, CFI = 1.00 and TLI 1.00 for both models.

The third step in the study (Model C) which included all study variables and observation time from 30 days to 5 years postoperatively, the χ^2-test was significant ($P < 0.01$). Because of the relatively large sample size (n = 9,315), we accepted the model despite the result from the χ^2-test. The model showed good fit indicated by the other fit indices: RMSEA = 0.03 (90% CI: 0.02-0.04), SRMR = 0.01, CFI = 1.00, TLI = 0.94.

In the fourth to seventh step of the analysis (Model D to H), the results regarding goodness of fit were similar to Model C. We considered the goodness of fit indices for all models acceptable. The complete results for all models are given in below.

Correlation matrix for observed variables in the study

	1	2	3	4	5	6	7	8	9	10	11	12	13
1. Red blood cell transfusion	1.000												
2. Anaemia	0.374*	1.000											
3. Age	0.265*	0.186*	1.000										
4. Sex (female)	0.318*	0.044*	0.164*	1.000									
5. Body mass index	- 0.169*	- 0.124*	- 0.131*	- 0.014	1.000								
6. Diabetes	0.046*	0.107*	0.015	0.017	0.171*	1.000							
7. Pulmonary disease	0.104*	0.078*	0.095*	0.030*	0.038*	0.031*	1.000						
8. Creatinine level	0.090*	0.187*	0.052*	- 0.143*	- 0.009	0.033*	0.039*	1.000					
9. Smoker status	- 0.070*	- 0.013	- 0.130*	- 0.120*	0.035*	0.010	0.118*	- 0.010	1.000				
10. Operative blood loss	0.171*	0.040*	0.021	- 0.005	0.028*	- 0.007	0.055*	0.051*	0.003	1.000			
11. Cardiopulmonary bypass time (min)	0.286*	0.072*	0.043*	0.014	- 0.011	- 0.012	0.126*	0.016	- 0.038*	0.426*	1.000		
12. EuroSCORE II Operation category[a]	0.277*	0.086*	0.108*	0.136*	- 0.062*	- 0.045*	0.143*	0.002	- 0.083*	0.288*	0.697*	1.000	
13. Long-term mortality	0.145*	0.171*	0.173*	0.008	- 0.064*	0.062*	0.141*	0.136*	0.027†	0.075*	0.095*	0.104*	1.000

† P-value < 0.05

* P-value < 0.01

a EuroSCORE II operation category: Isolated coronary artery bypass (CABG), non-CABG, 2 procedures or ≥ 3 procedures

Results for SEM models: Model A

Direct, indirect and total effects of variables in model A

	Direct effects			*Indirect effects*			*Total effects*		
Variables	B	β	P-value	B	β	P-value	B	β	P-value
RBC transfusion									
Anaemia	0.46	0.37	< 0.001	N/A	N/A	N/A	0.46	0.37	< 0.001
Long-term mortality									
RBC transfusion	0.06	0.09	< 0.001	N/A	N/A	N/A	0.06	0.09	< 0.001
Anaemia	0.10	0.14	< 0.001	0.03	0.04	< 0.001	0.13	0.17	< 0.001

B unstandardized coefficient
β standardized coefficient
N/A Not applicable
RBC Red blood cell

Goodness of fit indices for model A

Fit statistic	Value
Likelihood ratio	
χ^2 model vs. saturated, 0 df	Not applicable
χ^2 baseline vs. saturated, 3 df	< 0.001
Root mean squared error of approximation	0.00
90% confidence interval, lower bound	0.00
90% confidence interval, upper bound	0.00
Comparative fit index	1.00
Tucker-Lewis index	1.00
Standardized root mean squared residual	0.00
Coefficient of determination	0.15

df Degrees of freedom
χ^2 Chi-square test

Results for SEM models: Model B

Direct, indirect and total effects of variables in model B

	Direct effects			*Indirect effects*			*Total effects*		
Variables	B	β	P-value	B	β	P-value	B	β	P-value
Operative blood loss									
Anaemia	0.51	0.04	< 0.001	N/A	N/A	N/A	0.51	0.04	< 0.001
RBC transfusion									
Anaemia	0.46	0.37	< 0.001	0.01	0.01	< 0.001	0.46	0.37	< 0.001
Operative blood loss	0.15	0.16	< 0.001	N/A	N/A	N/A	0.02	0.16	< 0.001
Long-term mortality									
RBC transfusion	0.05	0.08	< 0.001	N/A	N/A	N/A	0.05	0.08	< 0.001
Anaemia	0.10	0.14	< 0.001	0.02	0.03	< 0.001	0.13	0.17	< 0.001
Operative blood loss	0.004	0.07	< 0.001	0.001	0.01	< 0.001	0.005	0.08	< 0.001

B unstandardized coefficient
β standardized coefficient
N/A Not applicable
RBC Red blood cell

Goodness of fit indices for model B

Fit statistic	Value
Likelihood ratio	
χ^2 model vs. saturated, 0 df	Not applicable
χ^2 baseline vs. saturated, 6 df	< 0.001
Root mean squared error of approximation	0.00
90% confidence interval, lower bound	0.00
90% confidence interval, upper bound	0.00
Comparative fit index	1.00
Tucker-Lewis index	1.00
Standardized root mean squared residual	0.00
Coefficient of determination	0.16

df Degrees of freedom
χ^2 Chi-square test

Results for SEM models: Model C – complete model

Direct, indirect and total effects of variables in model C

	Direct effects			*Indirect effects*			*Total effects*		
Variables	B	β	P-value	B	β	P-value	B	β	P-value
Anaemia									
Age	0.01	0.15	< 0.001	N/A	N/A	N/A	0.01	0.15	< 0.001
Sex (female)	0.04	0.04	< 0.001	N/A	N/A	N/A	0.04	0.04	< 0.001
BMI	- 0.01	- 0.13	< 0.001	N/A	N/A	N/A	- 0.01	- 0.13	< 0.001
Diabetes	0.13	0.12	< 0.001	N/A	N/A	N/A	0.13	0.12	< 0.001
Pulmonary disease	0.06	0.06	< 0.001	N/A	N/A	N/A	0.06	0.06	< 0.001
Creatinine	0.001	0.18	< 0.001	N/A	N/A	N/A	0.001	0.18	< 0.001
Smoker	0.01	0.01	0.32	N/A	N/A	N/A	0.01	0.01	0.32
Operative blood loss									
Anaemia	0.18	0.014	0.18	N/A	N/A	N/A	0.18	0.01	0.18
Age	0.005	0.01	0.31	0.001	0.002	0.18	0.01	0.01	0.22
Sex (female)	0.04	0.003	0.76	0.01	0.001	0.20	0.04	0.004	0.72
BMI	0.04	0.03	0.001	- 0.002	- 0.002	0.18	0.04	0.03	0.001
Diabetes	- 0.22	- 0.02	0.13	0.02	0.002	0.18	< 0.20	< 0.01	0.17
Pulmonary disease	0.19	0.01	0.17	0.01	0.001	0.19	0.21	0.01	0.15
Creatinine	0.004	0.04	< 0.001	< 0.001	0.002	0.18	0.005	0.05	< 0.001
Smoker	N/A	N/A	N/A	< 0.001	< 0.001	0.42	0.001	< 0.001	0.42
CPB time	0.05	0.37	< 0.001	N/A	N/A	N/A	0.05	0.37	< 0.001
EuroSCORE II*	- 0.01	- 0.01	< 0.001	N/A	N/A	N/A	- 0.40	- 0.07	< 0.001
RBC transfusion									
Anaemia	0.35	0.29	< 0.001	0.01	0.01	0.18	0.36	0.29	< 0.001
Operative blood loss	0.01	0.09	< 0.001	N/A	N/A	N/A	0.01	0.09	< 0.001
Age	0.01	0.13	< 0.001	0.002	0.04	< 0.001	0.01	0.17	< 0.001
Sex (female)	0.32	0.28	< 0.001	0.01	0.01	< 0.001	0.34	0.30	< 0.001
BMI	- 0.01	- 0.12	< 0.001	- 0.004	- 0.03	< 0.001	- 0.02	- 0.15	< 0.001

Diabetes	0.04	0.03	< 0.001	0.05	0.03	< 0.001	0.09	0.06	< 0.001
Pulmonary disease	0.04	0.03	0.001	0.02	0.02	< 0.001	0.06	0.04	< 0.001
Creatinine	0.001	0.06	< 0.001	0.001	0.05	< 0.001	0.001	0.11	< 0.001
Smoker	N/A	N/A	N/A	0.003	0.003	0.32	0.003	0.003	0.32
CPB time	0.003	0.20	< 0.001	< 0.001	0.03	< 0.001	0.003	0.23	< 0.001
EuroSCORE II*	0.02	0.04	0.003	- 0.003	- 0.006	< 0.001	0.02	0.03	0.015
Long-term mortality									
RBC transfusion	0.02	0.03	0.012	N/A	N/A	N/A	0.02	0.03	0.012
Anaemia	0.07	0.09	< 0.001	0.01	0.01	0.007	0.08	0.10	< 0.001
Operative blood loss	0.003	0.06	< 0.001	< 0.001	0.003	0.015	0.003	0.06	< 0.001
Age	0.004	0.13	< 0.001	0.001	0.02	< 0.001	0.004	0.15	< 0.001
Sex (female)	- 0.01	- 0.02	0.084	0.01	0.01	< 0.001	- 0.004	- 0.01	0.58
BMI	- 0.003	- 0.04	< 0.001	- 0.001	- 0.01	< 0.001	- 0.004	- 0.06	< 0.001
Diabetes	0.04	0.05	0.01	0.01	0.01	< 0.001	0.05	0.06	< 0.001
Pulmonary disease	0.08	0.10	< 0.001	0.01	0.01	< 0.001	0.09	0.11	< 0.001
Creatinine	0.001	0.10	< 0.001	< 0.001	0.02	< 0.001	0.001	0.12	< 0.001
Smoker	0.02	0.04	< 0.001	0.001	0.001	0.32	0.02	0.04	< 0.001
CPB time	< 0.001	0.01	0.41	< 0.001	0.03	< 0.001	< 0.001	0.04	0.005
EuroSCORE II*	0.02	0.05	0.001	- 0.001	- 0.003	0.019	0.01	0.04	0.003

B unstandardized coefficient
β standardized coefficient
BMI Body mass index
CPB Cardiopulmonary bypass
N/A Not applicable
RBC Red blood cell
* EuroSCORE II operation category: Isolated coronary artery bypass (CABG), non-CABG, 2 procedures or ≥ 3 procedures

Goodness of fit indices for model C

Fit statistic	Value
Likelihood ratio	
χ^2 model vs. saturated, 4 df	< 0.001
χ^2 baseline vs. saturated, 42 df	< 0.001
Root mean squared error of approximation	0.03
90% confidence interval, lower bound	0.02
90% confidence interval, upper bound	0.04
Comparative fit index	1.00
Tucker-Lewis index	0.94
Standardized root mean squared residual	0.01
Coefficient of determination	0.40

df Degrees of freedom
χ^2 Chi-square test

Results for SEM models: Model D – observation time between 30 days and 1 year postoperatively

Direct, indirect and total effects of variables in model D

	Direct effects			*Indirect effects*			*Total effects*		
Variables	B	β	P-value	B	β	P-value	B	β	P-value
Anaemia									
Age	0.01	0.15	< 0.001	N/A	N/A	N/A	0.01	0.15	< 0.001
Sex (female)	0.04	0.04	< 0.001	N/A	N/A	N/A	0.04	0.04	< 0.001
BMI	- 0.01	- 0.13	< 0.001	N/A	N/A	N/A	- 0.01	- 0.13	< 0.001
Diabetes	0.13	0.12	< 0.001	N/A	N/A	N/A	0.13	0.12	< 0.001
Pulmonary disease	0.06	0.06	< 0.001	N/A	N/A	N/A	0.06	0.06	< 0.001
Creatinine	0.001	0.18	< 0.001	N/A	N/A	N/A	0.001	0.18	< 0.001
Smoker	0.01	0.01	0.32	N/A	N/A	N/A	0.01	0.01	0.32
Operative blood loss									
Anaemia	0.18	0.01	0.18	N/A	N/A	N/A	0.18	0.01	0.18
Age	0.005	0.01	0.31	0.001	0.002	0.18	0.01	0.01	0.22
Sex (female)	0.04	0.003	0.76	0.01	0.001	0.20	0.04	0.004	0.72
BMI	0.04	0.03	0.001	- 0.002	- 0.002	0.18	0.04	0.03	0.001
Diabetes	- 0.22	- 0.02	0.13	0.02	0.002	0.18	- 0.20	- 0.01	0.17
Pulmonary disease	0.19	0.01	0.17	0.01	0.001	0.19	0.21	0.01	0.15
Creatinine	0.004	0.04	< 0.001	< 0.001	0.002	0.18	0.005	0.05	< 0.001
Smoker	N/A	N/A	N/A	0.001	< 0.001	0.42	0.001	< 0.001	0.42
CPB time	0.05	0.37	< 0.001	N/A	N/A	N/A	0.05	0.37	< 0.001
EuroSCORE II*	- 0.40	- 0.07	< 0.001	N/A	N/A	N/A	- 0.40	- 0.07	< 0.001
RBC transfusion									
Anaemia	0.35	0.29	< 0.001	0.001	0.001	0.18	0.36	0.29	< 0.001
Operative blood loss	0.01	0.09	< 0.001	N/A	N/A	N/A	0.01	0.09	< 0.001
Age	0.01	0.13	< 0.001	0.002	0.04	< 0.001	0.01	0.17	< 0.001
Sex (female)	0.32	0.28	< 0.001	0.01	0.01	< 0.001	0.34	0.30	< 0.001
BMI	- 0.01	- 0.12	< 0.001	- 0.004	- 0.03	< 0.001	- 0.02	- 0.15	< 0.001

Diabetes	0.04	0.03	< 0.001	0.05	0.03	< 0.001	0.09	0.06	< 0.001
Pulmonary disease	0.04	0.03	0.001	0.02	0.02	< 0.001	0.06	0.04	< 0.001
Creatinine	0.001	0.06	< 0.001	0.001	0.05	< 0.001	0.001	0.11	< 0.001
Smoker	N/A	N/A	N/A	0.003	0.003	0.32	0.003	0.003	0.32
CPB time	0.003	0.20	< 0.001	< 0.001	0.03	< 0.001	0.003	0.23	< 0.001
EuroSCORE II*	0.02	0.04	0.003	- 0.003	- 0.006	< 0.001	0.02	0.03	0.015
Long-term mortality									
RBC transfusion	0.01	0.02	0.10	N/A	N/A	N/A	0.01	0.02	0.10
Anaemia	0.02	0.05	< 0.001	0.002	0.01	0.067	0.02	0.06	< 0.001
Operative blood loss	0.002	0.06	< 0.001	< 0.001	0.002	0.11	0.02	0.06	< 0.001
Age	0.001	0.04	0.001	< 0.001	0.01	< 0.001	0.001	0.05	< 0.001
Sex (female)	0.004	0.01	0.30	0.003	0.01	0.022	0.01	0.02	0.061
BMI	- 0.001	- 0.03	0.005	< - 0.001	- 0.01	< 0.001	- 0.001	- 0.04	< 0.001
Diabetes	0.01	0.03	0.001	0.003	0.01	< 0.001	0.02	0.04	< 0.001
Pulmonary disease	0.03	0.08	< 0.001	0.002	0.005	< 0.001	0.03	0.08	< 0.001
Creatinine	< 0.001	0.07	< 0.001	< 0.001	0.01	< 0.001	< 0.001	0.09	< 0.001
Smoker	0.005	0.02	0.11	< 0.001	< 0.001	0.33	0.005	0.02	0.098
CPB time	< 0.001	0.05	0.002	< 0.001	0.03	< 0.001	< 0.001	0.07	< 0.001
EuroSCORE II*	< 0.001	0.002	0.87	- 0.001	- 0.003	0.007	< - 0.001	- 0.001	0.95

B unstandardized coefficient
β standardized coefficient
BMI Body mass index
CPB Cardiopulmonary bypass
N/A Not applicable
RBC Red blood cell
* EuroSCORE II operation category: Isolated coronary artery bypass (CABG), non-CABG, 2 procedures or ≥ 3 procedures

Goodness of fit indices for model D

Fit statistic	Value
Likelihood ratio	
χ^2 model vs. saturated, 4 df	< 0.001
χ^2 baseline vs. saturated, 42 df	< 0.001
Root mean squared error of approximation	0.03
90% confidence interval, lower bound	0.02
90% confidence interval, upper bound	0.04
Comparative fit index	0.99
Tucker-Lewis index	0.94
Standardized root mean squared residual	0.01
Coefficient of determination	0.38

df Degrees of freedom
χ^2 Chi-square test

Results for SEM models: Model E – Observation time between 1 and 5 years postoperatively, n = 9,127

Direct, indirect and total effects of variables in model E

	Direct effects			*Indirect effects*			*Total effects*		
Variables	B	β	P-value	B	β	P-value	B	β	P-value
Anaemia									
Age	0.01	0.15	< 0.001	N/A	N/A	N/A	0.01	0.15	< 0.001
Sex (female)	0.04	0.04	< 0.001	N/A	N/A	N/A	0.04	0.04	< 0.001
BMI	- 0.01	- 0.12	< 0.001	N/A	N/A	N/A	- 0.01	- 0.12	< 0.001
Diabetes	0.12	0.11	< 0.001	N/A	N/A	N/A	0.12	0.11	< 0.001
Pulmonary disease	0.05	0.05	< 0.001	N/A	N/A	N/A	0.05	0.05	< 0.001
Creatinine	0.001	0.17	< 0.001	N/A	N/A	N/A	0.001	0.17	< 0.001
Smoker	0.007	0.01	0.40	N/A	N/A	N/A	0.01	0.01	0.40
Operative blood loss									
Anaemia	0.14	0.01	0.27	N/A	N/A	N/A	0.14	0.01	0.27
Age	0.01	0.01	0.26	0.001	0.002	0.28	0.01	0.01	0.19
Sex (female)	0.04	0.004	0.73	0.01	< 0.001	0.29	0.04	0.004	0.70
BMI	0.05	0.04	< 0.001	- 0.002	- 0.001	0.28	0.05	0.04	< 0.001
Diabetes	- 0.21	- 0.02	0.13	0.02	0.001	0.28	- 0.19	- 0.001	0.17
Pulmonary disease	0.19	0.01	0.17	0.01	0.001	0.29	0.19	0.01	0.15
Creatinine	0.003	0.03	0.001	< 0.001	0.002	0.28	0.004	0.04	< 0.001
Smoker	N/A	N/A	N/A	0.001	< 0.001	0.51	0.001	< 0.001	0.51
CPB time	0.04	0.35	< 0.001	N/A	N/A	N/A	0.04	0.35	< 0.001
EuroSCORE II*	- 0.30	- 0.06	< 0.001	N/A	N/A	N/A	- 0.30	- 0.06	< 0.001
RBC transfusion									
Anaemia	0.36	0.29	< 0.001	0.001	0.001	0.28	0.36	0.29	< 0.001
Operative blood loss	0.10	0.09	< 0.001	N/A	N/A	N/A	0.01	0.09	< 0.001
Age	0.01	0.13	< 0.001	0.002	0.04	< 0.001	0.01	0.17	< 0.001
Sex (female)	0.33	0.29	< 0.001	0.01	0.01	< 0.001	0.34	0.30	< 0.001
BMI	- 0.01	- 0.12	< 0.001	- 0.004	- 0.03	< 0.001	- 0.02	- 0.15	< 0.001

Diabetes	0.04	0.03	0.001	0.04	0.03	< 0.001	0.08	0.06	< 0.001
Pulmonary disease	0.04	0.03	0.003	0.02	0.02	< 0.001	0.06	0.04	< 0.001
Creatinine	0.001	0.06	< 0.001	0.001	0.05	< 0.001	0.001	0.11	< 0.001
Smoker	N/A	N/A	N/A	0.002	0.002	0.40	0.002	0.002	0.40
CPB time	0.003	0.20	< 0.001	< 0.001	0.03	< 0.001	0.003	0.23	< 0.001
EuroSCORE II*	0.02	0.03	0.007	- 0.003	- 0.01	< 0.001	0.02	0.03	0.022
Long-term mortality									
RBC transfusion	0.01	0.02	0.061	N/A	N/A	N/A	0.01	0.02	0.061
Anaemia	0.06	0.08	< 0.001	0.005	0.01	0.046	0.06	0.09	< 0.001
Operative blood loss	0.002	0.04	< 0.001	0.001	0.002	0.065	0.002	0.04	< 0.001
Age	0.003	0.12	< 0.001	< 0.001	0.02	< 0.001	0.004	0.14	< 0.001
Sex (female)	- 0.02	- 0.03	0.023	0.01	0.01	0.005	- 0.01	- 0.01	0.16
BMI	- 0.002	- 0.04	0.001	- 0.001	- 0.01	< 0.001	- 0.003	- 0.05	< 0.001
Diabetes	0.03	0.04	< 0.001	0.01	0.01	< 0.001	0.04	0.05	< 0.001
Pulmonary disease	0.06	0.08	< 0.001	0.004	0.01	< 0.001	0.06	0.08	< 0.001
Creatinine	< 0.001	0.08	< 0.001	< 0.001	0.02	< 0.001	0.001	0.10	< 0.001
Smoker	0.02	0.04	0.001	< 0.001	0.001	0.40	0.02	0.04	< 0.001
CPB time	< - 0.001	- 0.007	0.66	< 0.001	0.02	< 0.001	< 0.001	0.01	0.37
EuroSCORE II*	0.02	0.05	0.001	< - 0.001	- 0.002	0.11	0.01	0.05	0.001

B unstandardized coefficient
β standardized coefficient
BMI Body mass index
CPB Cardiopulmonary bypass
N/A Not applicable
RBC Red blood cell
* EuroSCORE II operation category: Isolated coronary artery bypass (CABG), non-CABG, 2 procedures or ≥ 3 procedures

Goodness of fit indices for model E

Fit statistic	Value
Likelihood ratio	
χ^2 model vs. saturated, 4 df	< 0.001
χ^2 baseline vs. saturated, 42 df	< 0.001
Root mean squared error of approximation	0.03
90% confidence interval, lower bound	0.02
90% confidence interval, upper bound	0.04
Comparative fit index	1.00
Tucker-Lewis index	0.95
Standardized root mean squared residual	0.01
Coefficient of determination	0.38

df Degrees of freedom
χ^2 Chi-square test

Results for SEM models: Model F – isolated coronary artery bypass grafting, n = 6,134

Direct, indirect and total effects of variables in model F

	Direct effects			*Indirect effects*			*Total effects*		
Variables	B	β	P-value	B	β	P-value	B	β	P-value
Anaemia									
Age	0.06	0.16	< 0.001	N/A	N/A	N/A	0.01	0.16	< 0.001
Sex (female)	0.05	0.05	< 0.001	N/A	N/A	N/A	0.05	0.05	< 0.001
BMI	- 0.01	- 0.11	< 0.001	N/A	N/A	N/A	- 0.01	- 0.11	< 0.001
Diabetes	0.13	0.13	< 0.001	N/A	N/A	N/A	0.13	0.13	< 0.001
Pulmonary disease	0.05	0.04	< 0.001	N/A	N/A	N/A	0.05	0.04	< 0.001
Creatinine	0.001	0.19	< 0.001	N/A	N/A	N/A	0.001	0.19	< 0.001
Smoker	0.03	0.04	0.003	N/A	N/A	N/A	0.03	0.04	0.003
Operative blood loss									
Anaemia	0.12	0.02	0.053	N/A	N/A	N/A	0.12	0.02	0.053
Age	0.002	0.01	0.34	0.001	0.004	0.056	0.003	0.16	0.20
Sex (female)	0.15	0.03	0.012	0.01	0.001	0.085	0.15	0.03	0.009
BMI	0.04	0.08	< 0.001	- 0.001	- 0.003	0.060	0.04	0.08	< 0.001
Diabetes	0.05	0.01	0.45	0.02	0.003	0.058	0.06	0.01	0.31
Pulmonary disease	0.03	0.005	0.70	0.006	0.001	0.090	0.03	0.01	0.64
Creatinine	0.03	0.09	< 0.001	< 0.001	0.01	0.055	0.04	0.10	< 0.001
Smoker	N/A	N/A	N/A	0.003	0.001	0.10	0.003	0.001	0.10
CPB time	0.03	0.32	< 0.001	N/A	N/A	N/A	0.03	0.32	< 0.001
RBC transfusion									
Anaemia	0.39	0.31	< 0.001	0.004	0.004	0.056	0.39	0.31	< 0.001
Operative blood loss	0.37	0.15	< 0.001	N/A	N/A	N/A	0.37	0.15	< 0.001
Age	0.005	0.10	< 0.001	0.003	0.05	< 0.001	0.01	0.15	< 0.001
Sex (female)	0.36	0.30	< 0.001	0.02	0.02	< 0.001	0.38	0.32	< 0.001
BMI	- 0.02	- 0.13	< 0.001	- 0.003	- 0.02	< 0.001	- 0.02	- 0.15	< 0.001
Diabetes	0.03	0.02	0.036	0.05	0.04	< 0.001	0.08	0.06	< 0.001

Pulmonary disease	0.06	0.04	< 0.001	0.02	0.01	0.001	0.08	0.05	< 0.001
Creatinine	0.001	0.06	< 0.001	< 0.001	0.07	< 0.001	0.001	0.13	< 0.001
Smoker	N/A	N/A	N/A	0.01	0.05	0.003	0.01	0.01	0.003
CPB time	0.003	0.12	< 0.001	0.001	0.05	< 0.001	0.004	0.17	< 0.001
Long-term mortality									
RBC transfusion	0.02	0.03	0.026	N/A	N/A	N/A	0.02	0.03	0.026
Anaemia	0.06	0.09	< 0.001	0.01	0.01	0.016	0.07	0.10	< 0.001
Operative blood loss	0.01	0.04	0.01	0.001	0.005	0.028	0.01	0.04	0.002
Age	0.003	0.12	< 0.001	0.001	0.02	< 0.001	0.004	0.14	< 0.001
Sex (female)	- 0.01	- 0.02	0.13	0.01	0.02	0.001	- 0.003	- 0.005	0.70
BMI	- 0.001	- 0.02	0.25	- 0.001	- 0.01	< 0.001	- 0.002	- 0.03	0.038
Diabetes	0.04	0.06	< 0.001	0.01	0.01	< 0.001	0.05	0.07	< 0.001
Pulmonary disease	0.07	0.08	< 0.001	0.005	0.01	< 0.001	0.08	0.09	< 0.001
Creatinine	< 0.001	0.09	< 0.001	< 0.001	0.02	< 0.001	0.001	0.11	< 0.001
Smoker	0.02	0.03	0.019	0.002	0.004	0.005	0.02	0.03	0.009
CPB time	< - 0.001	- 0.01	0.66	< 0.001	0.02	< 0.001	< 0.001	0.01	0.37

B unstandardized coefficient
β standardized coefficient
BMI Body mass index
CPB Cardiopulmonary bypass
N/A Not applicable
RBC Red blood cell

Goodness of fit indices for model F

Fit statistic	Value
Likelihood ratio	
χ^2 model vs. saturated, 3 df	0.57
χ^2 baseline vs. saturated, 38 df	< 0.001
Root mean squared error of approximation	0.00
90% confidence interval, lower bound	0.00
90% confidence interval, upper bound	0.20
Comparative fit index	1.00
Tucker-Lewis index	1.00
Standardized root mean squared residual	< 0.01
Coefficient of determination	0.36

df Degrees of freedom
χ^2 Chi-square test

Results for SEM models: Model G – 2 or more surgical procedures, n = 1,952

Direct, indirect and total effects of variables in model G

	Direct effects			*Indirect effects*			*Total effects*		
Variables	B	β	P-value	B	β	P-value	B	β	P-value
Anaemia									
Age	0.01	0.15	< 0.001	N/A	N/A	N/A	0.01	0.15	< 0.001
Sex (female)	< - 0.001	< - 0.001	0.98	N/A	N/A	N/A	< - 0.001	< - 0.001	0.98
BMI	- 0.01	- 0.10	< 0.001	N/A	N/A	N/A	- 0.01	- 0.10	< 0.001
Diabetes	0.13	0.10	< 0.001	N/A	N/A	N/A	0.13	0.10	< 0.001
Pulmonary disease	0.06	0.06	0.011	N/A	N/A	N/A	0.06	0.06	0.011
Creatinine	0.001	0.15	< 0.001	N/A	N/A	N/A	0.001	0.15	< 0.001
Smoker	0.002	0.002	0.92	N/A	N/A	N/A	0.002	0.002	0.92
Operative blood loss									
Anaemia	0.55	0.04	0.082	N/A	N/A	N/A	0.55	0.04	0.082
Age	0.01	0.01	0.62	0.003	0.01	0.092	0.01	0.02	0.44
Sex (female)	0.20	0.01	0.50	< - 0.001	< 0.001	0.98	0.20	0.01	0.50
BMI	0.06	0.04	0.082	- 0.01	- 0.004	0.10	0.05	0.03	0.12
Diabetes	- 0.40	- 0.02	0.31	0.07	0.004	0.10	- 0.33	- 0.02	0.40
Pulmonary disease	0.25	0.016	0.44	0.03	0.002	0.15	0.28	0.02	0.38
Creatinine	0.004	0.04	0.088	0.001	0.01	0.092	0.01	0.04	0.046
Smoker	N/A	N/A	N/A	0.001	< 0.0001	0.92	0.001	< 0.001	0.92
CPB time	0.06	0.39	< 0.001	N/A	N/A	N/A	0.006	0.39	< 0.001
RBC transfusion									
Anaemia	0.28	0.27	< 0.001	0.004	0.004	0.10	0.29	0.27	< 0.001
Operative blood loss	0.007	0.10	< 0.001	N/A	N/A	N/A	0.01	0.10	< 0.001
Age	0.007	0.18	< 0.001	0.002	0.04	< 0.001	0.01	0.22	< 0.001
Sex (female)	0.29	0.29	< 0.001	0.001	0.001	0.84	0.29	0.29	< 0.001
BMI	- 0.01	- 0.10	< 0.001	- 0.003	- 0.02	< 0.001	- 0.01	- 0.12	< 0.001
Diabetes	0.04	0.03	0.12	0.03	0.02	< 0.001	0.08	0.06	0.007

Pulmonary disease	- 0.01	- 0.01	0.73	0.02	0.02	0.009	0.01	0.01	0.62
Creatinine	< 0.001	0.05	0.021	< 0.001	0.05	< 0.001	0.001	0.09	< 0.001
Smoker	N/A	N/A	N/A	0.001	0.001	0.92	0.001	0.001	0.92
CPB time	0.002	0.16	< 0.001	< 0.001	0.04	< 0.001	0.002	0.20	< 0.001
Long-term mortality									
RBC transfusion	0.02	0.02	0.44	N/A	N/A	N/A	0.02	0.02	0.44
Anaemia	0.09	0.11	< 0.001	0.01	0.01	0.34	0.09	0.11	< 0.001
Operative blood loss	0.002	0.03	0.14	< 0.001	0.002	0.45	0.002	0.04	0.12
Age	0.005	0.15	< 0.001	0.001	0.02	0.001	0.01	0.17	< 0.001
Sex (female)	- 0.003	- 0.003	0.88	0.005	0.01	0.44	0.002	0.003	0.91
BMI	- 0.01	- 0.10	< 0.001	- 0.001	- 0.01	0.006	- 0.01	- 0.12	< 0.001
Diabetes	0.04	0.03	0.12	0.01	0.01	0.002	0.05	0.05	0.039
Pulmonary disease	0.08	0.10	< 0.001	0.01	0.01	0.022	0.09	0.11	< 0.001
Creatinine	0.001	0.12	< 0.001	< 0.001	0.02	< 0.001	0.001	0.14	< 0.001
Smoker	0.05	0.06	0.005	< 0.001	< 0.001	0.92	0.05	0.05	0.005
CPB time	< 0.001	0.03	0.15	< 0.001	0.02	0.081	< 0.001	0.05	0.018

B unstandardized coefficient
β standardized coefficient
BMI Body mass index
CPB Cardiopulmonary bypass
N/A Not applicable
RBC Red blood cell

Goodness of fit indices for model G

Fit statistic	Value
Likelihood ratio	
χ^2 model vs. saturated, 3 df	0.34
χ^2 baseline vs. saturated, 38 df	< 0.001
Root mean squared error of approximation	0.00
90% confidence interval, lower bound	0.00
90% confidence interval, upper bound	0.04
Comparative fit index	1.00
Tucker-Lewis index	1.00
Standardized root mean squared residual	< 0.01
Coefficient of determination	0.40

df Degrees of freedom
χ^2 Chi-square test

Results for SEM models: Model H – Transfusion of at least 2 units of RBC, n = 3,507

Direct, indirect and total effects of variables in model H

	Direct effects			*Indirect effects*			*Total effects*		
Variables	B	β	P-value	B	β	P-value	B	β	P-value
Anaemia									
Age	0.01	0.15	< 0.001	N/A	N/A	N/A	0.01	0.15	< 0.001
Sex (female)	0.06	0.06	< 0.001	N/A	N/A	N/A	0.06	0.06	< 0.001
BMI	- 0.01	- 0.13	< 0.001	N/A	N/A	N/A	- 0.01	- 0.13	< 0.001
Diabetes	0.12	0.11	< 0.001	N/A	N/A	N/A	0.12	0.11	< 0.001
Pulmonary disease	0.06	0.06	< 0.001	N/A	N/A	N/A	0.06	0.06	< 0.001
Creatinine	0.001	0.18	< 0.001	N/A	N/A	N/A	0.001	0.18	< 0.001
Smoker	0.002	0.003	0.80	N/A	N/A	N/A	0.002	0.003	0.80
Operative blood loss									
Anaemia	0.19	0.01	0.18	N/A	N/A	N/A	0.19	0.01	0.18
Age	0.005	0.01	0.37	0.001	0.002	0.18	0.01	0.01	0.26
Sex (female)	0.04	0.003	0.76	0.01	0.001	0.19	0.10	0.01	0.46
BMI	0.04	0.03	0.003	- 0.002	- 0.002	0.18	0.04	0.03	0.001
Diabetes	- 0.22	- 0.01	0.15	0.02	0.002	0.18	- 0.20	- 0.01	0.20
Pulmonary disease	0.25	0.02	0.10	0.01	0.001	0.19	0.26	0.02	0.086
Creatinine	0.005	0.04	< 0.001	< 0.001	0.002	0.18	0.005	0.05	< 0.001
Smoker	N/A	N/A	N/A	0.001	< 0.001	0.80	< 0.001	< 0.001	0.80
CPB time	0.1	0.37	< 0.001	N/A	N/A	N/A	0.05	0.37	< 0.001
EuroSCORE II*	- 0.41	- 0.07	< 0.001	N/A	N/A	N/A	- 0.41	- 0.07	< 0.001
RBC transfusion									
Anaemia	0.36	0.30	< 0.001	0.002	0.001	0.18	0.37	0.30	< 0.001
Operative blood loss	0.01	0.10	< 0.001	N/A	N/A	N/A	0.01	0.10	< 0.001
Age	0.01	0.12	< 0.001	0.002	0.05	< 0.001	0.01	0.17	< 0.001
Sex (female)	0.31	0.27	< 0.001	0.02	0.02	< 0.001	0.33	0.29	< 0.001
BMI	- 0.01	- 0.11	< 0.001	- 0.004	- 0.03	< 0.001	- 0.02	- 0.15	< 0.001

Diabetes	0.04	0.03	< 0.001	0.04	0.03	< 0.001	0.08	0.06	< 0.001
Pulmonary disease	0.03	0.02	0.01	0.03	0.02	< 0.001	0.06	0.04	< 0.001
Creatinine	0.001	0.06	< 0.001	0.001	0.06	< 0.001	0.001	0.12	< 0.001
Smoker	N/A	N/A	N/A	0.001	0.001	0.80	0.001	0.001	0.80
CPB time	0.003	0.21	< 0.001	< 0.001	0.04	< 0.001	0.003	0.24	< 0.001
EuroSCORE II*	0.02	0.04	0.002	- 0.004	- 0.007	< 0.001	0.02	0.03	0.009
Long-term mortality									
RBC transfusion	0.02	0.03	0.013	N/A	N/A	N/A	0.02	0.03	0.01
Anaemia	0.07	0.09	< 0.001	0.01	0.01	0.008	0.08	0.10	< 0.001
Operative blood loss	0.003	0.06	< 0.001	< 0.001	0.003	0.016	0.003	0.06	< 0.001
Age	0.004	0.12	< 0.001	0.001	0.02	< 0.001	0.004	0.14	< 0.001
Sex (female)	- 0.01	- 0.01	0.22	0.01	0.01	< 0.001	0.001	0.001	0.92
BMI	- 0.003	- 0.05	< 0.001	- 0.001	- 0.01	< 0.001	- 0.004	- 0.06	< 0.001
Diabetes	0.04	0.05	< 0.001	0.01	0.01	< 0.001	0.05	0.06	< 0.001
Pulmonary disease	0.08	0.10	< 0.001	0.01	0.01	< 0.001	0.09	0.10	< 0.001
Creatinine	0.001	0.10	< 0.001	< 0.001	0.02	< 0.001	0.001	0.12	< 0.001
Smoker	0.02	0.04	< 0.001	< 0.001	< 0.001	0.80	0.02	0.04	< 0.001
CPB time	< 0.001	0.01	0.41	< 0.001	0.03	< 0.001	< 0.001	0.04	< 0.001
EuroSCORE II*	0.02	0.04	0.001	- 0.001	- 0.003	0.025	0.02	0.05	< 0.001

B unstandardized coefficient
β standardized coefficient
BMI Body mass index
CPB Cardiopulmonary bypass
N/A Not applicable
RBC Red blood cell
* EuroSCORE II operation category: Isolated coronary artery bypass (CABG), non-CABG, 2 procedures or ≥ 3 procedures

Goodness of fit indices for model H

Fit statistic	Value
Likelihood ratio	
χ^2 model vs. saturated, 4 df	< 0.001
χ^2 baseline vs. saturated, 42 df	< 0.001
Root mean squared error of approximation	0.04
90% confidence interval, lower bound	0.03
90% confidence interval, upper bound	0.05
Comparative fit index	0.99
Tucker-Lewis index	0.92
Standardized root mean squared residual	0.01
Coefficient of determination	0.40

df Degrees of freedom
χ^2 Chi-square test

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