Tuomas Tauriainen

COMPLICATIONS ASSOCIATED WITH PREOPERATIVE ANEMIA, PERIOPERATIVE BLEEDING AND BLOOD TRANSFUSIONS AFTER ISOLATED CORONARY ARTERY BYPASS GRAFTING
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Academic Dissertation to be presented with the assent of the Doctoral Training Committee of Health and Biosciences of the University of Oulu for public defence in Auditorium 1 of Oulu University Hospital (Kajaanintie 50), on 26 May 2017, at 12 noon

UNIVERSITY OF OULU, OULU 2017
Cardiovascular diseases are the leading cause of death worldwide, and coronary artery disease accounts for the majority of them. The treatment of choice for complex coronary artery disease is coronary artery bypass grafting. However, as surgery in general, cardiac surgery is associated with an increased risk of perioperative bleeding and utilization of blood products.

The present study aimed to investigate the impact of preoperative anemia, perioperative bleeding and retained blood syndrome as well as blood transfusion on the outcomes after isolated coronary surgery. The severity of perioperative bleeding was assessed mainly using the E-CABG and UDPB stratification criteria.

Our analyses showed that severe bleeding is associated with a significantly increased risk of stroke. Furthermore, severe bleeding increased the risk of several adverse events even in low-risk patients. Retained blood syndrome was observed to be a common complication after coronary surgery and was associated with an increased risk of postoperative complications. Preoperative anemia seems to have no significant impact on patient early and late survival. Instead, the frequent exposure to blood products may be the determinant of poorer survival observed among anemic patients.

Perioperative blood loss and exposure to allogeneic blood has been shown to increase adverse events. Therefore, prevention of bleeding and measures to optimize patient blood management could improve patient outcomes after cardiac surgery.

*Keywords:* bleeding, bleeding severity, blood loss, blood transfusion, cardiac surgery, coronary artery bypass grafting, low-risk, outcome, preoperative anemia, red blood cell, retained blood, stroke
Tauriainen, Tuomas, Preoperatiiviseen anemiaan, perioperatiiviseen verenvuotoon ja verensiirtoihin liittyvät komplikaatiot sepelvaltimoiden ohitusleikkauskessa.

Oulun yliopiston tutkijakoulu; Oulun yliopisto, Lääketieteellinen tiedekunta; Oulun yliopistollinen sairaala

Acta Univ. Oul. D 1412, 2017

Oulun yliopisto, PL 8000, 90014 Oulun yliopisto

Tiivistelmä

Sydän ja verisuonitaudit ovat maailmanlaajuisesti yleisin kuoleman aiheuttaja, joista sepelvaltimoiden ohitusleikkaus on käyppä hoito vakavassa sepelvaltimotaudissa. Kuten kirurgiassa yleisestikin, erityisesti sydänkirurgia on yhdistetty suurentuneeseen verenheitoon ja veritapaturmien eikä liitokitse.

Tutkimukseni tavoitteena oli selvittää preoperatiivisen anemiaan, perioperatiivisen verenheitoon, verensiirtojen annon, sekä leikkausalueelle jääneen veren itsenäisiä vaikutuksia potilaiden lopputulemiin sepelvaltimoiden ohitusleikkauskseen. Veritapatureita ja perioperatiivisen verenheiton määärät arvioitiin pääasiallisesti käytävän E-CABG ja UDPB verenheitotapotiehiksi.


Perioperatiivisen verenheiton ja altistuimen veritapauksille on osoitettu lisäävän haittatahduksia. Siispä verenheiton vähentäminen ja veritapauksien säästämisen voisi parantaa potilaiden ennustetta sydänkirurgiassa.

Asiasanat: aivoinfarkti, leikkausalueelle jäänyt veri, matala leikkausriski, preoperatiivinen anemia, punasolu, sepelvaltimoiden ohitusleikkaus, sydänkirurgia, verensiirrot, verenheiton vakavuus, verenheitto
Acknowledgements

The present study was conducted at the Oulu University Hospital between the years 2015 and 2017.

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I appreciate the help and enjoyable company of Eeva-Maija Kinnunen, MD, PhD, and Joni Koski-Vähälä, MS, during the data acquisition process, and I especially value the advice given me by Eeva-Maija on doctoral training. You have been good friends.

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Finally, I wish to thank my parents for supporting me in my career as well as in my fast paced and sports oriented lifestyle. My life would not be the same without you.

5.4.2017

Tuomas Tauriainen, Oulu
List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
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<tbody>
<tr>
<td>ACT</td>
<td>Activated clotting time</td>
</tr>
<tr>
<td>ADP</td>
<td>Adenosine diphosphate</td>
</tr>
<tr>
<td>aPTT</td>
<td>Activated partial thromboplastin time</td>
</tr>
<tr>
<td>ASA</td>
<td>American Society of Anesthesiologists</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>CABG</td>
<td>Coronary artery bypass grafting</td>
</tr>
<tr>
<td>CAD</td>
<td>Coronary artery disease</td>
</tr>
<tr>
<td>CCS</td>
<td>Canadian Cardiovascular Society</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CPB</td>
<td>Cardiopulmonary bypass</td>
</tr>
<tr>
<td>EACA</td>
<td>Epsilon-aminocaproic acid</td>
</tr>
<tr>
<td>E-CABG</td>
<td>European registry of Coronary Artery Bypass Grafting</td>
</tr>
<tr>
<td>eGFR</td>
<td>Estimated glomerular filtration rate</td>
</tr>
<tr>
<td>EPO</td>
<td>Erythropoietin</td>
</tr>
<tr>
<td>FFP</td>
<td>Fresh frozen plasma</td>
</tr>
<tr>
<td>GPIIb/IIIa</td>
<td>Glycoprotein IIb/IIIa</td>
</tr>
<tr>
<td>HR</td>
<td>Hazard ratio</td>
</tr>
<tr>
<td>IABP</td>
<td>Intra-aortic balloon pump</td>
</tr>
<tr>
<td>ICU</td>
<td>Intensive care unit</td>
</tr>
<tr>
<td>IMA</td>
<td>Internal mammary artery</td>
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<tr>
<td>INR</td>
<td>International normalized ratio</td>
</tr>
<tr>
<td>LMWH</td>
<td>Low molecular weight heparin</td>
</tr>
<tr>
<td>LVEF</td>
<td>Left ventricular ejection fraction</td>
</tr>
<tr>
<td>MEA</td>
<td>Multiple electrode aggregometer</td>
</tr>
<tr>
<td>NOAC</td>
<td>Novel oral anticoagulant</td>
</tr>
<tr>
<td>NYHA</td>
<td>New York Heart Association</td>
</tr>
<tr>
<td>OASIS</td>
<td>Organization to Assess Strategies for Ischemic Syndromes</td>
</tr>
<tr>
<td>OPCAB</td>
<td>Off-pump coronary artery bypass</td>
</tr>
<tr>
<td>OR</td>
<td>Odds ratio</td>
</tr>
<tr>
<td>PAI-1</td>
<td>Plasminogen activator inhibitor-1</td>
</tr>
<tr>
<td>PCC</td>
<td>Prothrombin complex concentrate</td>
</tr>
<tr>
<td>PCI</td>
<td>Percutaneous coronary intervention</td>
</tr>
<tr>
<td>PLATO</td>
<td>PLATElet inhibition and patient Outcomes</td>
</tr>
<tr>
<td>POAF</td>
<td>Postoperative atrial fibrillation</td>
</tr>
<tr>
<td>PPS</td>
<td>Postpericardiotomy syndrome</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>PS</td>
<td>Phosphatidylserine</td>
</tr>
<tr>
<td>PT</td>
<td>Prothrombin time</td>
</tr>
<tr>
<td>RBC</td>
<td>Red blood cell</td>
</tr>
<tr>
<td>RBS</td>
<td>Retained blood syndrome</td>
</tr>
<tr>
<td>rFVIIa</td>
<td>Recombinant activated Factor VII</td>
</tr>
<tr>
<td>ROTEM</td>
<td>Rotational thromboelastometry</td>
</tr>
<tr>
<td>RR</td>
<td>Risk ratio</td>
</tr>
<tr>
<td>SCA</td>
<td>Society of Cardiovascular Anesthesiologists</td>
</tr>
<tr>
<td>SIRS</td>
<td>Systemic inflammatory response syndrome</td>
</tr>
<tr>
<td>STS</td>
<td>Society of Thoracic Surgeons</td>
</tr>
<tr>
<td>TACO</td>
<td>Transfusion-associated circulatory overload</td>
</tr>
<tr>
<td>TEG</td>
<td>Thromboelastography</td>
</tr>
<tr>
<td>TF</td>
<td>Tissue factor</td>
</tr>
<tr>
<td>TRALI</td>
<td>Transfusion related acute lung injury</td>
</tr>
<tr>
<td>TRIM</td>
<td>Transfusion related immune modulation</td>
</tr>
<tr>
<td>TXA</td>
<td>Tranexamic acid</td>
</tr>
<tr>
<td>UDPB</td>
<td>Universal Definition of Perioperative Bleeding</td>
</tr>
<tr>
<td>UH</td>
<td>Unfractionated heparin</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
</tr>
</tbody>
</table>
Original publications

The present dissertation is based on four studies that are referred to throughout the text with their Roman numerals.


Contribution of the author on the dissertation:

I Data acquisition, drafting the article, final approval of the version to be submitted.

II Data acquisition, final approval of the version to be submitted.

III Study plan, data acquisition, statistical analysis, drafting the article, final approval of the version to be submitted.

IV Study plan, data acquisition, statistical analysis, drafting the article, final approval of the version to be submitted.
## Contents

**Abstract**  
**Tiivistelmä**

**Acknowledgements**  
**List of abbreviations**  
**Original publications**  
**Contents**  

1 **Introduction**  

2 **Review of the literature**

2.1 Perioperative bleeding in adult cardiac surgery  
2.1.1 Characteristics of perioperative bleeding  
2.1.2 Retained blood syndrome  
2.1.3 Resternotomy for bleeding  
2.1.4 Risk factors for perioperative blood loss  

2.2 Red blood cells and coagulation  

2.3 Transfusion of allogeneic blood products  
2.3.1 Transfusion of red blood cells  
2.3.2 Transfusion of platelets  
2.3.3 Transfusion of plasma and plasma products  

2.4 General adverse events related to transfusion of allogeneic blood products  
2.4.1 Transfusion, mortality and morbidity in cardiac surgery  
2.4.2 Liberal versus restrictive transfusion policies  

2.5 Costs of blood product transfusions  

2.6 Outcomes associated with preoperative anemia in cardiac surgery  
2.6.1 Intraoperative anemia  

2.7 Optimization of patient blood management  
2.7.1 Preoperative factors  
2.7.2 Intraoperative factors  
2.7.3 Postoperative factors  

2.8 Summary of the literature  

3 **Aims of the research**  

4 **Materials and methods**

4.1 Study designs and patient populations  
4.2 Data collection
1 Introduction

Cardiovascular diseases are the leading cause of death worldwide and account for up to 31% of overall deaths. Coronary artery disease (CAD) causes 42% of cardiovascular deaths. (http://www.who.int/mediacentre/factsheets/fs317/en/, accessed 24th of Dec, 2016). Patients with CAD have narrowed or occluded arteries supplying the myocardium, which induces the classic symptoms of exercise intolerance, squeezing chest pain and shortness of breath. The treatment of choice for CAD, apart from best medical management, is coronary revascularization with percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CABG). The choice between invasive treatments depends on the coronary anatomy and the location of the stenoses. Guidelines recommend surgery to improve survival of patients with a significant left-main disease (>50% stenosis) and/or triple/double vessel disease (>50% stenosis) with impaired left ventricular function or in the presence of proximal left anterior descending stenosis >50%. Bypass surgery is also beneficial to relieve unacceptable symptoms in patients with 1 or more coronary artery stenosis >70% amenable to revascularization. (Kohl et al. 2014).

Patients undergoing cardiac surgery are exposed to several factors affecting their outcomes, some of the most significant of which include preoperative anemia, bleeding and transfusion of blood products (Ranucci et al. 2013). The prevalence of preoperative anemia among patients undergoing cardiac surgery is rather high, ranging between 24% and 54% (Hung et al. 2011 & Miceli et al. 2014). Recent studies suggested that preoperative anemia is associated with adverse outcomes in patients undergoing cardiac surgery (Hung et al. 2011, Karkouti et al. 2008, Kulier et al. 2007, Ranucci et al. 2012).

A substantial proportion of patients undergoing CABG is exposed to significant perioperative bleeding (Dyke et al. 2014), and up to 92% of them have been observed to require allogeneic blood products during or after surgery (Stover et al. 1998). Some of the most important factors associated with an increased risk of perioperative bleeding are the use of potent anticoagulant or antiplatelet drugs before surgery (Davidson 2014), female gender (Biancari et al. 2016 & Ahmed et al. 2013), preoperative anemia (Biancari et al. 2016, Magovern et al. 1996, Mehran et al. 2010), low body mass index (BMI) (Carroll et al. 2006 & Dixon et al. 2014), critical preoperative state (Society of Thoracic Surgeons blood conservation guidelines, 2007), urgency of the operation (Santarpino et al. 2015), decreased renal function (Biancari et al. 2016, Litmathe et al. 2003, Magovern et al. 1996)

Numerous risk factors for perioperative bleeding, a growing shortage of blood products and the possible harm associated with RBC administration, has led to the development of methods aiming to reduce bleeding and transfusions and of guidelines for optimization of blood conservation (Society of Thoracic Surgeons blood conservation clinical practice guidelines, 2007 & 2011). Indeed, utilization of a blood conservation program has been reported to significantly improve patient outcomes and reduce hospital costs after cardiac surgery (LaPar et al. 2013).

Since blood loss is ultimately treated with RBCs, the individual effects of preoperative anemia, perioperative bleeding and transfusion of blood products are difficult to disentangle. This dissertation aimed to investigate the individual impact of these risk factors on patient outcomes after isolated CABG. Furthermore, we sought to clarify the incidence and impact of retained blood syndrome (RBS) on postoperative adverse events (Boyle et al. 2015)
2 Review of the literature

2.1 Perioperative bleeding in adult cardiac surgery

2.1.1 Characteristics of perioperative bleeding

Stratification of the severity of perioperative bleeding

Perioperative bleeding is a common and potentially life threatening complication. Although all patients undergoing cardiac surgery are exposed to a certain risk of perioperative hemorrhage, it is not clear at which point bleeding begins to have a clinically significant impact. Perioperative bleeding is important particularly in patients undergoing CABG because they are usually exposed pre- and postoperatively to antithrombotic and/or anticoagulant medication (Airaiksinen et al. 2011 & Purkayastha et al. 2006). Therefore, evaluation of the severity of perioperative bleeding is essential in order to provide a reliable stratification of the amount of bleeding and its clinical prognostic implications.

One of the limiting factors in the evaluation of the impact of bleeding on the outcome after cardiac surgery was the lack of a valid method to stratify its severity. Indeed, grading of the severity of bleeding has become an important outcome measure in many studies focusing on PCI (Ben-Gal et al. 2010, Becker et al. 2011, Chesebro et al. 1987, Cohen et al. 1997, Gallo et al. 2009, OASIS-2 investigators 1999, Yusuf et al. 2001). Only recently two classifications of the severity of perioperative bleeding in cardiac surgery have been proposed, the Universal Definition of Perioperative Bleeding (UDPB) and the E-CABG bleeding grades (Dyke et al. 2014 & Biancari et al. 2015).

Since it is difficult to quantify the absolute amount of blood loss during and after cardiac surgery, the severity of perioperative bleeding can be indirectly assessed using measures to control bleeding and improve oxygen delivery (Mariscalco et al. 2016). Indeed, the amount of blood product transfusions required, the use of procoagulants, delayed closure of the sternum, the amount of postoperative chest tube output and the need for re sternotomy have been used as indicators of hemorrhage (Dyke et al. 2014 & Biancari et al. 2015). However, transfusion of RBCs may be the easiest and most useful tool to evaluate perioperative bleeding, since blood loss can persist after the removal of chest drains.
Both the UDPB and E-CABG bleeding grades have been reported to be valuable tools in estimating the severity of perioperative bleeding (Kinnunen et al. 2014 & Mariscalco et al. 2016). The advantage of the E-CABG bleeding classification resides in its simplicity, which enables the use of the E-CABG grades also in retrospective studies. Although, the UDPB classification might be able to differentiate perioperative bleeding more accurately, it is much more complex. The UDPB classification requires information on several procoagulants and blood products, thus reducing its usability in clinical and/or in research environments. Table 1 summarizes current classifications of bleeding severity in patients undergoing PCI and cardiac surgery.

Most currently used bleeding classifications in the literature do not apply to patients undergoing cardiac surgery (Brascia et al. 2016). Indeed, the E-CABG and the UDPB bleeding grades are shown to be the most reliable predictors of adverse events when compared with several bleeding stratification methods designed for interventional cardiology. Interestingly, the E-CABG bleeding classification had the best predictive ability of early mortality and acute kidney injury after coronary surgery (Brascia et al. 2016). Due to the fact that the E-CABG and UDPB classifications are intended to be used in cardiac surgery, they can assess a larger variation of blood loss more reliably, while avoiding the limitations of bleeding grades designed for interventional cardiology.
<table>
<thead>
<tr>
<th>Class</th>
<th>UDPB Dyke et al. 2014</th>
<th>E-CABG Bianc ari et al. 2015</th>
<th>Plato Becker et al. 2011</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insignificant</td>
<td>Transfusion of 0 RBC units</td>
<td>Transfusion of 0-1 RBC units</td>
<td>Transfusion of 2-3 RBC units</td>
</tr>
<tr>
<td></td>
<td>12h chest tube output &lt;600ml</td>
<td></td>
<td>&gt;30 g/L drop in hemoglobin</td>
</tr>
<tr>
<td>Mild / (Minimal)</td>
<td>Transfusion of 1 RBC units</td>
<td>Transfusion of 2-4 RBC units</td>
<td>Medical intervention</td>
</tr>
<tr>
<td></td>
<td>12h chest tube output 601-800ml</td>
<td>Transfusion of platelets</td>
<td>Clinically significant disability</td>
</tr>
<tr>
<td>Moderate / (Minor)</td>
<td>Transfusion of 2-4 RBC units</td>
<td>Transfusion of 2-4 FFP units</td>
<td>Re-exploration/tamponade</td>
</tr>
<tr>
<td></td>
<td>Transfusion of platelets</td>
<td>Transfusion of platelets</td>
<td>Delayed sternal closure</td>
</tr>
<tr>
<td></td>
<td>Prothrombin complex concentrate use</td>
<td>Prothrombin complex concentrate use</td>
<td>12h chest tube output 801-1000ml</td>
</tr>
<tr>
<td></td>
<td>Transfusion of cryoprecipitate</td>
<td></td>
<td>Transfusion of 5-10 RBC units</td>
</tr>
<tr>
<td></td>
<td>12h chest tube output 801-1000ml</td>
<td></td>
<td>And/or re-exploration for bleeding</td>
</tr>
<tr>
<td></td>
<td>Transfusion of 5-10 FFP units</td>
<td></td>
<td>&gt;50 g/L drop in hemoglobin</td>
</tr>
<tr>
<td></td>
<td>Re-exploration/tamponade</td>
<td></td>
<td>Fatal bleeding</td>
</tr>
<tr>
<td></td>
<td>Delayed sternal closure</td>
<td></td>
<td>Intrapericardial bleeding/tamponade</td>
</tr>
<tr>
<td></td>
<td>12h chest tube output 1001-2000ml</td>
<td></td>
<td>Intracranial bleeding</td>
</tr>
<tr>
<td>Massive / (Major, life threatening)</td>
<td>Transfusion of &gt;10 RBC units</td>
<td>Transfusion of &gt;10 RBC units</td>
<td>Transfusion of &gt; 3 RBC units</td>
</tr>
<tr>
<td></td>
<td>Transfusion of &gt;10 FFP units</td>
<td></td>
<td>Fatal bleeding</td>
</tr>
<tr>
<td></td>
<td>rFVIIa use</td>
<td></td>
<td>Intrapericardial bleeding/tamponade</td>
</tr>
<tr>
<td></td>
<td>12h chest tube output &gt;2000ml</td>
<td></td>
<td>Intracranial bleeding</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Hypotension/inotrope use/surgery</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&gt;50 g/L drop in hemoglobin</td>
</tr>
</tbody>
</table>
Incidence of minor and severe perioperative bleeding

Severe perioperative bleeding and RBC transfusions have been associated with increased mortality (Karkouti et al. 2004) and morbidity in cardiac surgery (Shaw et al. 2013 & Scott et al. 2008). However, only a few studies on the incidence of perioperative bleeding have used a bleeding severity classification suitable for cardiac surgery. Furthermore, the majority of the studies mainly focused on severe bleeding (Ben-Gal et al. 2010, Karkouti et al. 2004, Ranucci et al. 2013). After all, a large number of patients suffer from moderate bleeding as well, which is significantly associated with several adverse events in patients undergoing cardiac operations (Mariscalco et al. 2016). Interestingly, blood loss even in hemodynamically stable patients has been shown to worsen outcomes (Magruder et al. 2016). Therefore blood conservation strategies could reduce the risk of undesirable events. According to Dyke et al. (2014), adjusted 30-day mortality was low in patients who experienced insignificant (0.8%), mild (1.7%) or moderate (3.7%) bleeding, whereas in severe-massive perioperative bleeding the 30-day mortality was 7.8%.

The incidence of massive bleeding after cardiac surgery as defined by the UDPB or E-CABG criteria is lower than 2% (Dyke et al. 2014, Magruder et al. 2016, Mariscalco et al. 2016), whereas the incidence of mild and moderate bleeding is more variable. Indeed, insignificant and/or mild bleeding can be observed in 40% to 65% of patients. (Dyke et al. 2014, Mariscalco et al. 2016, Tettey et al. 2009). Magruder et al. (2016) reported on patients whose chest tube output was under 50ml/h for each of three consecutive hours after the index operation. The incidence of these patients with minimal bleeding was 9.9%, which implies that a large proportion of patients can be operated on with reduced perioperative bleeding. The distribution of bleeding severity, with similar classification criteria to UDPB among patients undergoing cardiac surgery is presented in Table 2.
Table 2. Incidence of bleeding in patients undergoing cardiac surgery.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Insignificant</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Massive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mariscalco et al. 2016 (E-CABG)</td>
<td>44.5%</td>
<td>41.0%</td>
<td>11.8%</td>
<td>1.7%</td>
<td></td>
</tr>
<tr>
<td>Dyke et al. 2014 (UDPB)</td>
<td>51.4%</td>
<td>14.9%</td>
<td>24.0%</td>
<td>8.2%</td>
<td>1.6%</td>
</tr>
<tr>
<td>Ranucci et al. 2013 (12h chest tube output &gt;900ml/ reoperation)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11.3%</td>
</tr>
<tr>
<td>Karkouti et al. 2004 (Transfusion of &gt;5 RBC units)</td>
<td></td>
<td></td>
<td></td>
<td>10.9%</td>
<td></td>
</tr>
</tbody>
</table>

2.1.2 Retained blood syndrome

Excessive perioperative bleeding, with or without occluded or misplaced chest tubes can lead to accumulation of blood around the heart and lungs, which may generate a continuum of complications, which Boyle et al. (2015) named as retained blood syndrome. This condition has received increasing interest among researchers during the last few years. Indeed, recent evidence suggests that poor outcomes observed in patients with increased perioperative bleeding (Christensen et al. 2012), might in fact be caused by retained blood syndrome (Balzer et al. 2016).

Intrathoracic blood accumulation is common after cardiac surgery and the procedures for its removal are easy to define. Retained blood can be removed with a resternotomy/rethoracotomy, pericardial fenestration, thoracentesis or by inserting a pleural drainage tube. The aforementioned interventions in addition to surgery to relieve late fibrotic conditions such as constrictive pericarditis and fibrothorax, form the diagnostic criteria of RBS.

Retained blood syndrome has been proposed to have acute, sub-acute and chronic phases (Boyle et al. 2015). The acute phase of RBS consists of cardiac tamponade and/or hemothorax presenting during the first hours after the operation (Boyle et al. 2015) and is related to excessive early postoperative bleeding (Christensen et al. 2012). In the sub-acute phase, near the time of hospital discharge, the principal findings of RBS are bloody fluid collections in the pleura or the pericardium, which later may transform into inflammatory effusion. Constrictive pericarditis and fibrothorax characterize the chronic phase of RBS several months or years after the index operation. It has been suggested that the sub-acute and chronic manifestations are induced by persistent inflammation triggered by retained blood and subsequent coagulation reactions. (Boyle et al. 2015).

According to the most recent reports, the early outcomes of patients requiring procedures for retained blood removal are poor. Balzer et al. (2016) reported that
RBS in patients undergoing cardiac surgery increased significantly the risk of in-hospital mortality (Odds Ratio [OR] 4.04, 95% CI 2.59-6.35), intensive care unit (ICU) stay > five days (OR 4.60, 95% CI 3.45-6.18), prolonged mechanical ventilation time (OR 3.60, 95% CI 2.69-4.85), need for hemodialysis (OR 4.45, 95% CI 3.19-6.23) and in-hospital length of stay > 13 days (OR 3.85, 95% CI 2.88-5.21).

**Inflammatory mechanisms and retained blood syndrome**

Since the individual manifestations of RBS associated especially with the sub-acute and chronic phases have been studied and managed as separate entities, it is important to acknowledge their common cause; retained blood.

Coagulation and inflammation are shown to be strongly associated with each other, supporting the hypothesis behind RBS. One of the most important mediators of coagulation is tissue factor (TF), which is the main inductor of the extrinsic coagulation pathway and can be detected largely in the extravascular cells (Bach et al. 2006). The extrinsic pathway activates when TF comes into contact with blood, e.g. as a result of surgical trauma, infection, inflammation or chemical damage. Exposed TF binds with a free circulating factor VIIa, activating the coagulation cascade. Subsequently, activation of the coagulation cascade increases thrombin concentration in the blood, which induces platelet activation, fibrin formation and several pro-inflammatory events, e.g. recruitment and activation of white blood cells (especially monocytes, which have a particularly high expression of TF), complement activation and the release of cytokines and chemokines. The resulting pro-inflammatory state further up-regulates the expression of TF, which in turn enables generation of a positive feedback loop. (Foley et al. 2016). The on-going inflammation at the coagulation site increases local production of vascular endothelial growth factor (VEGF), which is the primary mediator of tissue permeability (Grove et al. 2002), i.e. it enables more coagulation factors to enter the extravascular space. Coagulation outside the vascular lumen, i.e. thrombin and fibrin generation, further strengthens the inflammatory state (Szaba et al. 2002).

**Post pericardiotomy syndrome and effusions of the chest**

There are several similarities between the symptoms of sub-acute RBS and the post pericardiotomy syndrome (PPS), which is usually characterized by pleural and/or pericardial effusions, pleuritic chest pain, friction rub and/or fever without signs of infection after cardiac surgery. The incidence of PPS varies according to different
diagnostic criteria, and ranges between 10% and 40%. The precise pathogenesis of PPS is poorly understood despite numerous proposed causes ranging from a latent viral infection to an autoimmune pathomechanism. (Imazio 2012). Mild forms of PPS are generally managed with systemic corticosteroids, however larger pericardial and pleural effusions require percutaneous drainage or surgical pericardial fenestration.

Mild pleural effusion may affect up to 91% of patients who underwent cardiac surgery (Landymore et al. 1990). The majority of these mild fluid collections subside with time (Lee et al. 2001 & Vargas et al. 1994), however reports addressing chronic and large effusions resistant to thoracentesis have been published (Charniot et al. 2007 & Lee et al. 2001). In the study by Lee et al. (2001), all patients requiring late thoracoscopic removal of persistent pleural effusions over two months after CABG, showed findings suggestive of an inflammatory process. Histologic examination of the pleural samples in the sub-acute effusions less than six months from the index operation demonstrated a dominance of inflammation with very little sign of fibrosis. Interestingly, chronic effusions were characterized by low inflammatory markers and considerably thickened and fibrotic pleura. Furthermore, fluid accumulations in the pleura shortly after cardiac surgery are shown to contain elevated levels of VEGF (Chibante et al. 2006), which suggest a potential explanation for persistent effusions.

Likewise, mild pericardial effusions are frequently observed after cardiac surgery (Pepi et al. 1994) and up to one half of them could substantially increase in volume during the first postoperative weeks (Ashikhmina et al. 2010). The incidence of significant pericardial effusion in the modern surgical era varies roughly between 1% (Ashikhmina et al. 2010 & Kuvin et al. 2002) and 8% (Lehto et al. 2015). Interestingly, the size and location of the effusions seem to be associated with the type of operation performed. Valve procedures are associated with more diffuse fluid accumulation in contrast with CABG (Pepi et al. 1994). According to Kuvin et al. (2002), the largest effusions were observed in patients undergoing CABG associated with valve operations. Importantly, the incidence of significant pericardial effusion can be significantly decreased using retrocardially placed drainage tubes (Eryilmaz et al. 2006) and/or posterior pericardiotomy (Biancari et al. 2010).

Up to 74% of patients with significant pericardial effusion can show evidence of cardiac tamponade, a life threatening complication requiring urgent evacuation. Indeed, close monitoring of patients discharged with mild pericardial effusions is
necessary, since tamponade can develop even long after the operation (Ashikhmina et al. 2010).

It can be speculated that pleural and pericardial effusions after cardiac surgery might be caused by congestive heart failure, but most early postoperative effusions have been reported to contain red blood cells (Light et al. 2002 & Sadikot et al. 2000). Moreover, postoperative pleural effusions share the characteristics of exudate fluid with a large amount of inflammatory mediators (Ashikhmina et al. 2010 & Sadikot et al. 2000), thus excluding the possibility of heart failure as a cause of these effusions. The pathogenesis of PPS is still unknown, although an immunological mechanism is suspected to be one of its key mediators. Interestingly, pleural and pericardial effusions have been speculated to be associated with PPS (Imazio 2012), which could be included as a part of the retained blood syndrome (Boyle et al. 2015).

**Atrial fibrillation and retained blood**

The most frequent complication after cardiac surgery is postoperative atrial fibrillation (POAF), which occurs in 20% to 40% of patients (LaPar et al. 2014 & Pivatto et al. 2014). It is known to cause a number of adverse events, such as hemodynamic instability, stroke, acute kidney injury and early mortality (Mariscalco et al. 2014, Lahtinen et al. 2004) along with increased hospital resource utilization (LaPar et al. 2014). Despite substantial research efforts, the pathogenesis of POAF is still unclear. Recently, an association between chest tube occlusion and increase in AF has been reported (Karimov et al. 2013). Subsequently, an improvement in chest tube drainage early after the operation has been shown to reduce the incidence of POAF over 50%. (Ege et al. 2004 & Eryilmaz et al. 2006). According to Biancari et al. (2010), similar results can be achieved with posterior pericardiotomy, which enables the pericardial fluid to drain into the left pleural space. The benefit of effective clearance on POAF risk might be due to reduced inflammatory response and compression of the heart.

**Chest tube drainage**

Externalization of blood from around the heart and lungs immediately after cardiac surgery is of main importance to avoid compression, which can be deleterious to the recovering patient. This is usually performed using large bore chest tubes, which also enables the assessment of on-going bleeding and the quality of


hemostasis. According to Halm et al. (2007), fluid drainage from the surgical space is most effective with a straight or coiled drainage tube. Avoidance of dependent loops, where the collection bag or suction device is positioned higher than any part of the tube, is advisable to improve outflow. Effective usage of pericardial and pleural drains after surgery is reported to mitigate the incidence of retained blood syndrome (Boyle et al. 2015).

The obvious limitation with conventional chest tubes is the fact that they can function only as long as their lumen remains open. Indeed, chest tube occlusion is a relatively common complication after cardiac operations. In a survey aimed at cardiac surgeons, 100% of the responders had observed clogged chest tubes during their career (Shalli et al. 2009). Up to 36% of pleural and/or pericardial tubes are reported to be occluded at some point after cardiac surgery. Chest tube occlusion has been associated with non-elective operation, increased blood product utilization, long aortic cross-clamp time and prolonged total operational time (Karimov et al. 2012). The aforementioned results suggest that chest tube occlusion might have an association with increased perioperative bleeding.

Regular inspection of chest tube function in the intensive care unit (ICU) is crucial to identify excessive blood loss, air leakage or clogging in a timely fashion. However, detecting a clog can be difficult since the occlusions are more frequently located in the intrathoracic part of the tube (Karimov et al. 2012).

Prevention of chest tube occlusion

In addition to reduced incidence of RBS, maintaining the patency of the chest tubes is a plausible method to improve patient outcomes, reduce hospital readmissions and unnecessary costs after cardiac surgery. Currently, approximately 20% of cardiac surgery patients require interventions for retained blood and its incidence is similar between all procedure types (Balzer et al. 2016 & Sirch et al. 2016).

Despite the rather high incidence of chest tube occlusion, clinical staff in cardiac surgery wards lack a standardized procedure of chest tube management (Boyle et al. 2015). Milking, stripping and tapping of the tubes can be attempted to clear occlusions (Day et al. 2008), but none of these commonly used methods of chest tube management is reported to be superior over the others. It has even been debated whether chest tube manipulation should be performed at all since it can be harmful. (Day et al. 2008 & Wallen et al. 2004). A negative intrathoracic pressure caused by chest tube stripping, i.e. squeezing and pulling the full length of the tube, might damage the underlying tissues entrapped at its orifice, subsequently causing
more bleeding. Chest tube stripping can generate negative pressures up to -400 cm H₂O compared to the commonly used, -20 cm H₂O suction from a chest drain device. (Halm et al. 2007). An expert opinion recommends gentle milking over the other methods to attempt clot dislodgement after its detection (Kirkwood 2002). Milking is usually considered to stand for squeezing, twisting or kneading the tube to generate short bursts of suction (Halm et al. 2007).

In the most extreme situations, removal of an occlusion can be attempted by inserting a small balloon catheter inside the chest tube, e.g. a Foley urinary catheter. The method in question requires proximal clamping of the tube to avoid formation of a pneumothorax. After disassembly of the suction system, the clamp is carefully opened to enable the catheter to pass through. The balloon is inflated and the occluding material is pulled through, as if performing an embolectomy. Even though this method provides the means to clear intrathoracic clogs, it increases the risk of damaging underlying structures, generating a pneumothorax and spreading infections. (Boyacioglu et al. 2014).

A promising new method to reduce chest tube clogging is active clearance devices. They can clean the total length of the tube safely and steriley, and are shown to improve fluid drainage (Arakawa et al. 2011). According to Sirch et al. (2016) the incidence of retained blood syndrome decreased by 43% after introduction of active clearance devices. Patients treated with an active clearance device required significantly fewer procedures to remove pericardial or pleural effusions. (Sirch et al. 2016).

2.1.3 Resternotomy for bleeding

Reoperation after cardiac surgery is mostly needed in the presence of excessive postoperative bleeding causing hemodynamic instability or cardiac tamponade and is performed on approximately 2% to 7% of patients in cardiac surgery (Kristensen et al. 2012 & Ranucci et al. 2008).

Patients undergoing surgical re-exploration are often morbid and considered to have a high operative risk (Ranucci et al. 2008). Increased age, low BMI, decreased renal function, non-elective surgery, operation other than isolated CABG, prolonged duration of CPB, the number of anastomoses and anticoagulant medication are the most frequently identified predictors of resternotomy for bleeding (Dacey et al. 1998, Karthik et al. 2004, Kristensen et al. 2012, Moulton et al. 1996, Vivacqua et al. 2011). Re-exploration early after cardiac surgery is a potentially life-saving procedure (Dimopoulou et al. 2001), but still is associated
with significant morbidity and mortality (Canadyova et al. 2012, Mehta et al. 2009, Sellman et al. 1997). Indications for re-exploration are shown in Table 3.

**Table 3. Criteria for re-exploration for bleeding by Kirklin and Barratt-Boyes.**

<table>
<thead>
<tr>
<th>Re-exploration criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Chest tube drainage of</td>
</tr>
<tr>
<td>More than 500ml during the first hour</td>
</tr>
<tr>
<td>More than 400ml during each of the first 2 hours</td>
</tr>
<tr>
<td>More than 300ml during each of the first 3 hours</td>
</tr>
<tr>
<td>More than 1000ml in total during the first 4 hours</td>
</tr>
<tr>
<td>More than 1200ml in total during the first 5 hours</td>
</tr>
<tr>
<td>2. Excessive bleeding that restarts</td>
</tr>
<tr>
<td>3. Sudden massive bleeding</td>
</tr>
</tbody>
</table>

**Antiplatelet/antifibrinolytic medication and re sternotomy**

The use of anticoagulant and antiplatelet medication is common especially in patients undergoing CABG. These drugs have been shown to increase the use of RBCs and other blood products (McDonald et al. 2005). However, only low molecular weight heparin (Jones et al. 2002 & McDonald et al. 2005) and potent antiplatelets (Biancari et al. 2012a) are associated with an increased incidence of re sternotomy. According to Jones et al. (2002), patients who received enoxaparin within 48 hours of the index operation had more than double the risk of re sternotomy for bleeding ([Hazard ratio] HR 2.6, 95% CI 1.1-5.9). Biancari and colleagues (2012a) reported similar results with clopidogrel exposure before the index operation ([Risk ratio] RR 1.88, 95% CI 1.37-2.58).

Two antifibrinolytic drugs, tranexamic acid and aprotinin, have been associated with a significant decrease in excessive bleeding and the rate of re sternotomy in cardiac surgery (Pagano et al. 2008 & Shi et al. 2013). However, the use of aprotinin was reported to be associated with increased mortality and morbidity after CABG (Schneeweiss et al. 2008), which led to its withdrawal from the market. Interestingly, the use of aprotinin in cardiac surgery has been recently reapproved due to significant errors in the initial analysis (The Pharmaceutical Journal, online, DOI: 10.1211/PJ.2013.11129008). The effect of antifibrinolytic drugs on perioperative bleeding is further discussed in the “Optimization of patient blood management” chapter.
**Individual surgeons’ impact on bleeding and resternotomy**

As in all fields of surgery, a junior cardiac surgeon must overcome the learning curve, during which the surgical performance is expected to improve over time. Indeed, as reported by Wolfe et al. (2007), a sudden increase in the number of re-explorations for bleeding was explained by employment of a new member of the surgical staff. The incidence of re-operations for bleeding later returned to previous levels. According to Biancari et al. (2012b), the resternotomy rate between individual surgeons ranged between 1.4% and 11.7%. The individual surgeon was an independent predictor of re-exploration for bleeding and excessive postoperative blood loss even in the absence of clopidogrel exposure. Dixon and colleagues (2014) reported similar results, where the individual surgeon was an independent predictor of the amount of chest tube drainage. This is, in turn, confirmed by the fact that bleeding originates very often from surgical sources such as the internal mammary artery harvesting site, side branches of the grafts and anastomosis among patients requiring re-exploration. These observations reinforce the utmost importance of a meticulous surgical technique as a means to reduce the excessive risk of adverse events associated with significant perioperative bleeding.

**Resternotomy and its associated adverse events**

Patients requiring re-exploration have an increased risk of adverse events. The rate of in-hospital mortality after reoperation for bleeding ranges between 6% and 38% (Canadyova et al. 2012 & Sellman et al. 1997) and the increase in the risk of mortality has been shown to persist even after adjustment for baseline comorbidities. (Mehta et al. 2009). The pooled adjusted risk of death is more than twice as high among patients undergoing re-exploration compared to controls (RR 2.56, 95%CI 1.46-4.50) (Biancari et al. 2012). Nevertheless, it is important to separate patients with stable and unstable indications for re-exploration, since “unplanned” interventions are associated with higher operative mortality (LaPar et al. 2014a). Only 14% of patients re-operated due to asystole survive until hospital discharge (Fiser et al. 2001). Interestingly, an underlying coagulopathic source of bleeding is reported to be associated with poorer outcomes after re-exploration for bleeding (Hall et al. 2001).

Despite the evidence of the prognostic importance of reoperation for bleeding, it is difficult to disentangle the independent effects of reoperation itself, bleeding and perioperative anemia, blood product transfusion and baseline risk profile on
postoperative mortality. Moulton et al. (1996) reported that the decision to re-operate had deleterious effects on outcomes in low risk patients, and in patients with mild postoperative bleeding. However, it is worth noting that patients requiring re-exploration generally receive more blood product transfusions and other hemostatic agents than conservatively treated patients (Unsworth-White et al. 1995), which are, in turn, significantly associated with increased adverse events after cardiac surgery (Engoren et al. 2002, Freeland et al. 2015, Jakobsen et al. 2012, Koch et al. 2006, Shaw et al. 2014, Surgenor et al. 2009, Whitson et al. 2010). Ranucci and coworkers (2008) reported that among patients exposed to resternotomy the increase in the risk of death was almost linearly associated with the amount of transfused RBC units.

Due to the fact that the bleeding rate can vary significantly between patients, the indications for re-exploration are frequently based on the volume of lost blood in a period of time. The outcomes of early resternotomy are shown to be better within the first 12 hours, after which the risk of adverse events is significantly increased. (Canadyova et al. 2012, Choong et al. 2007, Karthik et al. 2004, Ranucci et al. 2008). The risk of death is over five-fold higher in patients re-operated after the initial 12 hours (RR 5.22, 95% CI 2.43-11.21) (Biancari et al. 2012). Choong et al. (2007) speculated that the poorer outcomes associated with delayed resternotomy might be explained by longer ongoing hemodynamic instability, higher blood loss and increased blood product transfusions. It can be speculated, that resternotomy itself might be the source of adverse events in patients with low levels of bleeding. The combined effects of RBC transfusion and delayed re-exploration might account for the poorer outcomes observed in patients with severe bleeding.

Nevertheless, resternotomy has been associated with improved outcomes in bleeding patients who require more than 12 units of RBC transfusions (Ranucci et al. 2008). Indeed, patients with an uneventful recovery after re-exploration are shown to have similar long-term survival compared to patients without the need for re-operation (Biancari et al. 2012b). Interestingly, Mehta et al. (2009) reported that the incidence of death among patients requiring resternotomy decreased significantly during their study period of four years.

The need for resternotomy is associated with other adverse events such as prolonged intensive care unit (ICU) stay, mechanical ventilation time, stroke, renal failure, infections and prolonged in-hospital length of stay. (Biancari et al. 2012, Karthik et al 2004, Ranucci et al. 2008, Unsworth-White et al. 1995). Re-operation increases total hospitalization costs by approximately 6000 €. The extra costs are
mainly due to prolonged ICU stay (48%), increase in transfusions (20%) and the additional costs of anesthesia and surgery (31%). (Alström et al. 2011).

Resternotomy in the intensive care unit

Resternotomy can be performed also in the intensive care unit instead of the operating theatre. According to Kim et al. (2016), the patients re-operated in the ICU were more frequently in cardiac tamponade or cardiogenic shock and their bleeding originated from the heart. The total time from the decision to operate to readmission to the ICU is shorter among patients in the ICU group compared to resternotomy in the operating room. Postoperative blood loss, the amount of transfused blood products and mortality has been reported to be similar between both groups as well. (Kim et al. 2016).

Operating in the ICU raises questions about sterility. Kim et al. (2016) and LaPar et al. (2014) reported, that patients who underwent resternotomy in the ICU did not have an increased risk of sternal wound infections compared to standard cardiac surgery. The incidence of sternal wound infections is reported to range between 1% and 5% (Charalambous et al. 2006). However, the results are likely to be better in centers that perform routine re-explorations in the ICU. Resternotomy has been reported to be equally safe regardless of location (Kim et al. 2016 & LaPar et al. 2014), but avoiding transfer to the operating room can save precious time in emergency situations.

2.1.4 Risk factors for perioperative blood loss

The incidence of severe and massive bleeding after cardiac surgery ranges from 8% to 12% and 1.6% to 1.7%, respectively (Dyke et al. 2014 & Mariscalco et al. 2016). Since excessive bleeding is associated with an increased risk of adverse events, it is of utmost importance to recognize factors predictive of perioperative bleeding. The most commonly used risk scores to predict perioperative bleeding in cardiac surgery and PCI are summarized in Table 4 and 5.
### Table 4. Bleeding risk scores in cardiac surgery and interventional cardiology.

<table>
<thead>
<tr>
<th>WILL-BLEED (Biancari et al. 2016)</th>
<th>Score</th>
<th>TRACK (Biancari et al. 2016)</th>
<th>Score</th>
<th>TRUST (Biancari et al. 2016)</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>LMWH/fondaparinux/unfractionated heparin</td>
<td>1</td>
<td>Weight &lt;60 F, &lt;85 M</td>
<td>2</td>
<td>Weight &lt; 77 kg</td>
<td>1</td>
</tr>
<tr>
<td>Potent antiplatelet drugs pause &lt;5 days</td>
<td>2</td>
<td>Female sex</td>
<td>4</td>
<td>Female sex</td>
<td>1</td>
</tr>
<tr>
<td>Female sex</td>
<td>2</td>
<td>Age &gt; 67 years</td>
<td>6</td>
<td>Age &gt; 65 years</td>
<td>1</td>
</tr>
<tr>
<td>Acute coronary syndrome</td>
<td>2</td>
<td>Complex surgery</td>
<td>7</td>
<td>Non-isolated surgery</td>
<td>1</td>
</tr>
<tr>
<td>Anemia</td>
<td>3</td>
<td>Hematocrit &lt; 40%</td>
<td>1 point per each % &lt;40%</td>
<td>Hemoglobin level &lt;135 g/L</td>
<td>1</td>
</tr>
<tr>
<td>eGFR &lt;45 mL/min/1.73m2</td>
<td>3</td>
<td></td>
<td>Serum creatinine level &gt;120 μmol/L</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Critical preoperative state</td>
<td>5</td>
<td></td>
<td>Non-elective surgery</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Previous cardiac surgery</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Bleeding risk scores</td>
<td>PAPWORTH (Vuylsteke et al. 2011) Score</td>
<td>Litmathe et al. 2003 Score</td>
<td>Magovern et al. 1996 Score</td>
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<td>--------------------------------------</td>
<td>---------------------------------------</td>
<td>---------------------------</td>
<td>---------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI less than 25</td>
<td>1</td>
<td>Diabetes 1</td>
<td>Low BMI 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aortic valve stenosis and/or regurgitation</td>
<td>1</td>
<td>Female sex 1</td>
<td>Female sex 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age 75 years or older</td>
<td>1</td>
<td>Age &gt; 70 years 1</td>
<td>Age &gt; 74 years 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other than CABG/ single valve</td>
<td>1</td>
<td>Cardiogenic shock 3</td>
<td>Cardiogenic shock 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urgent/Emergent operation</td>
<td>1</td>
<td>Hemoglobin &lt; 110 g/L 3</td>
<td>Low red blood cell mass 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Creatinine &gt; 1.6 mg/dl 1</td>
<td>Creatinine &gt; 1.8 mg/dl 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Urgent/Emergent operation 2/4</td>
<td>Urgent/Emergent operation 3/4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Previous cardiac surgery 2</td>
<td>Previous cardiac surgery 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Left ventricle EF &lt; 35% 3</td>
<td>Left ventricle EF &lt; 30% 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diabetes 1</td>
<td>Diabetes 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Peripheral vascular disease 1</td>
<td>Peripheral vascular disease 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Albumin &lt; 4 g/dL 1</td>
<td>Albumin &lt; 4 g/dL 1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Gender

Recent evidence suggests that female gender could be associated with an increased risk of perioperative bleeding (Biancari et al. 2016 & Ahmed et al. 2013). It has been included as a risk factor for perioperative bleeding or blood product transfusion in numerous bleeding risk scores developed for interventional cardiology (Mehran et al. 2010 & Mehta et al. 2009a), cardiac surgery (Alghamdi et al. 2006, Litmathe et al. 2003, Magovern et al. 1996, Ranucci et al. 2009) and risk assessment in myocardial infarction (Subherwal et al. 2009 & Mathews et al. 2011). Indeed, female gender is reported to increase the risk of RBC exposure in cardiac surgery (OR 2.42, 95% CI 2.02-2.66) (Alghamdi et al. 2006) and periprocedural bleeding in patients undergoing PCI (2.6, 95% CI 1.74-3.91) (Ahmed et al. 2009). Research on the physiological background of gender as a predictor of bleeding in cardiac surgery is scarce.

On the other hand, female sex is associated with higher prevalences of anemia, low BMI, renal insufficiency and diabetes, which are other known predictors of perioperative bleeding (Ahmed et al. 2013). Paradoxically, female gender has also been associated with a hypercoagulable state, measured as faster fibrin/clot formation (Roeloffzen et al. 2010) and increased thrombocyte reactivity/aggregation in platelet function testing (Zuern et al. 2009). These results imply that women could be susceptible to perioperative ischemic events as well.

Ahmed and colleagues (2013) speculated, that due to more efficient platelet reactivity, women might have an elevated response to antiplatelet drugs. Indeed, women treated with GPIIb/IIIa inhibitors experienced significantly more episodes of major bleeding (Alexander et al. 2006).

Opposite results have been reported as well. Female gender was significantly associated with resistance to antiplatelet medication in the study by Silvain et al. (2012), and according to Lopes et al. (2015), male gender was found to be associated with a higher risk of bleeding events. In a study by Biancari et al. (2016), female gender has been reported to predict perioperative bleeding and/or blood transfusions (E-CABG bleeding grades 2-3) (OR 1.68, 95%CI 1.14-2.49). In their WILL-BLEED score, estimating the risk of severe perioperative blood loss in CABG, female gender was considered to have a predictive score of 2 out of 5 points. The WILL-BLEED score was found to be simple and accurate in detecting patients at risk of severe perioperative bleeding. The exact impact of gender on coagulation is not fully understood. Ahmed et al. (2013) speculated in their article on female gender as a predictor for bleeding risk in PCI, that the increase could be caused by
sex associated confounding factors, such as lower BMI, lower creatine clearance and anatomical differences. Furthermore, platelet function and pharmaco dynamic factors of antiplatelet drugs have been observed to be different in women.

**Anemia**

Anemia is considered one of the most important risk factor for increased perioperative bleeding within interventional cardiology or cardiac surgery (Biancari *et al.* 2016, Magovern *et al.* 1996, Mehran *et al.* 2010). As later discussed in the “Red blood cells and coagulation” paragraph, erythrocytes are suggested to have an active role in hemostasis. In certain circumstances, red blood cells have been reported to express phosphatidyl serine (PS) on their surface, which is a powerful catalyst of the coagulation cascade. Red blood cells are also able to secrete PS-rich microparticles increasing the potential for clot formation. (Du *et al.* 2013 & Morel *et al.* 2011).

In the case of anemia, the collisions between RBCs and thrombocytes are less frequent, decreasing the number of platelets in the proximity of the vessel wall (Tokarev *et al.* 2011). Fewer contacts with platelets, coagulation factors and TF could result in impaired clot formation and subsequent bleeding. Ninivaggi *et al.* (2012) and Horne *et al.* (2006) reported, that increasing hematocrit levels were associated with increased thrombin generation. On this basis, anemia may possibly exacerbate perioperative bleeding.

**Body mass index**

Patients with low BMI have been shown to have an increased risk of perioperative bleeding (Carroll *et al.* 2006, Dixon *et al.* 2014, Karkouti *et al.* 2001, Society of Thoracic Surgeons blood conservation clinical practice guidelines, 2007) and need of RBC transfusions (Schwann *et al.* 2001). Moreover, according to Nolan *et al.* (2011) the amount of postoperative chest tube drainage is inversely associated with BMI. It has been speculated that increased bleeding among low-weight patients could be caused by the relatively higher degree of hemodilution. The standard priming volume of bypass circuits could induce anemia in patients with lower blood volumes (Schwann *et al.* 2001).

On the other hand, an association between obesity and increased coagulation has been reported in the literature. Leptin and adiponectin are hormones secreted by fat tissue, which are reported to affect clot formation. Excessive leptin
concentrations are even able to promote platelet aggregation (Nakata et al. 1999). Furthermore, a decreased leptin effect is associated with prolonged and incomplete coagulation, whereas high leptin activity may result in a hypercoagulative state (Konstantinides et al. 2001). Decreased adiponectin concentration associated with type II diabetes and abdominal obesity (Faber et al. 2009) might also be able to contribute to hypercoagulability (Kato et al. 2006). Interestingly, obesity in addition to glucose intolerance is associated with an elevated level of circulating TF as well (Kopp et al. 2003).

The fibrinolytic system counteracts coagulation by degrading fibrin, and it is mediated by plasminogen activator. On the other hand, the main mediator of antifibrinolysis is plasminogen activator inhibitor-1 (PAI-1) (Faber et al 2009). An increased PAI-1 expression has been observed in morbidly obese patients with dyslipidemia (Scelles et al. 1992) and hyperinsulinemia (Samad et al. 2000). These studies provide compelling evidence on the association between obesity, diabetes and hypercoagulability and it seems that this procoagulant state might subside incrementally with decreasing body weight index, ultimately increasing the risk of bleeding in cardiac surgery.

**Antiplatelet and anticoagulant medication**

Patients referred for CABG are likely to receive some anticoagulation or antiplatelet medication before the operation (Davidson 2014). This exposes the patients to a higher risk of perioperative bleeding. When feasible, a strategy of timely withdrawal of anticoagulant and antiplatelet medication is recommended.

Aspirin is usually used in all patients undergoing CABG. It is an irreversible inhibitor of the cyclo-oxygenase-1 enzyme. Its pharmacodynamic properties are based on the decrease in thromboxane A₂ secretion from thrombocytes, which reduces platelet aggregation. Preoperative use of aspirin within 5 days before surgery is associated with increased perioperative bleeding, allogeneic blood transfusions and resternotomy (Hastings et al. 2015, Ma et al. 2014, Sun et al. 2008). However, lower daily doses, 100 mg - 325 mg, are shown not to increase blood loss or RBC transfusions (Aboul-Hassan et al. 2016, Myles et al. 2016, Sun et al 2008). Importantly, preoperative use of low dose aspirin (81 mg-160 mg) has been reported to have otherwise a favorable impact on the outcomes. Low-dose aspirin before CABG is associated with decreased postoperative mortality (Aboul-Hassan et al. 2016 & Deng et al. 2015) and reduced perioperative myocardial

Unfractionated heparin (UH) acts by accelerating the effects of the natural anticoagulant, antithrombin III. It is routinely used during cardiopulmonary bypass to prevent clot formation in the perfusion equipment. The impact of UH on coagulation is commonly measured using activated clotting time (ACT), which is recommended to be higher than 480s during extracorporeal circulation. At the end of perfusion, protamine sulfate is used to reverse the effects of unfractionated heparin. (Davidson et al. 2014). The problem with the use of UH is the risk of rebound bleeding after protamine administration, due to re-elevated heparin concentrations. According to Galeone et al. (2013), increased heparin levels were present in up to 49% of patients, measured 20 min to 3 hours after the initial reversal using protamine.

The mechanism of action of low molecular weight heparin (LMWH) is similar to unfractionated heparin, but due to its smaller molecular size the antithrombin-heparin complex inhibits factor Xa instead of thrombin (Hirsh et al. 2001). The evidence on bleeding risk related to LMWH use is scarce. In the (2011) Update to the Society of Thoracic Surgeons blood conservation clinical practice guidelines, LMWH is stated to increase postoperative bleeding and its withdrawal should be considered. According to Kincaid et al. (2003) LMWH administered within 12 hours of surgery is associated with lower postoperative hemoglobin levels and increased RBC transfusions compared to a continuous infusion of unfractionated heparin. On the other hand, Renda and colleagues (2007) showed that LMWH is a safe alternative to UH before CABG.

Clopidogrel, ticagrelor and prasugrel are potent antiplatelet drugs acting through the inhibition of platelet aggregation via the P2Y12 receptor. All three are associated with a reduced incidence of stroke, myocardial infarction and mortality compared to various other antiplatelet therapies in acute coronary syndrome. Clopidogrel is reported to yield superior results compared to treatment with only aspirin, although ticagrelor and prasugrel are shown to be more efficient than clopidogrel. On the other hand, the risk of bleeding might be higher in patients treated with ticagrelor and prasugrel compared to clopidogrel (Angiolillo 2012).

Exposure to ADP receptor antagonists before CABG is associated with increased perioperative bleeding, resternotomy for surgical hemostasis and blood product transfusions (Blais et al. 2013, Hansson et al. 2016, Smith et al. 2012). However, their effects depend on the timing of withdrawal. Hansson and colleagues (2016) reported that preoperative administration of clopidogrel and ticagrelor
within 0-72 hours of CABG increased significantly the incidence of major postoperative bleeding. According to Gherli et al. (2016), ticagrelor use within 2 days of CABG increases significantly the risk of platelet transfusions and perioperative bleeding. Interestingly, when comparing the risk of major bleeding between ticagrelor and clopidogrel, the risk is significantly higher with ticagrelor administered 0-4 days from the surgery (DiNicolantonio et al. 2013), however the results are opposite if the drugs are discontinued within 3-5 days (Hansson et al. 2016). Exposure to clopidorel within 5 days of cardiac surgery is associated with an increased risk of re-exploration for bleeding (Cao et al. 2014). According to the meta-analysis by Biancari et al. (2012a), pooled outcomes of observational studies showed that preoperative use of clopidogrel increases the risk of re-sternotomy for bleeding by three fold. Likewise, preoperative administration of prasugrel is also reported to increase the risk of perioperative bleeding after CABG (Drews et al. 2015 & Smith et al. 2012).

Warfarin’s mechanism of action is inhibition of vitamin-K dependent synthesis of coagulation factors in the liver. Warfarin is the treatment of choice in patients with a mechanical valve prosthesis. It is still used in a number of patients with atrial fibrillation. Preoperative discontinuation of warfarin more than two days before cardiac surgery or according to international normalized ratio (INR) levels does not increase bleeding complications in cardiac surgery (2010a). However, failure to discontinue warfarin before surgery may expose the patient to a significantly increased risk of bleeding (2010a).

Information regarding Novel Oral AntiCoagulants (NOACs) and bleeding risk in cardiac surgery are scarce (Davidson et al. 2014) with only a few reports published (Warkentin et al. 2012). According to Davidson et al. (2014), NOACs should be discontinued within 3-5 days of cardiac surgery to decrease the risk of bleeding.

Timely withdrawal of GPIIb/IIIa inhibitors before cardiac surgery is shown not to increase the risk of perioperative bleeding. Discontinuation of eptifibatide and tirofiban >8 hours before cardiac surgery does not increase the risk of perioperative bleeding (Bizzarri et al. 2001 & Waldron et al. 2016). Much like the aforementioned drugs, eptifibatide and abciximab withdrawn more than 2 to 4 (Dyke et al. 2000 & Włodzimski et al. 2016) and >12 hours, respectively, from surgery are not associated with increased risk of bleeding (Cheng et al. 2004). Recommendations for withdrawal of different antiplatelet and anticoagulant medications in elective and urgent operations are presented in further detail in table 6.
Table 6. Recommendations for withdrawal of antiplatelet and anticoagulant medications in elective and urgent CABG.

<table>
<thead>
<tr>
<th>Medication</th>
<th>Pause in elective surgery</th>
<th>Pause in urgent surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin 100 mg - 325 mg</td>
<td>Often no pause</td>
<td></td>
</tr>
<tr>
<td>Unfractionated heparin</td>
<td>Often no pause</td>
<td></td>
</tr>
<tr>
<td>Low molecular weight heparin</td>
<td>At least 12-24 hours</td>
<td>At least 24 hours</td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>At least 5 days</td>
<td>At least 24 hours</td>
</tr>
<tr>
<td>Ticagrelor</td>
<td>At least 1-3 or 5 days</td>
<td>At least 24 hours</td>
</tr>
<tr>
<td>Prasugrel</td>
<td>At least 7 days</td>
<td></td>
</tr>
<tr>
<td>Warfarin</td>
<td>At least 2-5 days</td>
<td></td>
</tr>
<tr>
<td>NOACs</td>
<td>At least 3-5 days</td>
<td></td>
</tr>
<tr>
<td>Eptifibatide</td>
<td>At least 2-4 hours</td>
<td></td>
</tr>
<tr>
<td>Tirofiban</td>
<td>At least 2-4 hours</td>
<td></td>
</tr>
<tr>
<td>Abciximab</td>
<td>At least 12 hours</td>
<td></td>
</tr>
</tbody>
</table>

References: Davidson 2014 & Hillis et al. 2011

Decreased renal function

Renal failure is a well-known risk factor for perioperative bleeding and is included in most bleeding risk scoring methods (Biancari et al. 2016, Litmathe et al. 2003, Magovern et al. 1996). Its association with hemorrhage has been primarily observed in non-surgical patients, and recent evidence substantiated its role in patients undergoing surgery as well (Acedillo et al. 2013). Elevated serum creatinine levels and decreased eGFR have been associated with RBC transfusions (Acedillo et al. 2013, Alghamdi et al. 2006, Moskowitz et al. 2004, Society of Thoracic Surgeons clinical practice guidelines, 2007) and reoperations in cardiac surgery (Kristensen et al. 2012). Winkelmeyer et al. (2003) showed that an increased risk of perioperative bleeding might be present even in milder degrees of renal disease, however their results did not reach statistical significance.

Impaired hemostasis in patients with chronic kidney disease is considered to be multifactorial owing to decreased thrombocyte function, anemia and exposure to anticoagulant or antiplatelet medication. The defect in platelet adhesion and aggregation is suggested to be caused by uremic substances in addition to increased production of nitric oxide and prostacyclin, which are known platelet inhibitors (Sohal et al. 2006). Interestingly, they reported that hemoglobin could be able to scavenge nitric oxide, therefore decreasing its deleterious effects on platelet function in non-anemic patients.
Zwaginga et al. (1990) reported that platelet and vessel wall interactions were decreased due to unknown agents in the uremic plasma. In addition, Benigni et al. (1993) showed that the binding ability of GPIIb/IIIa receptors was decreased in uremia. Furthermore, the use of antiplatelet drugs, oral anticoagulants (Sood et al. 2013) and aspirin (Holden et al. 2008 & Livio et al. 1986) is shown to increase the risk of bleeding in patients requiring dialysis. Moreover, as high as 10% to 50% of dialysis patients treated with heparin are observed to suffer from bleeding (Abramson et al. 1999).

**Extracorporeal circulation and prolonged operation time**

Extracorporeal circulation is the main cornerstone of cardiac surgery. Despite being almost irreplaceable, its use has been associated with increased perioperative bleeding. A typical cardiopulmonary bypass (CPB) circuit consists of a reservoir, oxygenator and heat exchanger, a pump for arterial circulation, blood filters and bubble detectors, a suction device, cardiac vent, a system for cardioplegia administration, and tubing. In the majority of procedures, venous canullas are inserted to vena cavae or the right atrium, and the blood is drained with the help of gravity. The blood reservoir is filled mainly by the venous line, but the suction device and cardiac vent drain their blood also into the reservoir. The arterial canulla is commonly inserted in the ascending aorta, which circulates the oxygenated blood from the reservoir. A separate system for cardioplegia infusion is either inserted to the sinus cornarius and/or the ascending aorta. (Ghosh et al. 2015).

Cardiopulmonary bypass is one of the rare occasions during which the intrinsic pathway is involved in the coagulation cascade. Blood contacting the perfusion equipment induces clot formation and breakdown, platelet activation and inflammation, subsequently depleting coagulation factors. This undesirable event is controlled by administration of unfractionated heparin (Besser et al. 2010), which is able to increase the risk of perioperative bleeding. Indeed, low thrombocyte and fibrinogen levels in addition to poor platelet adhesion and aggregation have been associated with CPB (McKenna et al. 1975). Nevertheless, clot formation activated by TF due to surgical trauma is thought to be the primary mediator of coagulation during extracorporeal circulation (De Somer et al. 2002).

Perhaps the most significant factor associated with increased risk of bleeding during CBP is hemodilution. At the beginning of perfusion, the reservoir and tubing must be filled with up to 2 liters of priming fluid (Besser et al. 2010), consisting of either a crystalloid solution or blood. The priming fluid is capable of generating a
considerable amount of hemodilutional anemia (Ghosh et al. 2015), the role of which in coagulation is discussed elsewhere in this dissertation. In fact, the primary cause of decreased coagulation factor concentrations during CPB has been reported to be hemodilution (Chandler 2005).

During some cardiac surgery operations, the patient’s core body temperature has to be lowered. In fact, even mild hypothermia, 34°-35°, is shown to reduce tissue oxygen consumption (Campos 2008) enabling the body and organs to tolerate varying degrees of hypoperfusion during the operation. (Besser et al. 2010). Nevertheless, an increase in hypothermia is associated with an incremental decrease in the efficacy of the coagulation cascade (Rungren et al. 2008). Low blood temperature inhibits platelet function and activated coagulation factors (Campos 2008) leading to slower clot formation, decreased thrombin generation (Whelihan et al. 2014) and impaired fibrinolysis (Campos 2008).

Interestingly, increased fibrinolysis during extracorporeal circulation is shown to be associated with a greater amount of postoperative blood loss (Ray et al. 1994) suggesting an increase in consumption of coagulation factors and platelets. Platelet dysfunction due to decreased expression of membrane glycoproteins has been reported to be associated with cardiopulmonary bypass as well (Kondo et al. 1993).

The length of the operation/CPB and aortic cross-clamp time directly represent the complexity of the procedure and are associated with increased need for blood product transfusions and perioperative bleeding. (Al-Sarraf et al. 2011, Carroll et al. 2006, Hall et al. 2001, Society of Thoracic Surgeons clinical practice guidelines, 2007). The mechanism behind this finding might be the increased exposure to foreign materials and mechanical stress (Carroll et al. 2006) producing an incremental consumption coagulopathy.

2.2 Red blood cells and coagulation

Recent evidence suggests that erythrocytes might have a more significant role in hemostasis than previously thought (Du et al. 2013, Morel et al. 2011, Peyrou et al. 1999, Van Der Meijden et al. 2012, Wohner 2008). The main mechanism by which RBCs contribute to hemostasis is the sheer volume of cells pushing thrombocytes (Tokarev et al. 2011) and coagulation factors (Vayá et al. 2013) against the vessel wall, increasing the likelihood of clot formation. Higher hematocrit levels can be caused, e.g. by increased number of RBCs or dehydration. An elevation in hematocrit has been associated with increased blood viscosity (Stammers et al. 2003), which is the leading cause of thrombotic complications in patients with
polycythemia vera (Boneau et al. 1994). Likewise, Valeri et al. (2001) reported that low levels of hematocrit produced a reversible platelet dysfunction manifesting as an increased bleeding time.

Although the role of RBCs in coagulation has been under investigation for almost a century, the correlation between erythrocyte aggregation and blood flow is poorly understood (Baskurt et al. 2007). In low shear conditions, red blood cell aggregation is reported to result in high viscosity and increased hydrodynamic resistance (Du et al. 2013). Interestingly, under certain conditions, erythrocytes can become rigid and may no longer be able to squeeze through the tightest capillaries (Schmid-Schönbein et al. 1969) generating a microthrombus.

However, the most recent evidence regarding phosphatidylserine expression on the surface of erythrocytes suggests that RBCs might also have an active role in coagulation. A rapid formation of thrombin is essential to enable adequate clot formation in the fast flowing blood, but this high-speed reaction is possible only on a procoagulant surface. It is provided by phosphatidylserine coated cells, which are most commonly platelets. According to Whelihan and coworkers (2012), 0.6% of healthy RBCs express PS on their surface. These cells could account for up to 40% of the thrombin generated during coagulation. Patients with abnormal erythrocyte membrane proteins or hemoglobin morphology frequently suffer from increased thrombus formation (Andrews et al. 1999). Interestingly, as a result of injury, PS expressed also inside a RBC can migrate to the cells’ surface and begin catalyzing the coagulation reaction (Du et al. 2013). Red blood cells can also excrete PS-rich microparticles (Morel et al. 2011) capable of initiating thrombin generation independently of the intrinsic pathway (Van Der Meijden et al. 2012).

Red blood cells have been reported to enhance coagulation activated by the extrinsic pathway event to a greater extent than platelets inside the physiological range of hematocrit (39%-50%) and thrombocytes (150-360 x10⁹/L) (Horne et al. 2006). Furthermore, Peyrou et al. (1999) reported that the efficacy of the reaction was linearly associated with increasing hematocrit values. The aforementioned results were partially confirmed in the study by Ninivaggi et al. (2012), where increasing hematocrit values elevated thrombin generation in platelet poor plasma only until the hematocrit level of 14%. Interestingly, Du and colleagues (2013) speculated that the results of Horne (2006) and Ninivaggi (2012) could possibly represent the separate ability of erythrocytes’ to induce both the intrinsic and the extrinsic coagulation pathways due to different TF concentrations.

On the contrary, transfusion of packed RBCs has been shown to improve coagulopathy by decreasing PT, APTT and INR values, although Sun et al. (2015)
speculated that the effect could be likely due to residual platelet and coagulation factor activity in packed RBC units.

2.3 Transfusion of allogeneic blood products

2.3.1 Transfusion of red blood cells

Transfusion of allogeneic blood products is frequently needed in patients undergoing cardiac surgery; up to 18% of all blood products used in operative medicine are used in this setting (Palo et al. 2006). In all living tissues, the alternation between aerobic and anaerobic metabolism is dependent on three factors: capacity of oxygen delivery, efficacy of oxygen extraction and the amount of oxygen consumed. In the case of anemia, insufficient oxygen carrying capacity can be compensated by increasing cardiac output and RBC transit time in the capillaries. (Society of Thoracic Surgeons blood conservation guideline task force et al. 2007). Other physiologic changes associated with anemia are elevated heart rate, stroke volume and the heart’s work load in addition to decreased peripheral vascular resistance (Roy et al. 1963). Moreover, the body can simultaneously increase the efficiency of tissue oxygen extraction and oxygen delivery. Indeed, these adaptations enable the body to tolerate surprisingly very low levels of anemia. The tissues are forced to rely on anaerobic metabolism once all compensatory mechanisms are exhausted. (Society of Thoracic Surgeons blood conservation guideline task force, 2007).

Critical oxygen delivery is defined as the point at which oxygen extraction is no longer able to compensate for the decreased oxygen supply and delivery. In an animal experiment, the critical oxygen delivery values were found to have a significant correlation with their corresponding critical hemoglobin values. After exposure to incremental levels of hemodilution, an average critical hemoglobin value of 2.8 ± 0.1 g/dL was obtained. These results were confirmed in other experiments as well (Schumacker et al. 1987).

Moreover, evidence regarding healthy patients with normovolemia suggests that tissue oxygenation could be maintained with hemoglobin levels as low as 6 g/dL to 7 g/dL (Doak et al. 1995). According to Viele et al. (1994), nearly all deaths among Jehovah’s witnesses undergoing cardiac surgery occurred with hemoglobin levels below 50 g/dL. Remarkably, some of the patients survived despite having such low hemoglobin levels. This evidence suggests that lower hemoglobin levels
could be tolerated in cardiac surgery, keeping in mind that patients undergoing CABG have diseased coronary arteries and limited compensatory mechanisms. The Society of Thoracic Surgeons blood conservation guideline task force (2007) recommends that RBCs should be transfused when hemoglobin levels drop below 6 g/dL, since it can be life saving. Postoperative transfusion is reasonable in most patients with hb <7 g/dL. In a setting of critical non-cardiac end organ ischemia, it is not unreasonable to transfuse patients even with hemoglobin levels of 10 g/dL. During cardiopulmonary bypass, a transfusion trigger of 6 g/dL of hemoglobin is reasonable. Moreover, in patients at risk of critical end organ ischemia, it is not unreasonable to maintain a hemoglobin level of 7 g/dL or more.

An optimal hematocrit level during bypass has not been decided, although some authors have suggested 14%-19% to be the lowest safe level. More recently, a hematocrit below 21%-24% has been associated with increased risk of renal failure. (Mangu et al. 2014).

2.3.2 Transfusion of platelets

In the coagulation system, platelets and fibrin are responsible for the formation of a primary hemostatic plug. As a result of vascular injury, the exposed subendothelial collagen triggers platelet activation. Platelet adhesion, on the other hand, is mediated by circulating von Willebrand factor and the GPIIb/IIIa complex located on the platelet’s surface. Activation and aggregation of the thrombocytes is further enhanced by P2Y12 receptor, binding to (adenosine diphosphate) ADP released by other platelets or damaged cells (Blockmans et al. 1995). Thrombocytes are also shown to enhance the humoral coagulation cascade (Hoffman et al. 1996).

In the American Association of Blood Banks (Kaufman et al. 2015), platelet transfusion during cardiac surgery is recommended in patients exhibiting thrombocyte dysfunction and/or thrombocytopenia and perioperative bleeding, however prophylactic transfusion of platelets should not be performed (Simon et al. 1984). A thrombocyte value of < 50 x 10^9/L, depending on the patient’s clinical status, is considered as an indication for platelet transfusion (Royal college of physicians of Edinburgh 1998). Guidelines for RBC transfusions are presented in further detail in Table 7.

Two of the most common complications associated with platelet transfusion are anaphylactoid reactions and a sudden onset of fever (Kiefel 2008). Another possible adverse event associated with platelet transfusions is an increased risk of a hypotensive reaction, which is characterized by respiratory distress.
Discontinuation of platelet transfusion is recommended to resolve these symptoms (Hume et al. 1996).

**Table 7. Guidelines for RBC transfusions.**

<table>
<thead>
<tr>
<th>Recommendation of the Society of Thoracic Surgeons blood conservation guidelines, 2007</th>
<th>Class</th>
<th>Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>For patients with hemoglobin level &lt; 60 g/dL, transfusion of RBCs is reasonable</td>
<td>IIa</td>
<td>C</td>
</tr>
<tr>
<td>For most patients with postoperative hemoglobin level &lt; 70 g/dL, transfusion of RBCs is reasonable</td>
<td>IIa</td>
<td>C</td>
</tr>
<tr>
<td>For patients with clinical evidence of bleeding, preferably confirmed by point-of-care tests, transfusion of non-red cell hemostatic products is reasonable</td>
<td>IIb</td>
<td>C</td>
</tr>
<tr>
<td>For patients with hemoglobin level &lt;10 g/dL and critical non-cardiac end-organ ischemia, transfusion of RBCs is not unreasonable</td>
<td>III</td>
<td>C</td>
</tr>
<tr>
<td>Transfusion of RBCs is not recommended when hemoglobin level is &gt;10 g/dL</td>
<td>IIa</td>
<td>C</td>
</tr>
<tr>
<td>For patients during cardiopulmonary bypass with hemoglobin value &lt;60 g/dL, and/or with mixed venous oxygen saturation &lt; 55%, transfusion of RBCs is reasonable</td>
<td>IIa</td>
<td>C</td>
</tr>
<tr>
<td>For patients during cardiopulmonary bypass with hemoglobin values &gt; 60 g/dL, in the presence of risk factors for impaired cerebral oxygen delivery and based on the patients clinical situation, transfusion of RBCs is reasonable</td>
<td>IIb</td>
<td>C</td>
</tr>
<tr>
<td>For patients during cardiopulmonary bypass with an increased risk of end-organ ischemia, transfusion of RBCs is reasonable to keep hemoglobin values &gt;70 g/dL</td>
<td>IIb</td>
<td>C</td>
</tr>
</tbody>
</table>

American Society of Anesthesiologists guidelines for RBC transfusion

In addition to above mentioned triggers:
- For patients with acute blood loss >1500ml or > 30% of blood volume
- For patients with evidence of rapid blood loss without immediate control
- The benefit of transfusions in patients with hemoglobin levels between 70 g/dL - 100 g/dL is unclear

### 2.3.3 Transfusion of plasma and plasma products

Transfusion of plasma, i.e. fresh frozen plasma (FFP) or Octaplas, and plasma products, e.g. cryoprecipitate, prothrombin complex concentrate (PCC) and isolated specific coagulation factors, are reserved for patients exhibiting severe bleeding and/or coagulation factor deficiencies (Roback et al. 2010). However, the benefit (and problem) with FFP and Octaplas transfusions is that they contain all possible coagulation factors in normal concentrations. In patients with isolated coagulation factor deficiencies, administration of FFP or Octaplas can lead to
unpredictable adverse events, due to an increase in the previously normal coagulation factor levels.

The decision to use plasma and plasma products, in addition to the amount to be transfused, should be guided by laboratory tests. Prothrombin time (PT), international normalized ratio (INR), activated partial thromboplastin time (APTT) and point-of-care tests are useful to determine the patients’ coagulation status. (Shah et al. 2015). The British committee for standards in hematology, blood transfusion task force suggested that FFP, should be used in the presence of severe bleeding associated with deficiency of multiple coagulation factors. However, specific transfusion triggers in surgical patients with excessive perioperative bleeding are not recommended (O’Shaughnessy et al. 2004). In the presence of normal PT, INR and/or APTT values, transfusion of FFP is not indicated (American Society of Anesthesiologists [ASA] guideline, 2006). The commonly used transfusion ratio of 1:3 (FFP to RBC) has not received negative or positive recommendations in the plasma transfusion guidelines (Roback et al. 2010).

Cryoprecipitate can be extracted from FFP and contains increased concentrations of fibrinogen, von Willebrand factor, fibronectin and factors FIII & XIII. It is mainly used in bleeding patients with low levels of fibrinogen (O’Shaughnessy et al. 2004). Interestingly, one unit of FFP contains similar amounts of fibrinogen as two units of cryoprecipitate. Before transfusion, the patient’s fibrinogen concentration should be obtained. Administration of FFP is indicated when the fibrinogen concentration is < 80-100 mg/dL. Transfusion is rarely recommended if the fibrinogen level is higher than 150 mg/dL (ASA guideline, 2006). The use of cryoprecipitate has been withdrawn from use in many European countries, including Finland, due to increased safety concerns. Cryoprecipitate transfusions have been associated with an increased risk of infection due to blood borne pathogens in addition to TRALI (Nascimento et al. 2014).

Prothrombin complex concentrate contains vitamin-K dependent coagulation factors, which are FII, FVII, FIX and FX. In addition, therapeutic concentrations of protein S and protein C are included. The indications of PCC transfusion are coagulation factor replenishment in bleeding patients with warfarin therapy and correction of perioperative coagulopathy verified by laboratory or point-of-care testing. Interestingly, PCC is considered faster and more effective than FFP in correcting deficiency of K-vitamin dependent coagulation factors (Tanaka et al. 2014). Indeed, the use of PCC has been associated with decreased blood loss and
fewer transfusions in cardiac surgery when compared to treatment with FFP (Cappabianca et al. 2016).

Today, transfusion of fibrinogen concentrations has become more common and is associated with decreased perioperative bleeding and reduced RBC transfusions in cardiac surgery (Levy & Goodnough 2015). The European trauma guidelines recommend transfusions of fibrinogen concentrate when plasma fibrinogen levels are less than 150 mg/dL – 200 mg/dL (Spahn et al. 2013). Administration of fibrinogen concentrate should be guided by point-of-care testing (Levy & Goodnough 2015).

Recombinant activated factor VII (rFVIIa) should be considered in patients with excessive microvascular bleeding as a rescue drug only after other treatment alternatives have been shown to be insufficient (ASA guideline 2006). An increase in thrombotic adverse events has been reported after off-label use of rFVIIa (O’Connell et al. 2006 & Raivio et al 2005). Guidelines for transfusion of fresh frozen plasma and cryoprecipitate are presented in Table 8.

Table 8. Guidelines for transfusion of fresh frozen plasma and cryoprecipitate.

<table>
<thead>
<tr>
<th>Recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Transfusion of fresh frozen plasma:</strong></td>
</tr>
<tr>
<td>In microvascular bleeding, when PT &gt;1.5 times normal, INR &gt; 2.0 or APTT more than two times normal</td>
</tr>
<tr>
<td>In microvascular bleeding, when patient has lost more than one blood volume</td>
</tr>
<tr>
<td>In urgent warfarin reversal</td>
</tr>
<tr>
<td>In correction of known coagulation factor deficiencies, when specific factors are unavailable</td>
</tr>
<tr>
<td>In heparin resistance, i.e. antithrombin III deficiency</td>
</tr>
<tr>
<td><strong>Transfusion of cryoprecipitate:</strong></td>
</tr>
<tr>
<td>In excessive microvascular bleeding, when fibrinogen values are &lt; 80-100 mg/dL</td>
</tr>
<tr>
<td>In excessive microvascular bleeding, in massively transfused patients, when fibrinogen values can not be measured</td>
</tr>
<tr>
<td>In patients with congenital fibrinogen deficiency</td>
</tr>
</tbody>
</table>

American Society of Anesthesiologists guideline for perioperative blood transfusion, 2006

2.4 General adverse events related to transfusion of allogeneic blood products

However, patients requiring RBCs have generally more comorbidities and undergo complex or urgent operations. The predictors of allogeneic blood transfusion are understandable very similar to factors associated with perioperative bleeding. The mechanisms by which allogeneic blood transfusions could cause adverse events are transfusion related immune modulation (TRIM), transfusion related acute lung injury (TRALI) and transfusion associated circulatory overload (TACO) (Crescenzi et al. 2012). The presence of retained white blood cells and other particles capable of initiating an immunologic response within allogeneic blood products could have a negative impact on the recipient’s immune system, ultimately leading to mild immunosuppression and adverse events (Blumberg et al. 2002). Indeed, allogeneic blood transfusions have been associated with an increased risk of infections after cardiac surgery, ranging from urinary tract infections to mediastinitis (Ang et al. 2012, Banbury et al. 2006, Vranken et al. 2014). The incidence of bacterial contamination in allogeneic blood is one in 3000/platelet units and one in 30,000/RBC units. The higher observed incidence of bacterial contamination with platelets is due to their warmer storage temperature of 22°C. (Jacobs et al. 2001). As a comparison, the exposure to viral agents through allogeneic blood transfusion is approximately 1-4 cases per million units (Dodd et al. 2000).

One of the most severe complications related to allogeneic blood transfusion is TRALI. Its etiology is thought to be two-staged. The development of TRALI requires activated leucocytes in the pulmonary microvasculature stimulated by an underlying infection or proinflammatory state caused by surgery (Bux et al. 2007). A reaction between transfused donor antibodies and recipient’s leucocytes induces endothelial damage leading to fluid accumulation into the alveoli. The condition is reported to occur more frequently after plasma transfusions (Wallis et al. 2003). The clinical characteristics of TRALI are a sudden onset of non-cardiogenic pulmonary edema and severe hypoxemia (Popovsky et al. 1985). Interestingly, transfusion of RBCs are reported to increase inflammatory markers in the lungs even in patients who do not meet the diagnostic criteria of TRALI (Tuinman et al. 2011). Transfusion related lung injury is relatively common after cardiac surgery and is associated with increased in-hospital death (Vlaar et al. 2011).

Transfusion associated circulatory overload is induced by excessive infusion speeds and/ or volumes in addition to underlying cardiac, pulmonary or renal dysfunctions. It does not have universally accepted diagnostic criteria, but the most commonly reported clinical signs are increased central venous pressure, pulmonary
congestion, dyspnea, and acute hypertension, which ultimately lead to heart failure (Andrzejewski et al. 2013).

### 2.4.1 Transfusion, mortality and morbidity in cardiac surgery

Interestingly, the association between allogeneic RBC transfusion and mortality has been observed to occur in two phases. During the short-term period, the risk of death is substantially elevated until the first year, after which it has been reported to decrease in magnitude (Bhaskar et al. 2012, Engoren et al. 2002, Surgenor et al. 2009). Patients requiring transfusions are up to 2.2 times more likely to be deceased within five years of the index operation (Bhaskar et al. 2012), and the increase in mortality risk has been shown to last over 10 years (Koch et al. 2006a). Long-term survival is observed to decrease after allogeneic blood transfusion in low-risk patients as well (Jakobsen et al. 2012). Furthermore, the risk of death (Koch et al. 2006 & Shaw et al. 2014) and adverse events have been reported to be dose-dependent according to the amount of RBCs transfused (Mariscalco et al. 2014 & Yu et al. 2014). Even transfusion of 1 or 2 units of RBCs has been reported to increase significantly the risk of adverse events and death (Paone et al. 2014).

The most frequently reported complications among cardiac surgery patients receiving allogeneic blood products are kidney injury, prolonged ventilation, postoperative infections, cardiac morbidity and thrombotic events (Freeland et al. 2015, Ghazi et al. 2015, Koch et al. 2006, Paone et al. 2014). Stroke is one of the most serious and life-altering complications after cardiac surgery and its incidence is approximately 1.5% (LaPar et al. 2015). According to Brascia et al. (2017), transfusion of RBCs was significantly associated with postoperative stroke after CABG (HR 2.26, 95% CI 1.85-2.77). Furthermore, Ghazi and colleagues (2015) showed that the incidence of deep venous thrombosis after cardiac surgery was almost three times higher in patients who received RBCs, reaching up to 40% of patients when platelets, FFP and cryoprecipitate were transfused together with erythrocytes.

The possible association with increased RBC storage time and adverse events has been under recent investigation. The mechanism of increased adverse events associated with old RBCs could be structural and functional changes induced by older age (Berezina et al. 2002). Transfusion of old RBCs (storage >21 days) has been shown to significantly increase mortality (Wang et al. 2012). On the other hand, Kinnunen et al. (2015) reported that the postoperative outcomes were similar regardless of RBC storage time with the exception of an increase in POAF in
patients who received at least one unit of older RBCs (storage >14 days). Indeed, Sartipy et al. (2015) reported that in their patient population of 47,000 patients, storage time of transfused RBCs did not affect the short or long-term mortality of patients undergoing cardiac surgery.

2.4.2 Liberal versus restrictive transfusion policies

The impact of blood conservation on patient outcomes is of major interest. Introduction of a blood conservation program can significantly reduce the proportion of patients receiving RBCs, platelets and plasma products in addition to the number of units transfused (LaPar et al. 2013, Mehra et al. 2015, Ternström et al. 2014). Moreover, the use of a blood conservation program has been significantly associated with reduced in-hospital mortality and transfusion related complications (LaPar et al. 2013).

However, several investigators have reported that the use of a restrictive transfusion trigger might not be advantageous (Carson et al. 2015, Carson et al. 2016, Curley et al. 2014, Hajjar et al. 2010, Murphy et al. 2015, Nakamura et al. 2015). Indeed, patients allocated to a restrictive RBC transfusion policy have been reported to have an increased mortality risk (Murphy et al. 2015) and cardiogenic shock (Nakamura et al. 2015). In a Cochrane systematic review comparing restrictive (most commonly 7 g/dL – 8 g/dL) and liberal (most commonly 9 g/dL – 10 g/dL) transfusion triggers, restrictive transfusion thresholds were associated with a decrease of 43% in RBC exposure without any significant changes in adverse events (Carson et al. 2016). Furthermore, Carson and colleagues (2015) reported similar 3-year mortality rates between the two transfusion policies.

The limitation with many studies investigating the effect of transfusion triggers is the notable difference in their methods. For example, in the study of Hajjar et al. (2010) a restrictive transfusion trigger was considered to be a hemoglobin value of 9 g/dL, which was used as a trigger for liberal transfusion in the study conducted by Murphy and colleagues (2015). It could be argued that some of the results represent a comparison between different degrees of liberal transfusion, since they differ from the recommendations of Society of Thoracic Surgeons blood conservation guidelines (2007). Recommended methods for blood conservation are presented in Table 9.
Table 9. Recommended methods of blood conservation according to the Society of Thoracic Surgeons blood conservation guidelines.

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>P2Y12 antagonist withdrawal</td>
<td>P2Y12 receptor antagonists should be discontinued before CABG if possible.</td>
</tr>
<tr>
<td>EPO</td>
<td>Recombinant EPO and iron supplementation is reasonable in preoperative anemic patients and patients who refuse blood. It is also reasonable in patients undergoing autologous blood donation. Epo is not unreasonable in patients at risk of postoperative anemia.</td>
</tr>
<tr>
<td>Antifibrinolytic drugs</td>
<td>Tranexamic acid and epsilon-aminocaproic acid are indicated to reduce the number of patients needing transfusions and the amount of postoperative blood loss. Antifibrinolics poured into the surgical wound is reasonable to reduce transfusions and chest tube output after CPB.</td>
</tr>
<tr>
<td>RFVIIa</td>
<td>RFVIIa use is not unreasonable in patients with intractable non-surgical bleeding that is not responding to standard hemostatic treatment after cardiac surgery using CPB.</td>
</tr>
<tr>
<td>Protamine</td>
<td>It is not unreasonable to use titrated or empiric low-dose protamine administration to reduce bleeding and transfusions.</td>
</tr>
<tr>
<td>Red cell saving</td>
<td>Routine use of red cell salvage is helpful for blood conservation in CPB operations. During CPB it is not unreasonable to autotransfuse cardiotomy suction blood.</td>
</tr>
<tr>
<td>Minimzed CPB and Off-pump</td>
<td>It is not unreasonable to use low-prime or minimized extracorporeal circuits to reduce hemodilution during CPB. Vacuum assisted venous drainage in addition to minimized circuits might be useful in reducing transfusions and bleeding. Off-pump surgery is reasonable to conserve blood if conversion to CPB is unlikely.</td>
</tr>
<tr>
<td>Topical hemostatic agents</td>
<td>Topical agents/ Tissue glue is not unreasonable to reduce bleeding in certain high-risk cardiac or aortic procedures.</td>
</tr>
</tbody>
</table>

2.5 Costs of blood product transfusions

Despite existing blood transfusion guidelines, the amount of RBCs used in everyday patient care vary significantly between hospitals. Indeed, the proportion of patients receiving transfusion of RBCs ranges between 27% and 92% in cardiac surgery (Stover et al. 1998).

Nevertheless, the increasing shortage of blood products and new safety measures, are likely to increase the costs of transfusions (Reeves et al. 2008).
Recent evidence in the literature suggests that excessive perioperative bleeding and transfusion related adverse events are associated with increased total hospitalization costs (Alström et al. 2011 & Christensen et al. 2009). Indeed, Murphy and colleagues (2007) reported that the costs of plasma and platelet units compared to one unit of RBCs are roughly 25% and 200% respectively. In turn, the cost estimates per one day spent in the ICU, high-dependency-unit and the general ward were roughly 900%, 700% and 350% of the price of an RBC unit. The total cost of cardiac surgery, without excessive bleeding related complications per patient, has been estimated to be approximately 8027€ (Christensen et al. 2009).

According to Stokes et al. (2016) patients managed using a liberal transfusion policy (<7.5 g/L vs. <9.0 g/L) received on average, one unit of RBCs more, compared to the restrictive group. This difference resulted in a significant, but modest increase in costs of RBC transfusions, accounting for £140 (165€) / patient. The mean cost of transfused RBCs in the liberal group, in which approximately three units were used per patient was £427 (504€). However, the total hospital costs after cardiac surgery have been reported to be reduced by several thousands / patient due to shorter ICU stays as a results of blood conservation (LaPar et al. 2013). Interestingly, Guinn and colleagues (2015) compared Jehovah’s Witnesses with patients who accept transfusions, and reported that the cost of treatment supplies was notably less in Witnesses due to lack of transfusions. However, the cost difference between restrictive and liberal transfusion triggers has been reported to diminish after three months from the initial hospitalization (Murphy et al. 2015). Nevertheless, in view of the number of cardiac procedures performed every year, a reduction of one RBC unit per patient results in a nationwide savings of millions (Stokes et al. 2016).

Contrary to restricting transfusions, many Jehovah’s Witnesses and other patients willing to participate, e.g. in autologous blood transfusion programs, are given preoperative epoetin-alpha with or without iron supplementation. As a comparison, the mean costs of epoetin-alpha and one unit of autologous blood per patient compared with the cost of one allogeneic blood unit was roughly 840% and 132%. Indeed, the use of epoetin-alpa has been reported to reduce patients receiving allogeneic blood by 60% (Coyle et al. 2000), but it is not considered cost-effective at the current market prices (Coyle et al. 2000 & Marchetti et al. 2000). The cost-effectiveness of cardiac surgery could be improved tremendously by adhering to blood conservation guidelines (LaPar et al. 2013).
2.6 Outcomes associated with preoperative anemia in cardiac surgery

Anemia is defined by the World Health Organization as a hemoglobin level <120 g/L in women and <130 g/L in men (http://www.who.int/vmnis/indicators/haemoglobin.pdf?ua=1, accessed on 11th of Dec, 2016). Recent evidence suggests that preoperative anemia is associated with adverse outcomes after cardiac (Hung et al. 2011, Karkouti et al. 2008, Kulier et al. 2007, Ranucci et al. 2012) and general surgery (Fowler et al. 2015 & Musallam et al. 2011). Importantly, the prevalence of preoperative anemia has been reported to be high among cardiac surgery patients ranging approximately between 24% and 54% (Hung et al. 2011 & Miceli et al. 2014). According to Karski et al. (1999), the most common etiologies for preoperative anemia among patients undergoing cardiac surgery are hospital acquired (37.3%), iron deficiency (29.3%) and anemia associated with chronic kidney disease (10.7%).

Patients with preoperative anemia are usually elderly with numerous baseline comorbidities (Boening et al. 2011, Kulier et al. 2007, Miceli et al. 2014, Ranucci et al. 2012, van Straten et al. 2009) and more likely to receive allogeneic blood products (Kim et al. 2015 & Ranucci et al. 2012).

Although anemic patients are usually considered to have a high-risk due to their increased comorbidity, several studies have reported preoperative anemia to be independently associated with increased mortality (Boening et al. 2011, Miceli et al. 2014, Ranucci et al. 2012) and morbidity (Kulier et al. 2007, Miceli et al. 2014, Ranucci et al. 2012). The most frequently reported complications are stroke, kidney injury, infections, prolonged mechanical ventilation time, increased length of ICU stay and low cardiac output syndrome (Kulier et al. 2007, Miceli et al. 2014, Ranucci et al. 2012).

2.6.1 Intraoperative anemia

Intraoperative anemia is unarguably a different entity compared to preoperative anemia, since it is associated with hemodilution and bleeding. Indeed, nadir hematocrit during CPB has been shown to be associated with increased in-hospital mortality (DeFoee et al. 2001). Moreover, in a cohort of 19,000 patients, Klein and colleagues (2016) reported that a decrease of 10 g/L in hemoglobin was significantly associated with an increased risk of mortality and the need for transfusions. A decrease in hemoglobin more than 50% from the baseline with a
nadir hemoglobin level >7 g/dL has been reported to increase the risk of a composite outcome of death, stroke and acute kidney injury.

The evidence on individual effects of anemia and RBC transfusions in the literature are scarce. Loor and coworkers (2013) reported that the simultaneous impact of intraoperative anemia and RBC transfusion had the highest risk of adverse events after cardiac surgery. Patients who experienced intraoperative anemia without requiring blood transfusions had a lower risk than those patients requiring blood transfusion. On the other hand, transfusion and preoperative anemia have been considered to have only a multiplying effect on major bleeding, which has been suggested to be the main determinant of adverse events in cardiac surgery (Ranucci et al. 2013).

Sustained postoperative anemia and even a decrease of 0.01 g/L (1mg/dL) in postoperative hemoglobin have been associated with a 22% increase in the risk of all-cause mortality and a 13% increase in adverse cardiac events Westenbrink et al. (2011).

2.7 Optimization of patient blood management

2.7.1 Preoperative factors

Withdrawal of anticoagulant and antiplatelet medication

As described earlier in the “Antiplatelet and anticoagulant medication” chapter, exposure to these drugs increases the risk of bleeding and re-exploration. The Society of Thoracic Surgeons clinical practice guidelines (2007) indicate that timely discontinuation of antiplatelet and anticoagulant medication before the operation is an important measure to reduce perioperative bleeding events. Appropriate withdrawal times and characteristics of the drugs in question are described earlier.

Hemoglobin optimization

Since anemia is associated with an increased risk of perioperative bleeding, it is reasonable to use a short course of preoperative erythropoietin (EPO) and iron supplements to restore RBC mass in patients who refuse transfusions, are considered to be in exceptional risk of postoperative anemia or plan to undergo
autologous RBC donation. Preoperative hemoglobin augmentation with EPO is considered off-label for other indications, due to the lack of large-scale studies in cardiac surgery. (Update to Society of Thoracic Surgeons clinical practice guidelines 2011). The limitation with erythropoietin is its slow pharmacological effect, which begins after 4-6 days of use reducing its potential in urgent and emergent operations.

Research in the field of preoperative hemoglobin optimization has been at a standstill, due to the black box warning issued by the US Food and Drug Administration against the use of EPO in surgical patients for preoperative hemoglobin augmentation > 120 g/L (D’Ambra et al. 2015). Indeed, daily erythropoietin treatment has been associated with increased mortality and arteriovenous access thrombosis (Phrommintikul et al. 2007) in addition to a composite outcome of death, stroke, myocardial infarction and hospitalization due to heart failure in patients with chronic kidney disease (McCullough et al. 2013). According to the aforementioned studies, patients with acute coronary syndromes might not benefit from EPO because of their hypercoagulable state (Update to Society of Thoracic Surgeons clinical practice guidelines 2011).

Nevertheless, the use of erythropoietin has been reported to have positive effects in patients undergoing cardiac surgery. Preoperative administration of EPO with iron supplementation has been significantly associated with reduced exposure to RBC transfusions in patients undergoing cardiac (Alghamdi et al. 2006a) and general surgery (Monk 2004), in addition to improved outcomes among Jehovah’s Witnesses (Tanaka et al. 2015). According to Weltert et al. (2015), even a single dose of EPO given two days before the operation is able to reduce the rate of allogeneic blood transfusions in anemic patients. The efficacy of hemoglobin optimization with per oral or intravenous iron therapy alone is poorly understood.

Preoperative coagulation testing

Preoperative coagulation testing can be used to detect coagulation defects before cardiac surgery (Moerloose 1996), and is considered as a good blood conservation method (Vasques et al. 2016).

Three of the most traditional tests for screening abnormalities in coagulation are platelet count, prothrombin time and activated partial thromboplastin time (Capoor et al. 2015, Koscielny et al. 2004, Moerloose 1996). Preoperative platelet count is a valid screening tool to assess the function of primary hemostasis. Indeed, low preoperative platelet count has been reported to be an independent predictor of
bleeding in patients undergoing coronary angiography. The most common use of aPTT is direct detection of hemophilia and indirect detection of von Willebrand disease, whereas an elevated PT indicates a disorder in vitamin-K dependent coagulation factors (Moerloose 1996). Interestingly, a positive history of hemorrhage has been considered more reliable in detecting patients at risk of perioperative bleeding than platelet count, PT or aPTT. Subsequently, routine preoperative screening with the aforementioned laboratory tests is not recommended (Ng et al. 2002, Moerloose 1996, Rohrer et al. 1988). Indeed, Capoor and colleagues (2015) showed in their million-patient sample that over 90% of PT and aPTT tests are ordered unnecessarily. The role of these laboratory tests might be in determining a preoperative reference point for later measurements (Moerloose 1996).

Moreover, the international normalized ratio is frequently tested from patients undergoing cardiac surgery. Tamim et al. (2016) reported on a significant association between preoperatively increased INR values and major bleeding in their cohort of more than 600,000 patients.

Point-of-care platelet function tests such as thromboelastography (TEG) or multiple electrode aggregometer (MEA) are designed to identify platelet derived coagulation disorders. These tests are considered simple and reliable in detecting and excluding coagulation abnormalities (Koscielny et al. 2004) and predicting perioperative bleeding (Rafiq et al. 2016). Indeed, preoperative platelet function testing can identify up to 97.7% of patients with impaired hemostatic status (Koscielny et al. 2004). The use of TEG and MEA is associated with decreased transfusion requirements (Corredor et al. 2015 & Ranucci et al. 2016) and costs in cardiac surgery (Agarwal et al. 2015). Interestingly, preoperative use of TEG can significantly reduce the need for re-exploration for bleeding after cardiac operations (Ranucci et al. 2016). Furthermore, a consensus statement regarding preoperative use of MEA recommended platelet transfusions and postponing the operation in cases with severe platelet dysfunction (Kong et al. 2015).

On the other hand, some authors have reported TEG to be a poor predictor of the amount of chest tube drainage and change in hemoglobin concentrations in patients undergoing cardiac surgery (Berger et al. 2015, Cherng et al. 1998, Rafiq et al. 2016).
2.7.2 Intraoperative factors

Cell salvage

An important part of blood product conservation is the use of cell savers. The routine use of these devices is considered safe and helpful in blood management (The Society of Thoracic Surgeons blood conservation guidelines 2007). Cell salvage comprises collecting, washing and re-transfusing the shed blood. Its collection is commonly achieved using standard suction devices, after which the blood is filtered and introduced into a container. However heparin must be added to stop the blood from clotting if the patient is not under full anticoagulation. Lastly, the blood is centrifuged and washed to separate excess cells, cytokines and blood components from the RBCs. (Ashworth et al. 2010).

Unprocessed shed mediastinal blood collecting onto the surgical field has been shown to contain increased concentrations of substances inducing coagulation and fibrinolysis. The aforementioned hemostatic events are known to activate an inflammation reaction (Lau et al. 2007) capable of leading to systemic inflammatory response syndrome (SIRS), which can result in severe multi-organ dysfunction. A significant increase in morbidity and mortality has been observed among patients suffering from SIRS (Agoustides 2012). Furthermore, surgical trauma detaches cellular debris and lipid particles from the tissues (Lau et al. 2007). On this basis, direct reinfusion of shed mediastinal blood without washing is not recommended and might be harmful (Society of Thoracic Surgeons blood conservation guidelines, 2007) due to the possibility of embolus formation (Lau et al. 2007).

The use of intraoperative cell salvage has been associated with significantly reduced exposure to red blood cells (RR 0.62, 95% CI 0.55-0.70, Carless et al. 2010) (Côte et al. 2016 & Wang et al. 2009) and reduction in the amount of RBCs transfused in cardiac surgery (Klein et al. 2008, Vermeijden et al. 2015 Vonk et al. 2013, Weltert et al. 2013). Furthermore, in a meta-analysis by Wang et al. (2009), red cell salvage was shown to reduce exposure not only to RBCs, but all allogeneic blood products. Intraoperative red cell salvage is associated with a saving of 1.2 units of RBCs per patient (Keeling et al. 1983). Mayer and colleagues (1985) showed that cell savers reduced the need for intra- and postoperative transfusions by approximately 33%.

However, some investigators have also reported adverse events associated with cell salvage. Intraoperative red cell saving has been suggested to impair coagulation
in all patients (Despotis et al. 1996) as well as high-risk individuals undergoing cardiac surgery (Shen et al. 2016). The effect could be caused by depletion of coagulation factors during centrifugation and washing of the salvaged blood. Furthermore, Attaran et al. (2011) concluded that, the routine use of cell salvage in all cardiac surgery is not beneficial and should be reserved only for selected cases. According to the Society of Thoracic Surgeons blood conservation guidelines (2007), the use of red cell saving devices is contraindicated in the presence of malignancy, infection or after using topical hemostatic agents.

**Surgical technique**

Minimized cardiopulmonary bypass and off-pump CABG are noteworthy options for blood management in cardiac surgery. Minimized extracorporeal circulation has been shown to reduce inflammation (Farag et al. 2016 & Fromes et al. 2002) and hemodilution (Fromes et al. 2002, The Society of Thoracic Surgeons 2007, Wiesenack et al. 2004) compared with the standard CPB. In the study by Wiesenack et al. (2004), 79% of patients undergoing conventional cardiopulmonary bypass received transfusions compared to only 39% in the minimized extracorporeal group.

The off-pump technique differs from conventional CABG since the heart is beating and cardiopulmonary bypass is not used. It is considered a reasonable method of blood conservation if conversion to on-pump operation is unlikely (Society of Thoracic Surgeons clinical practice guidelines, 2007). Indeed, recent evidence suggests that off-pump surgery is associated with a reduced need of RBC transfusions (Angelini et al. 2002, Forouzanna et al. 2011, Khan et al. 2004, Wijeysundera et al. 2005). Interestingly, off-pump surgery could have a positive effect on platelet function as well. Even though the OPCAB procedure decreased platelet count and affects their function, the adverse effects are significantly lower than in on-pump procedures. These smaller changes are shown not to affect bleeding time (Ballotta et al. 2007). According to Bidstrup et al. (2003), platelet function was even improved as a result of off-pump surgery.

**Topical agents**

There are numerous topical hemostatic agents available with different mechanisms of action. In a review by Barnard et al. (2009), Colgel, CoStasis, Cyanoacrylate Sealant, Tisseel, Beriplast, BioGlue and FloSeal were associated with improved
hemostasis compared to either other topical agents or surgical hemostasis alone in patients undergoing cardiac surgery.

Colgel and CoStasis are microfibrillar collagen products, which activate the coagulation and reinforce the generating clot. CoStasis includes also thrombin and can be mixed with the patient’s own plasma to improve fibrin formation. (Barnard et al. 2009). The use of Colgel has been associated with significantly reduced postoperative chest tube drainage after cardiac surgery (Sirlak et al. 2003). Blood contaminated by microfibrillar collagen should not be re-transfused to the patient due to the risk of end-organ ischemia (Robicsek 2004).

Fibrin sealants, e.g. Tisseel and Beriplast, are comprised of fibrinogen and thrombin, which enable clot formation. Fibrinogen and thrombin products can be used also in a solid form. Tachosil, a topical hemostatic plate, consists of collagen coated with fibrinogen and thrombin. According to Alizadeh et al. (2014), Tachosil is associated with reduced RBC transfusions. Interestingly, in type-A aortic dissection, the use of fibrin glue as a sealant between the graft and the aorta is shown to significantly improve patient survival (Kjaergard et al. 1996).

BioGlue contains bovine albumin and gluteraldehyde. Mixing the ingredients induces covalent bonding between the glue, tissues and artificial material, e.g. Dacron. Even though BioGlue has been reported to be efficacious in reducing bleeding (Coselli et al. 2003), it has been associated with several complications. LeMaire et al. (2007) reported an increased rate of nerve injury and myocardial necrosis in pigs exposed to Bioglue. LeMaire and colleagues (2002) showed that aortic reinforcement with BioGlue is associated with a higher risk of aortic stenosis in growing piglets. Furthermore, a case report with a suspicion of BioGlue induced anastomotic dehiscence and pseudoaneurysm formation has been published (Ngaage et al. 2005).

FloSeal is a combination of gelatin and thrombin. The contact with blood induces swelling of the gelatin enabling a tamponation. It is considered safe, and effective. Hemostasis has been reported to be achieved within three minutes in 72% of patients treated with FloSeal in cardiac surgery (Oz et al. 2000). In the Society of Thoracic Surgeons blood conservation guidelines (2007), the use of topical hemostatic agents is considered not unreasonable in certain high-risk procedures. However, reliable evidence on their effect is scarce.
Antifibrinolytic drugs and protamine

The three most commonly used antifibrinolytic drugs in cardiac surgery are epsilon-aminocaproic acid (EACA), tranexamic acid (TXA) and aprotinin. The former two are lysine analogues inhibiting plasminogen activity and fibrinolysis, however, tranexamic acid is considered approximately 10 times more effective. Aprotinin on the other hand, is a bovine protein, which is a protease inhibitor. It reduces fibrinolysis, thrombin generation and inflammation. (Society of Thoracic Surgeons clinical practice guidelines, 2007).

Both tranexamic acid and epsilon-aminocaproic acid have been reported to decrease postoperative bleeding and exposure to allogeneic blood products (Brown et al. 1997, Hardy et al. 1998, Speekenbrink et al. 1995). In the study conducted by Hardy and colleagues (1998) the incidence of severe bleeding decreased from 32% to 15% in patients treated with TXA or EACA and the drugs were not reported to affect serum creatinine levels perioperatively. Interestingly, aprotinin is considered to be the most effective of the three drugs, since its use has also been associated with a decrease in the rate of re-explorations for bleeding (OR 0.44, 95% CI 0.27-0.73). The OR for exposure to RBCs was (0.31, 95% CI 0.25-0.39) in patients receiving aprotinin and (0.5, 95% CI 0.34-0.76) after treatment with TXA (Laupacis et al. 1997). Tranexamic acid has been associated with decreased incidence of resternotomy as well (Shi et al. 2013).

The use of aprotinin in cardiac surgery was terminated due to safety concerns in 2007. The study conducted by Ferguson and colleagues (2008) stopped early because of an increased rate of adverse events related to aprotinin, which led to Bayer Pharmaceutical company suspending the sales of aprotinin (http://www.fda.gov/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/ucm142720.htm, accessed on 22th of Dec, 2016). The drug was associated with increased early mortality compared to TXA and EACA (Ferguson et al. 2008 & Henry et al. 2009). In the study conducted by Mangano et al. (2006), patients exposed to aprotinin had an increased risk of renal failure requiring dialysis, myocardial infarction/heart failure and stroke. In a Cochrane systematic review and meta-analysis, Henry et al. (2011) concluded that aprotinin significantly increased the risk of death compared to tranexamic acid and epsilon-aminocaproic acid (RR 1.39, 95% CI 1.02-1.89). Both lysine analogues were safe and effective in reducing bleeding during cardiac surgery.

Interestingly, the European commission has reinstated the license for aprotinin use in patients undergoing cardiopulmonary bypass. The Committee for Medicinal
Products for Human Use, reported on 2013 that the initial trials suggesting adverse events related to aprotinin were flawed. They noticed numerous methodological errors and reanalysis of the data showed no statistically significant correlation with aprotinin and early death in two studies. (The Pharmaceutical Journal, online, DOI: 10.1211/PJ.2013.11129008). This hypothesis is supported by the findings of Kluth and coworkers (2008) who reported that low-dose aprotinin does not increase adverse events after CABG. Moreover, Sedrakyan et al. (2004) showed that aprotinin administration was not associated with a change in the risk of mortality, myocardial infarction or renal failure. Interestingly, it reduced significantly the risk of stroke.

Protamine sulfate is routinely used to reverse the effects of systemic heparinization during cardiopulmonary bypass. Its dosing is important, since excessive doses can increase bleeding. In fact, Meesters et al. (2016) reported that high doses of protamine, 1.3:1 protamine to heparin ratio, are associated with significantly increased postoperative blood loss. On the other hand, a ratio of 0.8:1 was shown to decrease bleeding.

2.7.3 Postoperative factors

Postoperative cell salvage

Postoperative RBC saving is identical to intraoperative salvage, although the RBCs are collected from shed mediastinal blood. As discussed in the intraoperative cell salvage chapter, shed mediastinal blood contains different forms of debris and embolic material in addition to immunologic and hemostatic agents (Lau et al. 2007). Transfusion of unwashed chest drainage blood is therefore not recommended (Society of Thoracic Surgeons blood conservation guidelines, 2007).

Indeed, several studies have reported decreased transfusion requirements in patients who have received postoperative infusion of shed blood (Dalrymple-Hay et al. 1999 & de Varennes et al. 1996). However, in the study of Body et al. (1999), shed mediastinal blood was ineffective and increased the risk of infections in patients who did not receive allogeneic blood. Nevertheless, the Society of Thoracic Surgeons (2007) concluded in their guidelines that reinfusion of washed shed mediastinal blood is not unreasonable.
Procoagulants

The indications and effects of plasma products have been discussed in more detail in the “Transfusion of plasma and plasma products” chapter. Their use is reasonable in the presence of serious bleeding and coagulation factor deficiencies, but prophylactic use is not recommended. Despite the hemostatic effects of plasma products, they are associated with the same adverse events as RBC transfusion (Society of Thoracic Surgeons clinical practice guidelines, 2011).

Furthermore, administration of factor XIII has been reported to reduce bleeding in patients whose FXIII plasma levels are below normal (Gödje et al. 2006). The evidence on FXIII transfusion in reducing bleeding is scarce, although promising phase-1 studies have been published (Levy et al. 2009).

Activated recombinant factor VII is reserved for excessive bleeding unresponsive to other treatment modalities (Society of Thoracic Surgeons clinical practice guidelines, 2011), although the evidence on its efficacy is scarce. According to Ranucci et al. (2008a), rFVIIa was associated with a decreased risk of RBC exposure without an increase in adverse events. On the other hand, Gill and colleagues (2009) reported that patients receiving rFVIIa had increased but non-significant risks for death, stroke and other thrombotic events.

Postoperative coagulation testing

Testing the hemostatic capacity in bleeding patients is helpful to identify possible coagulation factor deficiencies and guide transfusions (Levi et al. 2015). In a Cochrane database systematic review and meta-analysis, with most of the studies including cardiac surgery patients, Wikkelso et al. (2016) showed that point-of-care coagulation tests (TEG and ROTEM) reduced significantly the risk of RBC transfusions. However, the quality of the study was considered low due to a high risk of bias. Furthermore, postoperative point-of-care coagulation testing has been reported to be associated with decreased need for re-exploration for bleeding (Deppe et al. 2016).

The advantage of point-of-care testing over routine laboratory tests is the ability to acquire results faster. The time difference between these tests could be as high as 45 minutes. Furthermore, when compared to routine testing, point-of-care tests offer a wider range of measures to assess the patient’s coagulation status, ranging from clot firmness to antiplatelet drug activity (Görlinger et al. 2013). In fact, Whiting et al. (2015) reported in their extensive study, that point-of-care
testing is more effective and able to save costs in patients undergoing cardiac surgery. The potential saving per patient ranged between 43 (£1) and 132 (£156) British Pounds.

On the other hand, the study conducted by Agarwal et al. (2015a), showed no correlation between abnormal coagulation tests and postoperative bleeding.

2.8 Summary of the literature

In this literature review, issues regarding anemia, perioperative bleeding and allogeneic blood transfusions in cardiac surgery were discussed. In conclusion, recent evidence suggests that pre- and intraoperative anemia, RBC transfusions, excessive bleeding and its derivatives (retained blood syndrome and resternotomy) might have a deleterious effect on patient outcomes, although their exact mechanisms are not completely understood. Additional research is required to identify methods to improve survival and reduce complications in patients at risk of perioperative bleeding.
3 Aims of the research

The aims of this study were to investigate the impact of preoperative anemia, perioperative bleeding and transfusions of red blood cells on patient outcomes after coronary artery bypass grafting. Furthermore, we sought to clarify the incidence and prognostic impact of retained blood syndrome (RBS) (Boyle et al. 2015) on morbidity and mortality. The aims of the individual original articles are stated below.

I to investigate the impact of severe bleeding and blood transfusion according to the UDPB, E-CABG and PLATO bleeding criteria on the development of stroke after isolated CABG.

II to investigate the incidence and prognostic impact of bleeding and transfusion of blood products according to the E-CABG and UDPB bleeding criteria in low-risk patients undergoing isolated CABG and having a EuroSCORE II < 2%.

III to investigate the incidence and impact of retained blood syndrome on postoperative outcomes in patients undergoing isolated CABG.

IV to investigate the independent roles of preoperative anemia and transfusion of red blood cells on outcomes in patients undergoing isolated CABG.
4 Materials and methods

4.1 Study designs and patient populations

All four studies were clinical observational studies. In studies I and II, we utilized the data from the prospective multicenter study E-CABG (Biancari et al. 2015), which collects a consecutive series of patients undergoing isolated CABG in several European hospitals. Both off-pump and on-pump procedures were included regardless of the urgency status. In studies III and IV, a retrospective series of 2764 patients undergoing isolated CABG between June 2006 and December 2013 at the Oulu University Hospital was analyzed.

In study I, 2357 consecutive patients undergoing isolated CABG were included from the E-CABG registry collected between January 2015 and September 2015 in 15 different European centers. Patients with preoperative carotid endarterectomy (n=97) or angioplasty (n=28) were excluded from the analysis. Bleeding severity was classified according to the E-CABG, UDPB and PLATO criteria for major or life threatening bleeding.

In study II, 1213 out of 3790 consecutive patients undergoing elective isolated CABG classified as low-risk (EuroSCORE II < 2%) without any recent episodes of unstable angina, ST elevation or non-ST elevation myocardial infarctions were included from the E-CABG registry. We also excluded patients if they were exposed to clopidogrel, ticagrelor or cangrelor within 5 days from the surgery, vitamin-K antagonists within 2 days from the surgery or if they were preoperatively exposed to NOACs. The data were collected prospectively in 16 different European centers between January 2015 and December 2015. The severity of perioperative bleeding was assessed according to the E-CABG and UDPB bleeding criteria.

In study III, 2764 consecutive patients undergoing isolated CABG from the Oulu University Hospital data registry were included. The severity of perioperative bleeding was assessed according to E-CABG bleeding criteria. In the present study, the definition of retained blood syndrome (Boyle et al. 2015) was the need for re-exploration for bleeding/hematoma washout, insertion of new pleural drain/thoracentesis for removal of bloody fluid from the pleural space or the need for pericardial fenestration/centesis for (pre)tamponade within 30 days of the index operation. Only frankly bloody fluid was considered as retained blood.

In study IV, 2761 out of 2764 consecutive patients undergoing isolated CABG from the Oulu University Hospital data were included. Anemia was defined
according to the WHO criteria as hemoglobin values < 120g/L in women < 130g/L in men. Data on the amount of transfused RBCs was retrieved from a prospective hospital registry from the operation day until hospital discharge or up to 30 days after the index operation. The severity of perioperative bleeding was stratified using the E-CABG bleeding criteria.

4.2 Data collection

In both registries, an electronic patient database was utilized. Complete pre- intra- and postoperative data were available for the majority of patients collected prospectively from the E-CABG registry. The E-CABG registry collects numerous variables on patient characteristics in accordance with the EuroSCORE II criteria (Nashef et al. 2012). Data on antithrombotic medication were acquired as well. The degree of kidney disease is estimated using Modification of Diet in Renal Disease formula (Levey et al. 1999). Moreover, data on comorbidities, cardiac function and symptoms (LVEF, NYHA, CCS), preoperative state (Killip Score, Frailty), operative risk scores (EuroSCORE II and Grace Score) and the severity of coronary disease (Syntax Score) are recorded. Factors regarding intra- and postoperative phases, blood product transfusions and possible complications are collected as well.

The retrospective Oulu University Hospital registry had complete data of pre- intra- and postoperative variables. Preoperative use of antithrombotic and anticoagulant medication and perioperative use of PCC and rFVIIa were collected retrospectively, however data on blood product transfusions were collected prospectively. The data on the amount of postoperative blood loss were retrieved from a prospective ICU database in both registries. The cause and date of death was acquired from Statistics Finland, which keeps a record of the death certificates of all inhabitants of Finland.

4.3 Patient management

In the E-CABG and Oulu University Hospital registries, withdrawal of ADP receptor antagonists > 5 days, vitamin-K dependent anticoagulants > 2 days and LMWH > 1 day before the index operation was considered adequate to avoid excessive perioperative bleeding. Aspirin was continued until the morning of the operation day in the E-CABG registry, however it was discontinued at least seven days before the surgery until 2012, in the Oulu University Hospital, after which it was continued until the operation day. Low molecular weight heparin was used
preoperatively only in patients with acute coronary syndromes. Treatment protocols for perioperative anticoagulation and RBC transfusion were not standardized between the participating centers of the E-CABG registry. In Oulu University hospital, intraoperative unfractionated heparin was administered at 3.0 mg/kg to maintain an ACT of longer than 450 seconds, which was reversed by protamine sulfate in a 1:1 ratio at the end of surgery. An intraoperative administration of tranexamic acid was at the discretion of the perfusionist. Aprotinin was not used in these patients.

In studies I and II, RBCs were transfused intraoperatively to maintain a hemoglobin concentration > 70 g/L and/or a hematocrit level > 20% during cardiopulmonary bypass. A hemoglobin concentration of 80 g/L was used as a postoperative transfusion trigger. In studies III and IV, a hemoglobin level < 90 g/L during the operation day and < 80 g/L in all subsequent days was used as a cut off point for transfusion of RBCs. Fresh frozen plasma/Octaplas, platelets and other procoagulants were transfused according to the needs of the individual patients measured by coagulation tests. Activated recombinant factor VII was used only in cases with massive bleeding unresponsive to other treatment modalities.

Patients were operated using on-pump or off-pump techniques. During the former, intermittent ante- and retrograde cold blood cardioplegia was used for myocardial protection. At the Oulu University Hospital, off-pump operation was performed using an Octopus (Medtronic, Minneapolis, MN) or Starfish stabilizer (Medtronic, Minneapolis, MN) with intracoronary shunts. Epiaortic ultrasound was performed according to the preference of the individual surgeon to assess the condition of the ascending aorta. It was considered unnecessary in patients with clearly palpable calcified plaques or porcelain aorta. A no-touch-aorta technique was used when the aorta was diseased and its clamping was considered not safe. In the latter case, revascularization was performed using a Y-graft technique. Intraoperative cell saving was performed in all patients. At the end of the operation, one 24 Fr pericardial drain in addition to one 30 Fr mediastinal drain were inserted in all patients. One to two 28 Fr pleural drains were inserted in cases of accidental pleural opening, preoperative pleural effusion or after prophylactic pericardial fenestration. The drains were connected to a 15 cm H2O wall suction via an underwater seal. The blood was collected to a sterile collection chamber and later discarded. The nurses were allowed to clear mediastinal drains by regularly milking them without the use of active clearance devices. In normal circumstances, the drains were removed within 24 hours after the surgery, but in cases of persistent
fluid accumulation, the drains were removed after the output decreased below 200 mL/day.

Postoperative anticoagulation was mainly achieved using LMWH and aspirin. Enoxaparin 40-80 mg was started on the evening of the operation day if chest tube drainage was < 1000 ml and administration of 100 mg of aspirin begun on the first postoperative day. Warfarin starting from the first postoperative day was used in patients with a history of chronic anticoagulation. Indication for de novo therapy was an onset of new persistent atrial fibrillation. ADP receptor antagonists were administered only in cases of allergy to aspirin or in cases with recently applied intracoronary stents.

Resternotomy was performed during the operation day in cases with excessive bleeding, signs of tamponade, cardiac arrest or for removal of hematoma. A drop in hemoglobin levels > 1 g/L compared to the previous measurement or after an onset of hemodynamic instability raised suspicion of postoperative bleeding. Hemoglobin levels were routinely monitored at least once a day and hemodynamic instability in addition to retained blood were ultimately diagnosed with ECHO-cardiography or x-ray imaging.

Pericardial fenestration was required in cases with deteriorated oxyhemodynamic status or in the presence of signs of tamponade. Sub-xiphoidal access was used in all of the cases. Thoracoscopic fenestration could be performed for effusions presenting after 2 weeks from the index operation if the patient’s cardiorespiratory condition allowed bronchial intubation.

4.4 Outcome endpoints

The primary outcome endpoints of these studies were the in-hospital, 30-day and late mortality, acute kidney injury according to the KDIGO criteria (KDIGO, CKD work group, 2013), sternal wound infections, the E-CABG complication grades, the E-CABG complications score, the incidence and severity of bleeding in low-risk patients (EuroSCORE II < 2%, E-CABG and UDPB criteria) and any onset of temporary or permanent ischemic stroke occurring during the in-hospital stay after CABG. Postoperative stroke was defined as any focal or global neurologic disorder not resolving within 24 hours of its onset. The diagnosis of stroke was made using computed tomography or magnetic resonance imaging and confirmed by a neurologist. Temporal stroke was defined as any focal or global neurologic symptoms disappearing before discharge.
The secondary outcome endpoints were the effect of bleeding and blood transfusions in low-risk patients (EuroSCORE II < 2%), the length of intensive care unit stay, incidences of atrial fibrillation, ventricular fibrillation or asystole, postoperative use of antibiotics, mediastinitis, low cardiac output syndrome (classified as postoperative cardiac index < 2.0L/min/m² measured at least twice), repeat revascularization, gastrointestinal complications requiring surgery, nadir postoperative hemoglobin level, the amount of 12 hour chest drain output and the need for permanent pacemaker implantation. Table 10. summarizes the E-CABG complication grades and scores (Biancari et al. 2015).

4.5 Ethical considerations

The study was approved by the Review Board of the Oulu University Hospital and the review boards of other hospitals participating to the E-CABG registry. Due to the clinical and observational nature of the study, individual patient consent was waived. The studies were not financially supported.
Table 10. E-CABG complication grades and score.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Postoperative complication</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>Postoperative use of antibiotics</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Atrial fibrillation</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Transfusion of platelets</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Transfusion of FFP or Octaplas</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Transfusion of 2-4 units of RBCs</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Deep leg wound infection</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Permanent pacemaker implantation</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>Pericardial effusion requiring fenestration</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Acute kidney injury not requiring dialysis</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Transfusion of 5-10 units of RBCs</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Resternotomy for bleeding</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Deep sternal wound infection</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Postoperative intra-aortic balloon pump</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>Transfusion of &gt;10 units of RBCs</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Renal failure requiring dialysis</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Mediastinitis</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Stroke</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Surgical or percutaneous procedure for technical failure</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Reoperation for hemodynamic instability</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Ventricular fibrillation/asystole</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Surgery for gastrointestinal complications</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Extracorporeal membrane oxygenation</td>
<td>9</td>
</tr>
<tr>
<td>4</td>
<td>In-hospital death</td>
<td>10</td>
</tr>
</tbody>
</table>

4.6 Statistical analyses

Statistical analyses were performed using SPSS statistical software (version 23.0, IBM Corporation, New York, USA) in all substudies. No attempt to replace missing values was made in any of these studies. All tests were two-sided with the alpha level set at 0.05 to represent statistical significance.

Throughout the studies, nominal variables are reported as count and percentage, and continuous variables are reported as the mean and standard deviation. Univariate analysis was performed using Chi-square and Fisher’s exact test for
nominal variables and Mann-Whitney-U test for continuous variables. In multivariate analysis, logistic, ordinal and linear regressions were used for dichotomous, categorical and continuous variables, respectively. The fit of the multivariate analyses were assessed using Hosmer-Lemeshow goodness-of-fit test and the discriminatory ability was assessed using the C-statistics test. Risk estimates were reported as ORs, HRs, RRs or beta-coefficients with 95% CIs. Patient survival was assessed using Kaplan-Meier and Cox proportion hazards methods.

In study I, the risk factors associated with postoperative stroke were identified using logistic regression with backward selection. Only variables with p-values < 0.05 in univariate analysis were included in the multivariate model. Another multivariate analysis was performed to adjust the risk of stroke with increasing E-CABG, UDPB and PLATO bleeding grades for any possible covariates associated with the development of stroke, i.e. preoperative use of unfractionated heparin, critical preoperative state, emergency operation, use of CPB, diseased ascending aorta and the amount of chest tube output 12 hours after the surgery. Furthermore, a few additional variables were forced into the model (amount of RBC transfusions, administration of fibrinogen, PCC, new onset of POAF, prolonged use of inotropes after surgery, use of IABP and the use of extracorporeal mechanical oxygenation).

In study II, the effect of different bleeding severity grades on outcomes was evaluated using a logistic regression model. Possible confounding factors identified in the univariate analysis with a p-value < 0.2 were included in the multivariate analysis (gender, BMI, baseline hemoglobin, baseline hematocrit, INR, baseline thromboplastin time, poor mobility, eGFR, left ventricular EF, prior PCI, Syntax score, the use of both mammary arteries and off-pump operation).

In study III, the impact of retained blood on patient outcomes was assessed using logistic, ordinal and linear regression models and adjusted for variables with a p-value < 0.05 in univariate analysis (BMI, age, dialysis, recent myocardial infarction, critical preoperative status and urgency of the operation). Predictors of RBS were also identified in multivariate analysis.

In study IV, a propensity score matching was employed to select two groups of patients with similar baseline and operational characteristics with and without preoperative anemia. The individual propensity score was acquired using a non-parsimonious logistic regression model with anemia status set as the dependent variable, meaning that all possible variables were included as covariates during propensity score acquisition. The following variables were included as covariates: gender, age, BMI, e GFR, platelet count, pulmonary disease, dialysis, preoperative
stroke, diabetes, extracardiac arteriopathy, atrial fibrillation, neurological dysfunction, previous cardiac surgery, previous PCI, LVEF ≤ 50%, critical preoperative status, recent myocardial infarction, preoperative IAPB, cardiac massage, recent ventricular arrhythmia, operational urgency, off-pump surgery, epiaortic ultrasound, diseased ascending aorta, radial artery graft, and bilateral mammary artery grafts. Subsequently, a 1:1 propensity score matching was performed with a caliper width of 0.02 of the standard deviation of the logit of the propensity score. Standardized differences evaluated the success of the matching, values differing < 10% representing an acceptable result. Univariate analyses between the matched groups were assessed using the McNemar test for dichotomous variables and the paired sample t-test for continuous variables. Logistic, ordinal and linear regression models in addition to Cox proportional hazards estimate of propensity matched pairs were adjusted for the E-CABG bleeding grades to investigate the impact of blood product transfusions and re-exploration for excessive bleeding on the outcomes. In another multivariate analysis, preoperative anemia was adjusted for the effect of the amount of RBCs transfused.
5 Results

5.1 Bleeding, transfusions and the risk of stroke

In study I, the incidence of postoperative stroke was 1.3% and it remained permanent in 0.9% of the patients. The amount of RBCs transfused during surgery (OR 1.09, 95% CI 1.01-1.17, p = 0.022), RBCs transfused during and after the operation (OR 1.10, 95% CI 1.03-1.18, p = 0.003) in addition to emergency procedure (OR 3.97, 95%CI 1.47-10.74, p = 0.007), preoperative use of unfractionated heparin (OR 4.49, 95% CI 1.91-10.60, p = 0.001), atherosclerosis of the ascending aorta (OR 4.62, 95% CI 1.37-15.65, p = 0.014) and the use of cardiopulmonary bypass (OR 4.85, 95% CI 1.05-22.36, p = 0.043) were all identified as independent predictors of postoperative stroke. After including the highest degree of carotid artery stenosis as a covariate in the multivariate model, the amount of RBC units transfused was the only independent predictor of stroke (OR 1.29, 95% CI 1.13-1.46, p < 0.0001). The results remained similar after including a bilateral carotid stenosis > 50% to the model.

Moreover, severe bleeding classified as E-CABG grades 2-3, UDPB grades 3-4 and PLATO life threatening bleeding were independent predictors of postoperative stroke, adjusted ORs (OR 5.91, 95% CI 2.43-14.36, OR 2.66, 95% CI 1.05-6.73, OR 3.70, 1.59-8.64, respectively). Incidence of postoperative stroke in patients with and without preoperative anemia, experiencing severe-massive bleeding according to the UDPB (p<0.0001), E-CABG (p<0.0001) and PLATO (p<0.0001) definition criteria is presented in Figure 1 and the adjusted risk estimates of postoperative stroke in patients with severe-massive bleeding in Table 11.
Fig. 1. Incidence of postoperative stroke in patients with and without preoperative anemia, experiencing severe-massive bleeding according to the UDPB, E-CABG and PLATO definition criteria.

Table 11. Adjusted risk estimates of postoperative stroke in patients with severe-massive bleeding.

<table>
<thead>
<tr>
<th>Bleeding classification methods</th>
<th>Reference category</th>
<th>Severe-massive bleeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>UDPB classes</td>
<td>0-2</td>
<td>3-4</td>
</tr>
<tr>
<td>Crude estimate</td>
<td>-</td>
<td>3.63 (1.60-8.25)</td>
</tr>
<tr>
<td>Adjusted estimate</td>
<td>-</td>
<td>3.18 (1.32-7.65)</td>
</tr>
<tr>
<td>E-CABG bleeding grades</td>
<td>0-1</td>
<td>2-3</td>
</tr>
<tr>
<td>Crude estimate</td>
<td>-</td>
<td>7.31 (3.36-15.90)</td>
</tr>
<tr>
<td>Adjusted estimate</td>
<td>-</td>
<td>7.04 (2.99-16.55)</td>
</tr>
<tr>
<td>PLATO bleeding grades</td>
<td>No or major bleeding</td>
<td>Life-threatening bleeding</td>
</tr>
<tr>
<td>Crude estimate</td>
<td>-</td>
<td>4.19 (1.91-9.19)</td>
</tr>
<tr>
<td>Adjusted estimate</td>
<td>-</td>
<td>3.10 (1.36-7.09)</td>
</tr>
</tbody>
</table>

5.2 Incidence and impact of bleeding and transfusion in low-risk patients

In study II, 3.4% of low-risk patients were observed to experience severe bleeding according to the E-CABG grades 2-3, whereas 18.5% of patients had an E-CABG grade 1. Twelve-hour chest tube output > 1000 ml was present in 3.6% of patients and the resternotomy rate was 2.3%. Transfusion of RBCs was administered to 27.5%
of the patients. There were no differences between the incidences of severe bleeding among the participating centers despite a varying proportion of low-risk patients.

In multivariate analysis, an association with increasing E-CABG bleeding grades and acute kidney injury (E-CABG grade 1, OR 1.81, 95% CI 1.23-2.65), the composite end-point E-CABG complication grade 3 (E-CABG grade 1, OR 2.88, 95% CI 1.41-5.89), delirium (E-CABG grade 1, OR 2.34, 95% CI 1.07-5.12) and a prolonged need for inotropes (E-CABG grade 1, OR 1.67, 95% CI 1.14-2.46) was observed. Furthermore, the E-CABG bleeding grades 2-3 were significantly associated with an increased risk of in-hospital mortality (OR 123.07, 95% CI 7.73-1960.36), stroke (OR 11.28, 95% CI 1.96-64.97), pericardial fenestration (OR 14.13, 95% CI 3.96-51.00), postoperative need for antibiotics (OR 2.48, 95% CI 1.01-6.06) and sternal wound infections (OR 3.22, 95% CI 1.10-9.32). Increased grades of UDPB predicted similar outcomes. The risk estimates of adverse events as adjusted by including baseline and operative covariates with p<0.02 in univariate analysis are presented in Table 12.

Table 12. Risk estimates of adverse events according to the E-CABG bleeding severity grades and multivariate analysis.

<table>
<thead>
<tr>
<th>Complication</th>
<th>E-CABG bleeding severity grades</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grade 0 Grade 1 Grades 2-3</td>
<td></td>
</tr>
<tr>
<td>In-hospital death</td>
<td>Ref. categ.</td>
<td>3.56, 0.17-72.93</td>
</tr>
<tr>
<td>Stroke</td>
<td>Ref. categ.</td>
<td>1.41, 0.27-7.41</td>
</tr>
<tr>
<td>Delirium</td>
<td>Ref. categ.</td>
<td>2.34, 1.07-5.12</td>
</tr>
<tr>
<td>Prolonged use of inotropes</td>
<td>Ref. categ.</td>
<td>1.67, 1.14-2.46</td>
</tr>
<tr>
<td>Acute kidney injury</td>
<td>Ref. categ.</td>
<td>1.81, 1.23-2.65</td>
</tr>
<tr>
<td>Postop. antibiotics</td>
<td>Ref. categ.</td>
<td>1.26, 0.76-2.11</td>
</tr>
<tr>
<td>Sternal wound infection</td>
<td>Ref. categ.</td>
<td>1.82, 0.88-3.79</td>
</tr>
<tr>
<td>Pericardial fenestration</td>
<td>Ref. categ.</td>
<td>1.06, 0.22-5.00</td>
</tr>
<tr>
<td>E-CABG complication grade 3</td>
<td>Ref. categ.</td>
<td>2.88, 1.41-5.89</td>
</tr>
</tbody>
</table>

5.3 Outcome after procedures for retained blood syndrome

In study III, 9.2% of the patients required at least one procedure for removal of retained blood. One procedure was performed on 7.6%, two procedures for 1.4%, three procedures for 0.1% and five procedures for 0.03% of the patients. The resternotomy rate for retained blood removal was 6.5% after a mean of 1.8±4.1
days and 24.9% of patients requiring re-exploration were operated ≥ 2 days from the index operation. After exclusion of patients undergoing re-explorations, the incidence of RBS was 2.6%. Pleural drainage was required in 1.8%, pericardial fenestration in 1.4% and thoracentesis in 0.9% of the patients. Hemodynamic signs of tamponade were present in 80% of patients undergoing pericardial fenestration. Constrictive pericarditis requiring surgery was diagnosed in two patients, one of whom had retained blood accumulation during the index operation.

In multivariate analysis, age (OR 1.02, 95% CI 1.00-1.03, p = 0.03), preoperative dialysis (OR 4.93, 95% CI 2.00-12.33, p = 0.001) and critical preoperative state (OR 1.77, 95% CI 1.18-2.66, p = 0.006) were identified as independent predictors of retained blood syndrome. Indeed, RBS was more common in patients with severe bleeding according to E-CABG bleeding criteria.

Retained blood syndrome was associated with lower nadir hemoglobin values (76±10 g/L vs. 84±13 g/L, p<0.0001) even after excluding re sternotomy for bleeding. Interestingly, chest tube drainage was significantly higher among patients with retained blood when compared to controls, but the difference did not persist in patients not requiring re sternotomy (965±732 mL vs. 456±305 mL, p < 0.0001) and (480±368 mL vs. 456±305 mL, p = 0.95) respectively.

In multivariate analysis, RBS was an independent predictor of in-hospital (OR 2.26, 95% CI 1.14-4.48) and 30-day mortality (OR 2.11, 95% CI 1.15-3.86), however after exclusion of re sternotomy, statistical significance did not persist. The procedures for retained blood without re sternotomy were associated with an increased risk of new atrial fibrillation (OR 1.80, 95% CI 1.04-3.09), mediastinitis or deep sternal wound infection (OR 6.01, 95% CI 2.68-13.47), need for antibiotics (OR 4.78, 95% CI 2.80-8.14), acute kidney injury (Beta coefficient -3.41, 95% CI -6.11- -0.71) and gastrointestinal complications requiring surgery (OR 5.94, 95% CI 1.86-18.97), which resulted in increased ICU length of stay (OR 2.32, 95% CI 1.82-2.81). Furthermore, RBS regardless of re sternotomy status increased significantly the risk of composite outcome end-point, the E-CABG complications grade 3 (adjusted OR with excluded re sternotomy, 2.80, 95% CI 1.48-5.32).

Re-exploration for bleeding was found to be associated with increased risk of in-hospital (adjusted OR 2.55, 95% CI 1.18-5.51), 30-day mortality (adjusted OR 3.00, 95% CI 1.56-5.74), low cardiac output syndrome (adjusted OR 1.73, 95% CI 1.16-2.59), acute kidney injury (adjusted OR 2.42, 95% CI 1.64-3.52), E-CABG complications grade 3 (adjusted OR 3.40, 95% CI 2.24-5.17) and increased E-CABG complications score (adjusted beta coefficient 2.16, 95% CI 1.53-2.78).
outcomes related to retained blood syndrome with or without resternotomy and re-exploration for bleeding is shown in Table 13.

The incidence of retained blood syndrome in patients with stable hemodynamic conditions, i.e. without critical preoperative status or postoperative hemodynamic instability, was 6.3% with 4.7% of patients undergoing re-exploration for bleeding. Procedures for RBS were significantly associated with an increased risk of mediastinitis or deep sternal wound infections (adjusted OR 3.04, 95% CI 1.21-7.64), acute kidney injury (adjusted OR 2.66, 95% CI 1.53-4.63), E-CABG complications grade 3 (adjusted OR 2.74, 95% CI 1.30-5.82) and E-CABG complications grade (adjusted beta coefficient 1.19, 95% CI 0.64-1.74). Table 14 represents the outcomes associated with RBS in patients without unstable hemodynamic status.
<table>
<thead>
<tr>
<th>Complication</th>
<th>Overall series</th>
<th>No procedure for RBS</th>
<th>Procedure for RBS</th>
<th>Univariate analysis P-value</th>
<th>Adjusted risk estimate (95%CI)</th>
<th>Resternotomy for bleeding</th>
<th>Univariate analysis P-value</th>
<th>Adjusted risk estimate (95%CI)</th>
<th>Procedure for RBS excluding resternotomy</th>
<th>Univariate analysis P-value</th>
<th>Adjusted risk estimate (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>In-hospital death</td>
<td>66 (2.4)</td>
<td>50 (2.0)</td>
<td>16 (6.3)</td>
<td>&lt;0.01</td>
<td>2.26, 1.14-4.48</td>
<td>13 (7.2)</td>
<td>&lt;0.01</td>
<td>2.55, 1.18-5.51</td>
<td>3 (4.1)</td>
<td>0.20</td>
<td>1.77, 0.48-6.66</td>
</tr>
<tr>
<td>30-day mortality</td>
<td>89 (3.2)</td>
<td>68 (2.7)</td>
<td>21 (8.3)</td>
<td>&lt;0.01</td>
<td>2.11, 1.15-3.86</td>
<td>19 (10.5)</td>
<td>&lt;0.01</td>
<td>3.00, 1.56-5.74</td>
<td>2 (2.7)</td>
<td>1.00</td>
<td>0.52, 0.10-2.58</td>
</tr>
<tr>
<td>ICU stay (days)</td>
<td>2.2±2.5</td>
<td>2.0±2.1</td>
<td>3.4±4.4</td>
<td>&lt;0.01</td>
<td>1.74, 1.45-2.04</td>
<td>3.7±4.1</td>
<td>&lt;0.01</td>
<td>1.53, 1.20-1.85</td>
<td>4.6±5.1</td>
<td>&lt;0.01</td>
<td>2.32, 1.82-2.81</td>
</tr>
<tr>
<td>Stroke</td>
<td>58 (2.1)</td>
<td>48 (1.9)</td>
<td>10 (3.9)</td>
<td>0.03</td>
<td>1.57, 0.75-3.32</td>
<td>8 (4.4)</td>
<td>0.02</td>
<td>1.83, 0.80-4.21</td>
<td>2 (2.7)</td>
<td>0.65</td>
<td>0.95, 0.22-4.18</td>
</tr>
<tr>
<td>New atrial fibrillation</td>
<td>946 (38.1)</td>
<td>843 (37.3)</td>
<td>103 (46.0)</td>
<td>0.01</td>
<td>1.33, 1.01-1.75</td>
<td>87 (44.1)</td>
<td>0.11</td>
<td>1.19, 0.86-1.64</td>
<td>33 (54.1)</td>
<td>0.01</td>
<td>1.80, 1.04-3.09</td>
</tr>
<tr>
<td>Ventricular fibrillation/asystole</td>
<td>49 (1.8)</td>
<td>39 (1.6)</td>
<td>10 (3.9)</td>
<td>0.01</td>
<td>1.97, 0.93-4.19</td>
<td>7 (3.9)</td>
<td>0.02</td>
<td>2.01, 0.88-4.97</td>
<td>3 (4.1)</td>
<td>0.11</td>
<td>1.88, 0.50-7.00</td>
</tr>
<tr>
<td>Low cardiac output syndrome</td>
<td>383 (13.9)</td>
<td>328 (13.1)</td>
<td>55 (21.7)</td>
<td>&lt;0.01</td>
<td>1.74, 1.24-2.45</td>
<td>37 (20.4)</td>
<td>&lt;0.01</td>
<td>1.73, 1.16-2.59</td>
<td>18 (24.7)</td>
<td>&lt;0.01</td>
<td>1.71, 0.94-3.08</td>
</tr>
<tr>
<td>Repeat CABG or PCI</td>
<td>14 (0.5)</td>
<td>9 (0.4)</td>
<td>5 (2.0)</td>
<td>&lt;0.01</td>
<td>5.72, 1.87-17.43</td>
<td>4 (2.2)</td>
<td>&lt;0.01</td>
<td>6.78, 2.04-22.49</td>
<td>1 (1.4)</td>
<td>0.25</td>
<td>3.49, 0.41-29.60</td>
</tr>
<tr>
<td>Postop. use of antibiotics</td>
<td>937 (33.9)</td>
<td>806 (32.1)</td>
<td>131 (51.6)</td>
<td>&lt;0.01</td>
<td>2.08, 1.58-2.74</td>
<td>80 (44.2)</td>
<td>&lt;0.01</td>
<td>1.51, 1.09-2.09</td>
<td>51 (69.9)</td>
<td>&lt;0.01</td>
<td>4.78, 2.80-8.14</td>
</tr>
<tr>
<td>Deep SWI/mediastinitis</td>
<td>72 (2.6)</td>
<td>55 (2.2)</td>
<td>17 (6.7)</td>
<td>&lt;0.01</td>
<td>3.12, 1.72-5.66</td>
<td>8 (4.4)</td>
<td>0.06</td>
<td>2.01, 0.89-4.54</td>
<td>9 (12.3)</td>
<td>&lt;0.01</td>
<td>6.01, 2.68-13.47</td>
</tr>
<tr>
<td>Complication</td>
<td>Overall series No.</td>
<td>Procedure for RBS No.</td>
<td>Univariate analysis P-value</td>
<td>Adjusted risk estimate (95%CI)</td>
<td>Procedure for RBS excluding resternotomy No.</td>
<td>Univariate analysis P-value</td>
<td>Adjusted risk estimate (95%CI)</td>
<td></td>
<td></td>
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<td>------------------------------------</td>
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</tr>
<tr>
<td>Surgery for gastroint. compl.</td>
<td>32 (1.2)</td>
<td>22 (0.9)</td>
<td>10 (3.9) &lt;0.01</td>
<td>3.80, 1.67-8.64</td>
<td>6 (3.3) &lt;0.01</td>
<td>3.17, 1.16-8.71</td>
<td>4 (5.5) &lt;0.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute kidney injury</td>
<td>457 (16.9)</td>
<td>377 (15.3)</td>
<td>80 (32.7) &lt;0.01</td>
<td>2.50, 1.81-3.46</td>
<td>54 (30.9) &lt;0.01</td>
<td>2.42, 1.64-3.52</td>
<td>26 (37.1) &lt;0.01</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Nadir hemoglobin (g/L)</td>
<td>83±13</td>
<td>84±13</td>
<td>74±10 &lt;0.01</td>
<td>-6.31, -7.80-4.83</td>
<td>75±8 &lt;0.01</td>
<td>-7.49, -9.17-5.71</td>
<td>78±11 &lt;0.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood loss at 12 hours (mL)</td>
<td>503±394</td>
<td>456±305</td>
<td>965±732 &lt;0.01</td>
<td>503±5.5-551.0</td>
<td>1167±752 &lt;0.01</td>
<td>707±653-761</td>
<td>480±368 0.95</td>
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</tr>
<tr>
<td>E-CABG bleeding grades</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
<td>-3.24, -3.54--2.93</td>
<td>&lt;0.01</td>
<td>-4.34, -4.78--3.90</td>
<td>&lt;0.01 -1.13, -1.58--0.68</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Grade 1</td>
<td>1117 (40.4)</td>
<td>1091 (43.5)</td>
<td>26 (10.2)</td>
<td>0</td>
<td>26 (10.2)</td>
<td>0</td>
<td>26 (35.6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 2</td>
<td>506 (18.3)</td>
<td>332 (13.2)</td>
<td>174</td>
<td>153 (84.5)</td>
<td>21 (28.8)</td>
<td>3.90</td>
<td>&lt;0.01 -1.13, -1.58--0.68</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 3</td>
<td>68 (2.5)</td>
<td>31 (1.2)</td>
<td>37 (14.6)</td>
<td>28 (15.5)</td>
<td>28 (15.5)</td>
<td>9 (12.3)</td>
<td>28.0, 1.48-5.32</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E-CABG compl. grade 3</td>
<td>186 (7.2)</td>
<td>172 (6.9)</td>
<td>54 (21.3) &lt;0.01</td>
<td>3.24, 2.24-4.64</td>
<td>40 (22.1) &lt;0.01</td>
<td>3.40, 2.24-5.17</td>
<td>14 (19.2) &lt;0.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E-CABG compl. score*</td>
<td>3.3±4.7</td>
<td>3.0±4.2</td>
<td>6.1±7.0 &lt;0.01</td>
<td>2.51, 1.96-3.06</td>
<td>5.7±6.8 &lt;0.01</td>
<td>2.16, 1.53-2.78</td>
<td>7.1±7.4 &lt;0.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 14. Outcomes associated with RBS in patients without unstable hemodynamic status.

<table>
<thead>
<tr>
<th>Complication</th>
<th>No procedure for RBS No. 1657</th>
<th>Procedure for RBS No. 112</th>
<th>Univariate analysis P-value</th>
<th>Adjusted risk estimate (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>In-hospital death</td>
<td>12 (0.7)</td>
<td>4 (3.6)</td>
<td>0.02</td>
<td>3.46, 0.66-18.09</td>
</tr>
<tr>
<td>30-day mortality</td>
<td>20 (1.2)</td>
<td>4 (3.6)</td>
<td>0.06</td>
<td>0.64, 0.08-5.46</td>
</tr>
<tr>
<td>ICU stay (days)</td>
<td>1.4±1.5</td>
<td>2.2±1.7</td>
<td>&lt;0.01</td>
<td>0.55, 0.32-0.77</td>
</tr>
<tr>
<td>Stroke</td>
<td>20 (1.2)</td>
<td>2 (1.8)</td>
<td>0.65</td>
<td>0.69, 0.09-5.36</td>
</tr>
<tr>
<td>New atrial fibrillation</td>
<td>589 (35.5)</td>
<td>39 (34.8)</td>
<td>0.88</td>
<td>0.92, 0.60-1.42</td>
</tr>
<tr>
<td>Ventricular fibrillation/asystole</td>
<td>10 (0.6)</td>
<td>2 (1.8)</td>
<td>0.17</td>
<td>3.02, 0.62-14.74</td>
</tr>
<tr>
<td>Low cardiac output syndrome</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Repeat CABG or PCI</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Postop. use of antibiotics</td>
<td>435 (26.3)</td>
<td>49 (43.6)</td>
<td>&lt;0.01</td>
<td>2.05, 1.37-3.08</td>
</tr>
<tr>
<td>Deep SWI/mediastinitis</td>
<td>34 (2.1)</td>
<td>7 (6.3)</td>
<td>&lt;0.01</td>
<td>3.04, 1.21-7.64</td>
</tr>
<tr>
<td>Surgery for gastrointestinal compl.</td>
<td>5 (0.3)</td>
<td>1 (0.9)</td>
<td>0.33</td>
<td>0, 0-0</td>
</tr>
<tr>
<td>Acute kidney injury</td>
<td>140 (8.6)</td>
<td>22 (20.2)</td>
<td>&lt;0.01</td>
<td>2.66, 1.53-4.63</td>
</tr>
<tr>
<td>Nadir hemoglobin (g/L)</td>
<td>86±12</td>
<td>77±10</td>
<td>&lt;0.01</td>
<td>-6.84, -9.06- -4.61</td>
</tr>
<tr>
<td>Blood loss at 12 hours (mL)</td>
<td>460±258</td>
<td>1025±780</td>
<td>&lt;0.01</td>
<td>551, 490-612</td>
</tr>
<tr>
<td>E-CABG bleeding grades</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 1</td>
<td>670 (40.4)</td>
<td>9 (8.0)</td>
<td></td>
<td>-3.78, -4.26 - -3.30</td>
</tr>
<tr>
<td>Grade 2</td>
<td>122 (7.4)</td>
<td>85 (75.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 3</td>
<td>6 (0.4)</td>
<td>7 (6.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E-CABG compl. grade 3</td>
<td>56 (3.4)</td>
<td>12 (10.7)</td>
<td>&lt;0.01</td>
<td>2.74, 1.30-5.82</td>
</tr>
<tr>
<td>E-CABG compl. score*</td>
<td>2.0±2.9</td>
<td>3.7±5.2</td>
<td>&lt;0.01</td>
<td>1.19, 0.64-1.74</td>
</tr>
</tbody>
</table>

5.4 The effect of preoperative anemia on the outcome after coronary surgery

The incidence of preoperative anemia in our database of patients undergoing coronary artery bypass grafting was 24%, 22.8% in men and 28.6% in women. In unadjusted analysis, preoperative anemia was associated with increased in-hospital, 30-day and long-term mortality (p < 0.0001 in all). Unadjusted eight-year survival in anemic and non-anemic patients was 62.4% and 80.2%.

In univariate analysis, preoperative anemia was associated with significantly increased risk of RBC exposure and lower nadir hemoglobin values (p < 0.0001 in both), however blood loss at 12 hours was comparable. Longer ICU stay, ventricular fibrillation or asystole, atrial fibrillation, acute kidney injury,
postoperative use of antibiotics ($p < 0.0001$ in all), postoperative stroke ($p = 0.001$), surgery for gastrointestinal complications ($p = 0.001$) and low cardiac output syndrome ($p = 0.003$) were more frequent in preoperative anemic patients.

Propensity score matching was performed, which resulted in 560 patient pairs with similar baseline and operational factors represented as standardized differences $< 10\%$. After matching, in-hospital and 30-day mortality was similar between anemic and non-anemic patients, however the long-term survival was better in non-anemic patients ($p = 0.047$). In these propensity score matched pairs, preoperative anemia was associated with increased exposure to RBCs and lower nadir hemoglobin levels ($7.6\pm0.9$ vs. $8.3\pm1.1$ g/dL) ($p < 0.0001$ in both), however the amount of 12 hour chest tube drainage and the rate of resternotomy were similar between the groups.

In further analyses of the propensity score matched patients, an adjustment for the E-CABG bleeding classification showed only an increased risk of acute kidney injury associating with preoperative anemia (OR $1.50$, 95% CI $1.10$-$2.03$). Outcomes in the overall series are represented in Table 15. Finally, a covariate-adjusted analysis was performed. It showed that anemia was significantly associated only with an increased use of antibiotics, any acute kidney injury and late mortality. Interestingly, the E-CABG bleeding grades were associated with a significantly increased risk of 30-day mortality ($p<0.0001$), stroke ($p=0.002$), ventricular fibrillation/asystole ($p=0.008$), low cardiac output syndrome ($p<0.0001$), repeat revascularization ($p=0.006$), use of postoperative antibiotics ($p<0.0001$), mediastinitis ($p<0.0001$), surgery for gastrointestinal complications ($p<0.0001$), renal replacement therapy ($p<0.0001$), any acute kidney injury ($p<0.0001$) and long term mortality ($p<0.0001$), when it was introduced into the model. A regression model with both anemia and E-CABG bleeding grades included showed that anemia still had a trend towards higher long-term mortality ($p=0.051$, HR $1.230$, 95%CI $0.999$-$1.514$).

A Cox proportional hazards estimate of the propensity matched pairs stratified by the E-CABG bleeding classification showed that preoperative anemia was not an independent predictor of late death (HR $1.10$, 95% CI $0.86$-$1.39$), Figure 2. Interestingly, in this regression model, E-CABG bleeding grades 1-3 were significantly and increasingly predictive of late mortality compared to E-CABG grade 0 (Grade 1 HR $1.93$, 95%CI $1.37$-$2.73$, Grade 2 HR $2.19$ 95%CI $1.50$-$3.18$, Grade 3 HR $5.59$, 95%CI $3.34$-$9.39$). Likewise, anemia adjusted for the amount of transfused RBCs was not associated with late death, whereas the number of RBCs was shown to increase the risk.
Fig. 2. Cox proportional hazards estimate of survival in patients with and without anemia as adjusted by the E-CABG bleeding severity classification (HR, 1.10, 95%CI 0.86-1.39).
Table 15. Outcomes in the overall series.

<table>
<thead>
<tr>
<th>Complication</th>
<th>Overall series</th>
<th>Propensity score matched pairs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Overall population</td>
<td>No preop. anemia</td>
</tr>
<tr>
<td></td>
<td>No. 2761</td>
<td>No. 2099</td>
</tr>
<tr>
<td>In-hospital death</td>
<td>66 (2.4)</td>
<td>38 (1.8)</td>
</tr>
<tr>
<td>30-day mortality</td>
<td>89 (3.2)</td>
<td>48 (2.3)</td>
</tr>
<tr>
<td>ICU stay (days)</td>
<td>2.2±2.5</td>
<td>1.9±2.0</td>
</tr>
<tr>
<td>Stroke</td>
<td>58 (2.1)</td>
<td>33 (1.6)</td>
</tr>
<tr>
<td>Repeat CABG or PCI</td>
<td>14 (0.5)</td>
<td>10 (0.5)</td>
</tr>
<tr>
<td>Post-operative use of antibiotics</td>
<td>935 (33.9)</td>
<td>613 (29.2)</td>
</tr>
<tr>
<td>Deep sternal wound infection</td>
<td>40 (1.4)</td>
<td>26 (1.2)</td>
</tr>
<tr>
<td>Mediastinitis</td>
<td>43 (1.6)</td>
<td>29 (1.4)</td>
</tr>
<tr>
<td>Surgery for gastrointest. compl.</td>
<td>32 (1.2)</td>
<td>16 (0.8)</td>
</tr>
<tr>
<td>Acute kidney injury</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Complication</td>
<td>Overall series</td>
<td>Propensity score matched pairs</td>
</tr>
<tr>
<td>---------------------------------------------</td>
<td>----------------</td>
<td>--------------------------------</td>
</tr>
<tr>
<td></td>
<td>Overall population</td>
<td>No. 2761</td>
</tr>
<tr>
<td>Grade 1</td>
<td>325 (12.0)</td>
<td>181 (8.7)</td>
</tr>
<tr>
<td>Grade 2</td>
<td>71 (2.6)</td>
<td>40 (1.9)</td>
</tr>
<tr>
<td>Grade 3</td>
<td>61 (2.3)</td>
<td>33 (1.6)</td>
</tr>
<tr>
<td>New renal replacement therapy</td>
<td>51 (1.9)</td>
<td>28 (1.3)</td>
</tr>
<tr>
<td>Acute kidney injury without dialysis</td>
<td>396 (14.3)</td>
<td>223 (10.6)</td>
</tr>
<tr>
<td>Survival</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>1-year</td>
<td>96.0% (2015)</td>
<td>88.7% (587)</td>
</tr>
<tr>
<td>5-year</td>
<td>88.4% (1287)</td>
<td>72.7% (319)</td>
</tr>
<tr>
<td>8-year</td>
<td>80.2% (471)</td>
<td>62.4% (82)</td>
</tr>
</tbody>
</table>
6 Discussion

6.1 Bleeding, transfusions and the risk of stroke

Study I provides evidence that bleeding necessitating transfusion of blood products is associated with a significantly increased risk of stroke. After adjustment for several other relevant risk factors for stroke in multivariate models, e.g. the use ofCPB and clamping of diseased ascending aorta, RBC transfusion retains its independent prognostic value.

Furthermore, preoperative administration of unfractionated heparin in addition to operative urgency was also an independent predictor of stroke. Indeed, the use of unfractionated heparin and urgent/emergent operation increased the likelihood of exposure to potent antiplatelet drugs. The potential increase in bleeding (Hessel 2015) and the incidence of stroke (Nissinen et al. 2009) have been also identified in patients requiring prolonged CPB.

Our findings confirm the results of a number of studies reporting an increased risk of stroke associated with transfusion of blood products (Kinnunen et al. 2015a, Mariscalco et al. 2015, Mikkola et al. 2012). However bleeding stratification according to the E-CABG, UDPB and PLATO was not available at the time of these studies. Indeed, study I showed that the risk of stroke is substantially elevated only in patients with severe bleeding requiring large amounts of red blood cells. As the definition of major bleeding according to the PLATO criteria is much more liberal than E-CABG or UDPB, the low incidence of stroke (0.3%) in patients without major PLATO bleeding is worth noting. The finding suggests that even transfusion of two or more units of RBCs and a notable drop in hemoglobin are enough to increase the risk of stroke. Interestingly, Whitlock et al. (2015) reported that transfusion of a single unit of RBCs increased the risk of stroke and/or myocardial infarction.

Administration of Octaplas and platelets are suggested to have even a larger effect on the risk of stroke (Kinnunen et al. 2015a & Mikkola et al. 2012). The thrombogenic effect of these blood products cannot be disentangled in the present study.

Indeed, it can be speculated that hypotension and anemia could be plausible mechanisms of cerebral ischemia especially in patients with pre-existent atherosclerosis of the intracranial arteries. Indeed, Bahrainwala and colleagues (2011) reported a larger drop in hemoglobin values intraoperatively in patients who
suffered from cerebral infarction. Moreover, their analysis showed that, low postoperative hemoglobin was an independent predictor of stroke. Similarly, Fowler and colleagues (2015) demonstrated in their meta-analysis that preoperative anemia was significantly associated with increased risk of stroke.

Study I had a number of limitations to be acknowledged. Even though, our analyses were adjusted for the use of IABP, prolonged inotropic support and the need for extracorporeal circulation, the risk of stroke could have been affected by less pronounced hypotensive events. Acquisition of data regarding the patients’ intra and postoperative hemodynamic status was not accomplished due to unexpected difficulties. Profound hypotension among patients with severe hemorrhage might confound the effects of RBC transfusions in patients who developed stroke. Second, epiaortic ultrasound was performed only in 10% of the patients, which reduced our ability to account for diseased ascending aorta. Third, only a small proportion of patients had the status of their carotid arteries investigated before CABG. However, a subanalysis of this patient population showed that RBC transfusion was the only independent predictor of stroke after adjustment for the severity of stenosis in the internal carotid arteries. Fourth, the main outcome of the present study was the occurrence of clinically evident stroke, although its incidence is notably lower than that of ischemic lesions identified using magnetic resonance imaging (Nah et al. 2014).

### 6.2 Incidence and impact of bleeding and transfusion in low-risk patients

The results of study II suggest, that severe bleeding according to the E-CABG bleeding grades 2-3 is rare in low-risk patients undergoing elective and isolated CABG. However, up to 18.5% of patients experienced mild bleeding, E-CABG grade 1. Despite the rather low incidence of severe bleeding, we were able to identify E-CABG bleeding grades 2-3 and UDPB grades 3-4 as significant risk factors associated with major adverse events in multivariate analysis. Our findings were similar to those of some previous studies on this issue (Kinnunen et al. 2014, Murphy et al. 2007, Ranucci et al. 2013). Patients exposed to more than 5 units of RBCs and/or re-exploration had an increased risk of early death, acute kidney injury, sternal wound infection and stroke. Indeed, resternotomy for bleeding and transfusion of blood products have been associated with increased mortality and morbidity in the literature (Mehta et al. 2009, Koch et al. 2006, Surgenor et al. 2006).
2009), especially the risk of stroke is shown to be elevated (Biancari et al. 2012 & Paone et al. 2014).

The association between mild perioperative bleeding, according to the E-CABG and UDPB grades, and the composite end-point E-CABG complication grade 3 in low-risk patients is of great interest. Our findings are similar to the results of Jakobsen and colleagues (2012), who reported that perioperative administration of RBCs significantly increased the risk of death in low-risk patients.

Furthermore, our study suggests that surgical sources of bleeding can cause excessive bleeding also in patients with low preoperative risk of bleeding complications. The incidence of re-exploration for bleeding in the present study was 2.3%. The finding emphasizes the importance of meticulous hemostasis and adequate means of blood conservation to reduce adverse events associated with resternotomy for excessive bleeding. Indeed, cardiac surgery has been shown to be feasible and safe also in patients refusing blood transfusion (Vasques et al. 2016).

However, the present study can not disentangle the individual roles of perioperative blood loss, anemia and blood product transfusions on adverse outcomes after coronary surgery. Due to the aforementioned limitation, the results of this study should be interpreted more on a general level. Indeed, bleeding and transfusions have been shown to increase the risk of complications synergistically (Ranucci et al. 2013, Loor et al. 2013).

A number of limitations related to the present study have to be acknowledged. Although this series is quite large, the rate of adverse events was low, possibly hindering our statistical power. Secondly, the lack of data on nadir hemoglobin levels acquired beyond the operation day prevented analysis regarding any liberal and restrictive transfusion thresholds. An attempt to control for significant pre-intra- and postoperative confounding factors was made, but the risk of bias was likely not entirely eliminated, i.e. some patients could have received RBCs due to their critical postoperative status.

6.3 Outcome after procedures for retained blood

Study III showed that the incidence of retained blood syndrome (9.2%) was lower than previously reported (Balzer et al. 2016 & Sirch et al. 2016). Moreover, the requirement for retained blood removal was more common in patients with severe bleeding according to the E-CABG bleeding criteria. These patients also experienced frequent postoperative complications. Balzer and colleagues (2016) reported an increased mortality associated with RBS (resternotomy included), a
risk confirmed by the present study. However, they reported a much higher in-hospital death (19.7% vs. 6.3%) compared to the present series.

Comparable to the present study, Balzer et al. (2016), Boyle et al. (2015) and Sirch et al. (2016) reported an increased incidence of significant morbidity associated with RBS. It is worth noting in the present study, the increased risk of composite outcome end-point, the E-CABG complication grade 3, among hemodynamically stable patients who underwent any procedure for retained blood removal regardless of the need of resternotomy.

We observed that drainage volume from the chest tubes was significantly larger in patients needing resternotomy as compared to control patients. After excluding the patients who underwent a re-exploration, the amount of drainage was similar between patients with and those without RBS. This finding suggests that in some cases, blood might be retained due to chest tube occlusion. Interestingly, in a survey conducted by Shalli et al. (2009), 100% of cardiac surgeons had encountered clogged chest tubes. Indeed, up to 36% of patients undergoing cardiac surgery might have an occluded chest drain at some point of their treatment (Karimov et al. 2013). A promising method to reduce chest tube occlusion is usage of active clearance devices, which are capable of reducing the incidence of RBS up to 57.9% (Sirch et al. 2016). Preventive measures to avoid retained blood was shown to reduce the incidence of postoperative atrial fibrillation (Biancari et al. 2010, Sirch et al. 2016, Eryilmaz et al. 2006).

A number of limitations regarding the present study should be acknowledged. The majority of our data was collected retrospectively and the nature (bloody or not bloody) of fluid accumulation after procedures for its removal was defined only by the operating surgeon. Second, we were unable to evaluate the impact of timing of procedures for RBS on the outcomes.

### 6.4 The effect of preoperative anemia on the outcome after coronary surgery

The results of study IV suggest, that preoperative anemia is not associated with poorer survival in cardiac surgery, after adjustment for a number of baseline covariates and perioperative bleeding according to the E-CABG bleeding criteria. Despite recent evidence in the literature, the individual effects of preoperative anemia and blood product transfusions are not well understood.
In the literature, old age, low baseline eGFR and low LVEF have been associated with preoperative anemia (KDIGO CDK work group 2012, Miceli et al. 2014, Nijst et al. 2016). In addition, the present study identified recent myocardial infarction and critical preoperative status to be associated with preoperative anemia as well. We speculate that the aforementioned finding might be secondary to a substantial infusion of fluids in this group of patients with critical conditions.

The only adverse event associated with preoperative anemia in our study was an increased risk of acute kidney injury, which might be caused by lower nadir hemoglobin levels. Indeed, the results of the present study suggest that transfusion of RBCs might be the main cause of adverse events observed in preoperatively anemic patients. Every cardiac operation exposes the patient to a certain degree of bleeding regardless of blood conservation methods, which could lead to allogeneic blood transfusions in cases with low preoperative hemoglobin levels, as represented by the lower nadir hemoglobin levels observed among anemic patients in the present study. On the other hand, a similar amount of blood loss in non-anemic patients might not require treatment. Even though anemia has been associated with an increased risk of perioperative bleeding (Biancari et al. 2016), we were able to produce groups with similar amounts of bleeding after propensity score matching, supporting the aforementioned hypothesis. In fact, the E-CABG bleeding grades 1-3 were found to be independent predictors of mortality in the same regression model with preoperative anemia.

Since the prevalence of preoperative anemia has been reported to be notably higher among cardiac surgery patients (Hung et al. 2011 & Miceli et al. 2014) than the general population (Martinsson et al. 2014 & Tettamanti et al. 2010), the effect of preoperative hemoglobin optimization is of great interest. Although its benefits are still unclear, preoperative administration of EPO and/or intravenous iron has been shown to reduce the need for RBC transfusions (Cladellas et al. 2012, Litton et al. 2013, Sowade et al. 1997) and to improve the outcome of Jehovah’s Witnesses undergoing cardiac surgery (Tanaka et al. 2015).

A number of limitations regarding the present study must be acknowledged. The data were collected in a retrospective fashion, although data regarding the amount of blood loss and the use of blood products were recorded prospectively. An analysis investigating only patients allocated to elective CABG would have enabled the exclusion of preoperative hemodilutional anemia, but the reduced number of patients in our database would have reduced the statistical power. Thirdly, the present study does not address the underlying causes of preoperative anemia, which might have a prognostic impact on outcomes. Finally, the decision
to use propensity score matching might raise a few questions since it is commonly used to compare different treatment strategies. However, the differences between anemic and non-anemic patients were substantial and included almost all baseline covariates, which could result in an overfitting of a standard adjusted multivariate analysis. Propensity score matching can be considered a valid method to isolate the effects of a disease from other covariates.
7 Conclusions

In the present study, the prognostic impact of preoperative anemia, excessive perioperative bleeding, retained blood syndrome and RBC transfusions were investigated in patients undergoing isolated CABG. Our results indicate that perioperative bleeding, transfusion of RBCs and retained blood syndrome are associated with a significantly increased risk of postoperative adverse events. However, preoperative anemia was shown not to affect survival when adjusted for blood transfusion. Although our results are significant, causality is difficult to determine. The conclusions related to the specific substudies are:

I Results from the present study provide evidence on the detrimental effect of perioperative bleeding and/or blood product transfusions on the development of stroke after CABG. The risk of stroke was greatest among patients who suffered from severe bleeding according to the E-CABG, UDPB and PLATO bleeding criteria. In light of the present results, preoperative hemoglobin optimization and reduction of perioperative bleeding might decrease the need for RBC transfusions, and subsequently reduce the incidence of neurological complications after coronary surgery.

II The present study showed that in the absence of significant perioperative bleeding and with no or minimal blood product transfusions, isolated CABG can be performed on low-risk patients with excellent results. The incidence of severe perioperative bleeding was rather uncommon, but was associated with an increased risk of major adverse events. Patient blood management is of great importance also in low-risk patients undergoing cardiac surgery.

III Retained blood requiring any procedure for its removal is a relatively common complication after isolated CABG and is associated with significantly increased risk of severe adverse events.

IV After adjustment for important baseline and operational characteristics, in addition to the severity of perioperative bleeding and the amount of transfused allogeneic blood products, preoperative anemia was not associated with an increased risk of adverse events with the exception of acute kidney injury. The increased exposure to blood transfusions among anemic patients might be the determinant of their decreased survival.
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List of original publications


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COMPLICATIONS ASSOCIATED WITH PREOPERATIVE ANEMIA, PERIOPERATIVE BLEEDING AND BLOOD TRANSFUSIONS AFTER ISOLATED CORONARY ARTERY BYPASS GRAFTING