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COAGULATION DURING AND AFTER CARDIOPULMONARY BYPASS WITH FOCUS ON HEPARIN, PROTAMINE, APROTININ AND PLATELET FUNCTION

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COAGULATION DURING AND AFTER CARDIOPULMONARY BYPASS WITH FOCUS ON HEPARIN, PROTAMINE, APROTININ AND PLATELET FUNCTION

THESIS FOR DOCTORAL DEGREE (PH.D.)

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“Obstacles are those frightful things you see when you take your eyes off your goal.”

– Henry Ford

CONTENTS

LIST OF ABBREVIATIONS

ACT	Activated clotting time
ADP	Adenosine diphosphate
ALAT	Alanine transaminase
AmSECT	The American Society of ExtraCorporeal Technology
ASAT	Aspartate aminotransferase
ATIII	Antitrombin III
BARC	Bleeding academic research consortium
BART	Blood conservation using antifibrinolytics in randomized trial
BMI	Body mass index
CABG	Coronary artery bypass grafting
CPB	Cardio pulmonary bypass
CK-MB	Creatine phosphokinase-MB
CT	Clotting time
CFT	Clot formation time
DAPT	Dual antiplatelet therapy
EuroSCORE	European system for cardiac operative risk evaluation
EACA	Epsilon aminocaproic acid
EACTA	European association for cardiothoracic anesthesiology
EACTS	European association for cardio-thoracic surgery
ECMO	Extra corporeal membrane oxygenation
EMA	European medicines agency
HTC	Heparin test cartridge
ICU	Intensive care unit
IU	International units
KIU	Kallikrein inhibiting units
MCF	Max clot firmness
NAPaR	Nordic aprotinin patient registry
OR	Operating room
PLATO	Platelet inhibition and patient outcomes
POC	Point of care test
PRBC	Packed red blood cells
RCT	Randomized controlled trial
SCA	The Society of Cardiovascular Anesthesiologists
STS	The Society of Thoracic Surgeons
TRAP	Thrombin receptor-activating peptide-6
TXA	Tranexamic acid
U	Unit
UDPB	Universal definition of perioperative bleeding

ABSTRACT

Background: Heparin and protamine dosing for anticoagulation during cardiopulmonary bypass (CPB) and its reversal after CPB, respectively, is usually given according to body weight. Excess protamine may impair coagulation. The HeProCalc algorithm calculates heparin and protamine doses during the procedure. Platelets play a crucial role in coagulation and haemostasis after cardiac surgery and platelet malfunction may explain excessive bleeding after CPB. Patients on clopidogrel and aspirin undergoing coronary surgery with CPB risk excessive bleeding. Between 2008-2012, the European Medicines Agency (EMA) suspended the marketing authorization of aprotinin, but Swedish hospitals received permission to continue its use.

Aims: To investigate whether the HeProCalc algorithm affects heparin and protamine dosing, postoperative blood loss, and transfusion rate (*Study I+II*), whether ROTEM®*platelet* provides additional information to ROTEM® analysis in guiding platelet transfusions after cardiac surgery and identifying factors triggering platelet administration (*Study III*), and to compare aprotinin use in coronary surgery during 2006–2007 vs. 2008–2014, before and after the EMA suspension (*Study IV*).

Methods: In *Study I and II* 40 and 210 patients, respectively, undergoing cardiac surgery with CPB were randomized to heparin and protamine dosage based on body weight only or the HeProCalc algorithm. In *Study III* ROTEM® and ROTEM®*platelet* were analysed before anaesthesia (T0, n=23), after CPB (T1, n=23) and after platelet transfusion (T2, n=10). In *Study IV* consecutive patients on clopidogrel and aspirin undergoing coronary surgery with CPB and received aprotinin 2006-2014 were included.

Results: *Study I*: Equal doses of heparin were given in both groups, but there were lower mean doses of protamine in the HeProCalc vs. the control group (211±56 vs. 330±61mg, $p<0.001$). Postoperative bleeding was less in the HeProCalc group (280±229 vs. 649±279mL, $p=0.074$). *Study II:* Total protamine dose was 210mg (interquartile range [IQR] 190-240) in the HeProCalc group vs. 350mg (IQR 300-380, $p<0.001$) in the control group. The ratio of total protamine to initial dose of heparin in the HeProCalc group was 0.62 vs. 1.0 ($p<0.001$). Bleeding after 12 hours was 320mL (IQR 250-460) in the HeProCalc group vs. 350mL (IQR 250-450, $p=0.754$) in the control group. Transfusion rate did not differ sign. between groups. *Study III:* ROTEM® and ROTEM®*platelet* tests were all significantly reduced between T0 and T1. ROTEM® parameters improved sign. after platelet transfusion. Only ROTEM®*platelet* TRAPTEM increased between T1 and T2 ($p=0.008$). Factors triggering platelet transfusion were long duration of surgery and time on CPB. *Study IV:* After 2007, aprotinin was restricted to patients on clopidogrel with platelet aggregation <85% on day of surgery. Use of aprotinin decreased from 113 patients/year 2006–2007 to 6 patients/year 2008–2014. Mean EuroSCORE I and mean CPB time were sign. higher in the second period, indicating greater operative and bleeding risk. No sign. differences were observed regarding mortality, complications, bleeding and transfusion rates between the two periods.

Conclusions: *Study I:* HeProCalc-based dosage of heparin and protamine allowed for reduced protamine use after CPB compared with conventional calculations. Furthermore, HeProCalc-based regimen for heparin reversal suggested less postoperative bleeding, although the difference between the groups was not statistically significant. *Study II:* Use of the HeProCalc algorithm reduced protamine dosage and the protamine/heparin ratio after cardiopulmonary bypass compared with conventional dosage based on weight without significant effect on postoperative blood loss or the transfusion rate. *Study III:* ROTEM®*platelet* TRAPTEM improvement indicated that platelet transfusion may reverse CPB induced platelet dysfunction. *Study IV:* Aprotinin use decreased sign. after 2007. Despite a higher operative risk and longer CPB duration, patients given aprotinin between 2008 and 2014 did not differ sign. in outcomes and transfusion rates compared with those treated with aprotinin during the prior 2 years.

Populärvetenskaplig sammanfattning

Genom att använda en hjärtlungmaskin övertar den hjärtats och lungornas uppgifter att pumpa runt blodet i kroppen respektive att syresätta blodet. Dock medför den kontinuerliga passagen av patientens blod genom hjärtlungmaskinen och att blodet kommer i kontakt med luft en kraftig aktivering av koagulations (blodets förmåga att levra sig) - och immunsystemet. För att förhindra att blodet koagulerar sig tillför man det blodförtunnande läkemedlet heparin innan patienten ansluts till hjärtlungmaskinen. Heparinet har en snabb effekt och man kan efter att hjärtlungmaskinen infrånkopplats neutralisera heparinets blodförtunnande effekt med läkemedlet protamin. Höga doser protamin försämrar dock till viss del blodets förmåga att koagulera, vilket kan öka blödningen och behovet av blodtransfusioner efter en hjärtoperation. För att undvika detta kan man individanpassa doseringen av protamin på olika sätt, t.ex. genom att använda dataprogrammet HeProCalc. Effekten av detta program studerades i **Delarbetena I och II**.

I **Delarbete III** studerades effekten av hjärtkirurgi med hjälp av hjärtlungmaskin på blodplättarnas funktion. Vid blödningsproblematik efter en hjärtoperation kan blodplättar tillföras via transfusion och blodpättarnas koagulationsförmåga mättes före och efter hjärtlungmaskinanvändningen samt efter transfusion av blodplättar.

Patienter med instabil kranskärlssjukdom tillförs blodplättshämmande läkemedlet t.ex. clopidogrel för att förhindra att en hjärtinfarkt uppstår innan patienten antingen genomgår en kranskärlsoperation eller en ballongvidgning. I de fall då en hjärtinfarkt är hotande och patienten måste genomgå en akut kranskärlsoperation kan man inte vänta tills clopidogrelets effekt försvunnit. Detta innebär en risk för livshotande blödning och stort transfusionsbehov i samband med operationen. Riskerna kan dock minskas genom att tillföra läkemedlet aprotinin i samband med operationen. En kanadensisk studie indikerade dock 2008 en association mellan aprotinin och ökad mortalitet vid hjärtkirurgi, varför aprotinin blev indraget i de flesta västerländska länder förutom Storbritannien och Sverige. Efter rigorös myndighetsgranskning ansågs studien vara opålitlig och aprotinin blev åter fritt att använda 2016. I **Delarbete IV** studerades effekten av aprotinin hos patienter som genomgick akut kranskärlsoperation under behandling med clopidogrel före och efter 2008.

Sammanfattningsvis visade **Delarbetena I och II** att användningen av dataprogrammet HeProCalc minskade protamindosen med ca 30% jämfört med traditionell dosering baserat på vikt. I **Delarbete III** påvisades en signifikant sänkt blodplättsfunktion mätt med tre olika delmetoder efter frånkopplad hjärtlungmaskin. Efter transfusion av blodplättar var det endast en av dessa tre delmetoder som uppvisade en signifikant förbättring. I **Delarbete IV** visade sig de patienter, som opererats 2008-2014 trots högre operativ risk och längre tid i hjärtlungmaskin, inte ha signifikant skilda kliniska resultat eller transfusionsbehov jämfört med de patienter som opererats 2006-2007.

LIST OF SCIENTIFIC PAPERS

I. **Kjellberg G**, Sartipy U, van der Linden J, Nissborg E, Lindvall G.
An Adjusted Calculation Model Allows for Reduced Protamine Doses without Increasing Blood Loss in Cardiac Surgery.
Thorac Cardiovasc Surg 2016;64(6):487-93

II. **Kjellberg G**, Holm M, Fux T, Lindvall G, van der Linden J.
Calculation Algorithm Reduces Protamine Doses Without Increasing Blood Loss or the Transfusion Rate in Cardiac Surgery: Results of a Randomized Controlled Trial.
J Cardiothorac Vasc Anesth 2019;33(4):985-992.

III. **Kjellberg G**, Holm M, Lindvall G, Gryfelt G, van der Linden J, Wikman A.
Platelet function analysed by ROTEMplatelet in cardiac surgery after cardiopulmonary bypass and platelet transfusion
(Submitted)

IV. **Kjellberg G**, Holm M, van der Linden J.
Outcome of isolated coronary surgery patients on dual antiplatelet therapy during the European suspension of aprotinin 2006-2014.
Chirurgia, 2018;*31*(6):242-246.

1 INTRODUCTION AND BACKGROUND

1.1.1 Excessive bleeding in cardiac surgery

Excessive perioperative bleeding in cardiac surgery is associated with risk for serious complications including infections,[1, 2] respiratory and renal failure,[3] neurological events,[3] and mortality.[3-6] The etiology of excessive bleeding in cardiac surgery is multifactorial. Medication with anticoagulants and platelet inhibitors, activation of inflammatory systems via blood contact with the surfaces of the cardiopulmonary bypass (CPB) circuit, the negative effects on blood coagulation by shear stress generated by pumps and cardiotomy suction devices, hypothermia, and hemodilution are some important factors that may influence hemostasis during and after CPB.[7] Moreover, adequate estimation of platelet function, appropriate measures to counteract dual antiplatelet therapy (DAPT), and optimization of heparinization during CPB and the following reversal of heparin with protamine after CPB are other challenges to attain postoperative hemostatic control.

1.1.2 Heart-lung machine and cardiopulmonary bypass

The first successful heart operation with support from a heart-lung machine was performed in the United States 1953 by John Gibbon when he managed to close an atrial septal defect in an 18-year-old woman. One year later, the Swedish surgeon Clarence Crafoord, together with the surgeon Viking Olof Björk, Åke Senning and engineers from AGA AB, Stockholm, Sweden, developed their own heart-lung machine, which was used when they performed a successful removal of a cardiac myxoma at Sabbatsberg's Hospital in Stockholm. There was an urgent need for effective anticoagulation during the clinical use of heart-lung machines, and at that time approval from ethical boards or medical agencies were not needed. Thus, Clarence Crafoord and his team just administered heparin to these patients to prevent perioperative thrombembolic complications. By doing that, Clarence Crafoord together with Erik Jorpes, contributed considerably to the clinical use of heparin during CPB, which still today is the gold standard for anticoagulation during CPB. During the next decade the use of heart-lung machines in cardiac surgery escalated all over the world thanks to adequate anticoagulation with heparin and enabled advanced cardiac surgery during cardiac arrest.

1.1.3 The heart-lung machine

The heart-lung machine used for CPB consists of several parts (**Figure 1**). Venous blood is drained from the heart usually via a cannula in the right atrium or in each of the caval veins and collected into an external venous blood reservoir, before the venous blood is pumped through an oxygenator by a roller or centrifugal pump. By passing the oxygenator the venous blood becomes oxygenated and carbon dioxide is removed. The gas flow rate and oxygen percentage of the sweep gas ventilating the oxygenator determine oxygen saturation and carbon dioxide pressure in outgoing arterial blood. The oxygenator is also connected to a heater/cooler system with which temperature of the passing blood is controlled. After oxygenation, the now arterialized blood returns to the patient via an arterial cannula, normally placed in the ascending aorta. Blood circulating in the CPB circuit with its tubing gets in contact with the main pump and with additional pumps and tubes that are used for suction of blood from the surgical field to the venous reservoir and for delivery of cardioplegia solution. Altogether blood passing the CPB circuit gets in contact with 2-3 m^2 of foreign surfaces.

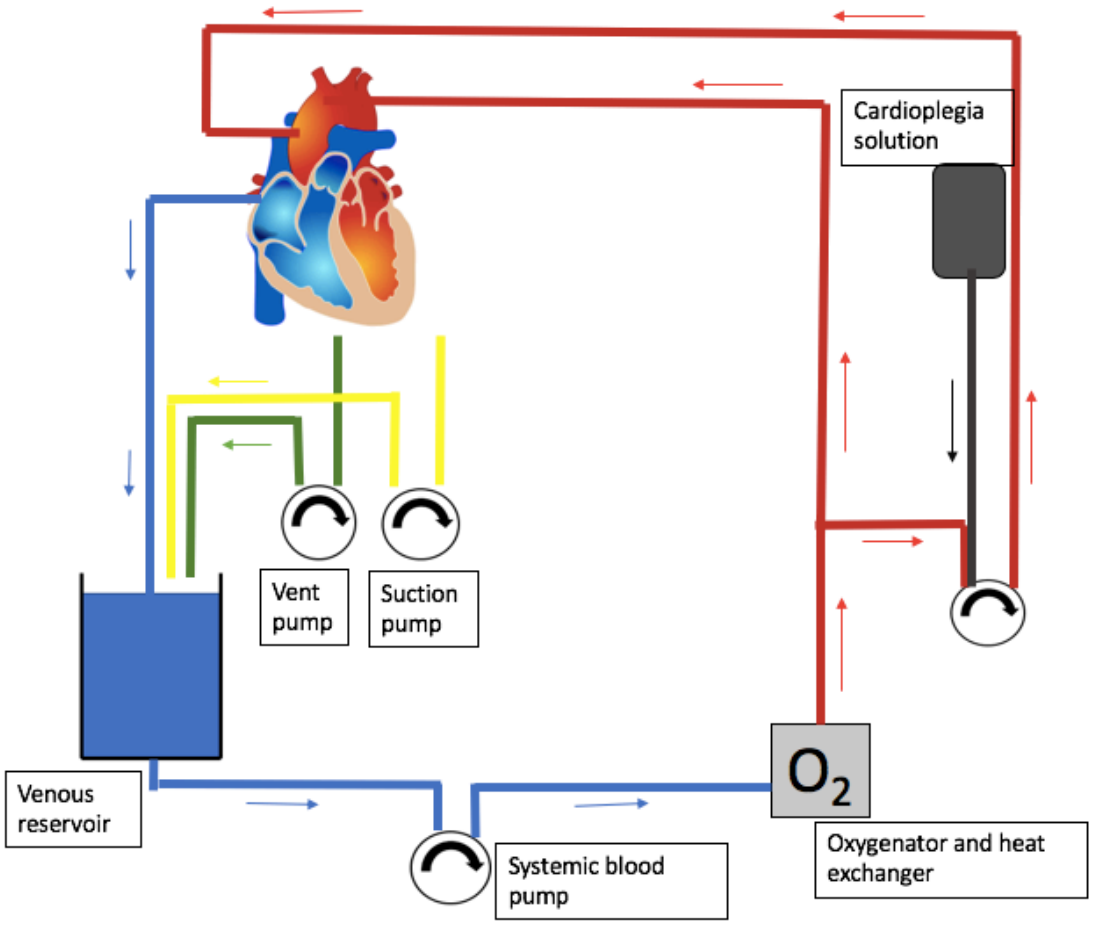


FIGURE 1. An illustration of the different components of the cardiopulmonary bypass system, which when in contact with blood contribute to the activation of inflammation- and the coagulation system. Blue color depicts venous desaturated blood, red arterial oxygen saturated blood, green blood from the aortic root/left ventricle from the bottom of the open surgical field, and yellow blood which is transported via the coronary suction device from the surgical wound to the venous reservoir.

The used pump flow rate is related to the patient´s body surface area, temperature and hematocrit, and normally a pump flow rate of 2.4 L/m^2/minute is used during normothermia. During surgery, there is sometimes need for hypothermia as a measure to decrease oxygen requirement. In situations requesting circulatory arrest, body temperature is often decreased to 28-24°C, although sometimes to <18°C, when selective cerebral perfusion is omitted, as the latter temperature is the threshold when the brain becomes isoelectric. Other reasons requiring a lower pump flow and hypothermia can be an unsatisfactory venous return or special surgical technical reasons, such as minimizing blood in the surgical field.

1.1.4 Inflammatory activation

CPB and surgical trauma will induce pronounced effects on the hemostasis, including systemic inflammatory response, leukocyte activation and the release of inflammatory mediators. Platelets activated by surgical trauma including contact with the inner surface of the CPB circuit will interact with activated leukocytes and amplify the inflammatory reaction to CPB. Factor XII will attach to high-molecular-weight kininogen (HMWK) and the complex then sticks to the non-physiologic surface. This complex releases FXIIa, kallikrein and bradykinin and the intrinsic coagulation cascade starts (**Figure 2**).

The coagulation and inflammatory systems interact closely trigger each other. Coagulation is activated as a response to inflammation, and simultaneously, thrombin and FXa act as proinflammatory agents. Altogether, the processes are caused both by blood contact with foreign surfaces lacking factors that normally inhibits complement activation and the surgical trauma but also by hypoperfusion, ischemia and tissue reperfusion[8] induced by CPB and aortic cross-clamping and de-clamping, respectively.

After end of CPB, protamine is given to neutralize heparin, forming a heparin-protamine complex, which will specially trigger cytokines, a process not observed during off-pump surgery[9, 10]. Furthermore, hypothermia, release of thrombin, tissue plasmin activator and FXII will stimulate the fibrinolytic process. The effects on the inflammatory system and hemostasis are enhanced by prolonged CPB as well as deep hypothermia.[10]

Clinical symptoms of extensive systemic inflammatory activation include loss of vascular tone and leakage of capillary fluid, resulting in need for volume substitution during CPB, which in turn will aggravate hemodilution. Vasodilation will induce the need for vasoconstrictive medication effecting the microcirculation in the tissues. These reactions may in some patients lead to post-CPB effects including low cardiac output, respiratory or renal failure.

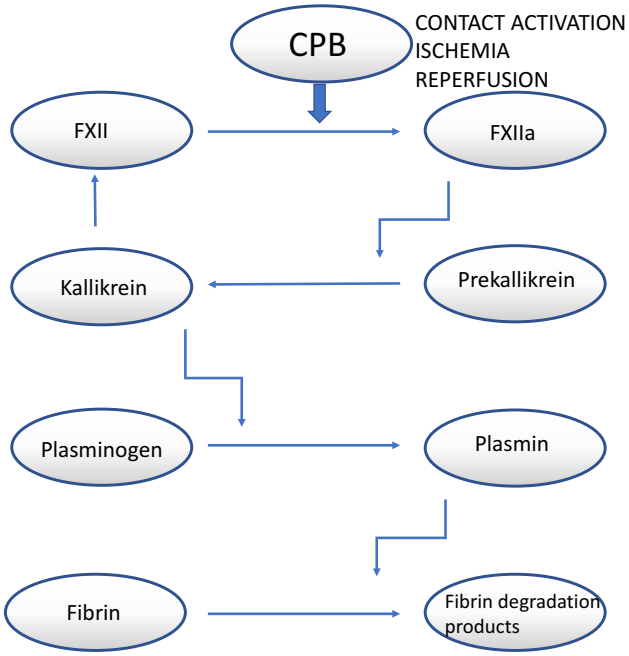


FIGURE 2. An illustration of some key coagulation and in inflammatory factors activated during cardio-pulmonary bypass (CPB).

1.1.5 Coagulation activation

The delicate balance of clot formation and bleeding is critically disturbed during cardiac surgery and CPB. The disruption is caused by blood being exposed to non-physiologic surfaces, sub-atmospheric pressure and shear stress. We attempt to set the coagulation system "on hold" during CPB using full heparinization. Still hemodilution caused by CPB prime effects not only platelets but all coagulation factors as well. Moreover, CPB prompts consumption of coagulation factors, e.g. fibrinogen levels decrease by 36% and platelet count by 45%,[11] which together all contribute to the fact that cardiac surgery involving CPB is associated with increased blood loss and a high frequency of transfusion of allogeneic blood products.

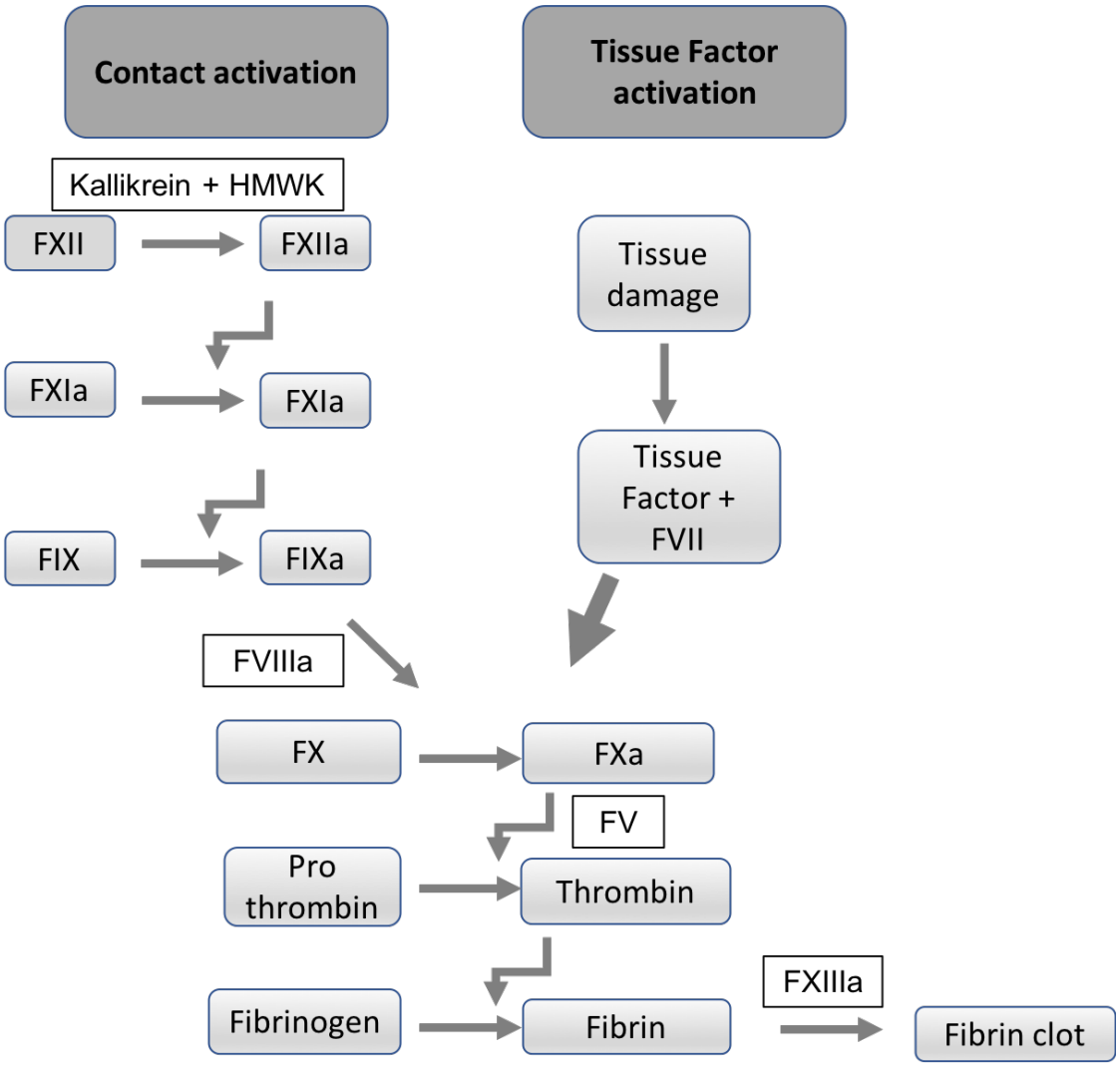


FIGURE 3. An illustration of the two pathways including activation of coagulation factors that in the end lead to fibrin clot. TF, Tissue Factor; HMWK, High Molecular Weight Kininogen.

As shown in **Figure 3** above, activation of FXII is caused both by activation of the inflammation system and the blood contact with the CPB circuit, whereas tissue damage leads to activation of tissue factor and FVII. Both pathways in the end lead to fibrin clots. Boisclair et al. showed that the main trigger of coagulation during cardiac surgery and CPB was the tissue factor-factor VIIa activation in response to the cutting of tissue.[12]

Use of hypothermia during CPB is associated with increased postoperative bleeding, decreased platelet aggregation and thrombin release. Both acidosis and hypothermia promote coagulopathy, the three factors included in the "lethal triad of trauma". Moreover, hypothermia will decrease synthesis of clotting factors and delay initiation of thrombi and clots.[13]

1.1.6 Platelet activation

Many of the reactions in the coagulation process take place on the surface of platelets, which makes them essential in this context. Despite their tiny size, 2-3 μm in diameter, platelets represent a considerably amount of total cell mass in the body due to their concentration of 150-300 x 10^9/L in blood. Their life span ranges between 5-10 days and young platelets are most reactive.[14]

Exposure to air, negative pressure and shear stress activate platelets and initiate conformational change, depletion of granule content and expression of various receptors that enhance aggregation and further activation. This pronounced reaction results in a decrease both in platelet count and function[11, 15], illustrated by a decrease in 70%, 65% and 54% in the Adenosine diphosphate (ADP) test, cyclooxygenase (COL) test, and Thrombin receptor-activating peptide-6 (TRAP) test, respectively, with Multiplate®.[11] Hemodilution affects platelets, concentration of coagulation factors and hemoglobin, resulting in less margination of platelets towards the vessel wall that further aggravates the impaired coagulation.[16] Other reasons of decreased platelet count include mechanical disruption and adhesion to the non-physiologic surface along with sequestration in organs. Importantly, reduction in platelet count together with dysfunction is the principal cause of bleeding and coagulopathy after cardiac surgery.[17] Furthermore, thrombin release caused by surgical trauma will activate platelets with ensuing effect on coagulation. Moreover, platelet count in circulating blood decreases markedly during CPB [7, 18] and a low postoperative platelet count has been linked to increased postoperative blood loss.[19, 20] Remaining platelets after cardiac surgery show a diminished response capacity to stimulation and although they recover within a few days, this is challenging as their contribution to coagulation early after surgery is critical.

Additional to platelet dysfunction related to CPB and cardiac surgery is the fact that some of these patients are on DAPT with one ADP-receptor blocker, e.g. clopidogrel, ticagrelor, or prasugrel, and aspirin. Preferably, the ADP-receptor blockers are discontinued well in time before surgery and according to current European association for cardio-thoracic surgery (EACTS)/ European association for cardio-thoracic anesthesiology (EACTA) guidelines[21] surgery should preferably be postponed at least 3, 5 and 7 days after intake of ticagrelor, clopidogrel, and prasugrel, respectively. However, in emergency surgery this is not possible. Consequently, patients who need urgent coronary surgery while on DAPT are at risk for increased bleeding.[22] Several observational studies have shown that clopidogrel exposure within 5–7 days prior to coronary surgery to be linked to an augmented risk of major bleeding, reoperation, and transfusions.[23] On the other hand, withholding DAPT prior to urgent surgery

puts the patient at risk of thrombotic events.[22] Point-of-care test (POC)-testing of platelet function may be of use to identify coronary patients at risk of excessive blood loss.[24-27]

The different platelet inhibitors used for DAPT may have different effects on platelet transfusion. In contrast to clopidogrel and prasugrel that bind irreversibly to circulating platelets, platelet bound ticagrelor may also inhibit transfused platelets, making platelet transfusion less effective.[28]

Platelet transfusion per se might imply a risk of adverse events such as allergic reactions, infections and stroke according to a meta-analysis of six randomized, double blinded, placebo controlled trials involving aprotinin versus placebo, whereby the effects of platelet transfusion was compared in those transfused (n=284) with those not transfused (n=1436).[29 30] Van Hout et al.[31] reported that cardiac surgery patients receiving platelet transfusion in the operating room (OR) suffered less postoperative blood loss, but more often needed vasoactive drugs, prolonged ventilation, prolonged intensive care and blood products postoperatively. However, in that small recent publication, where 169 patients were retrospectively propensity matched with 507 controls,[31] platelet transfusion was not associated with reoperation for bleeding, thromboembolic events, infections, organ failure, or mortality. There is according to Levy et al.[32], who reviewed 8 studies, no consensus regarding guidelines for transfusion of platelets. This impedes the ability to draw conclusions and highlights the requirement for further studies to evaluate the proper dose and triggers for platelet transfusion in perioperative patients.[32]

The EACTS/EACTA guidelines[21] only state, based on the clinical and practice guideline from the American Association of Blood Banks[33], that platelets *may be* transfused in patient bleeding postoperatively if the platelet count is less than 50 x 10^9 /L or if the patient is on antiplatelet drugs.

1.2 DRUGS WITH IMPACT ON COAGULATION AND INFLAMMATION

1.2.1 Heparin

To prevent microscopic clot formation or, in worst case, a life-threatening massive thrombus formation in the CPB circuit, adequate anticoagulation is required. Since the discovery by McLean in 1916, heparin remains the most commonly used drug for anticoagulation during CPB even 100 years later. Swedish biochemist J. Erik Jorpes at Karolinska Institutet is credited with identifying the chemical structure of heparin in 1935, which made it possible for the Swedish company Vitrum AB to launch the first heparin product for intravenous use in 1936.

The polysaccharide heparin, which is the strongest macromolecular acid in the body, has a molecular weight ranging from 3000 to 40 000 Dalton with a mean mass of approximately 15 000 Dalton. The varying molecular weight between heparin batches also may explain why their potency often differ. In cardiac surgery, heparin is administrated via a central venous line and the maximum activated clotting time (ACT) prolongation is achieved within five minutes. As heparin is a large molecule with a strong negative charge, extravasal distribution is unlikely to take place. Heparin exerts its effect by potentiating anti-thrombin (ATIII) a thousand times, thus also depressing effects of activated factors, IX, X, XI, XII, XIII and thrombin.[7] Even though heparin exerts its effect by potentiating ATIII 1,000-fold, the variation in response cannot be explained only by the ATIII level.[34] Levels of ATIII may be low preoperatively due to a daily treatment with low-molecular heparin or may decrease during surgery as a result of hemodilution and consumption during CPB. The binding of heparin to ATIII requires a heparin molecule with length of 18 saccharides, while a heparin molecule with only five saccharides is enough to inhibit factor Xa (**Figure 4**). Heparin also stimulates the release of tissue factor pathway inhibitor (TFPI) from endothelia cells.[35]

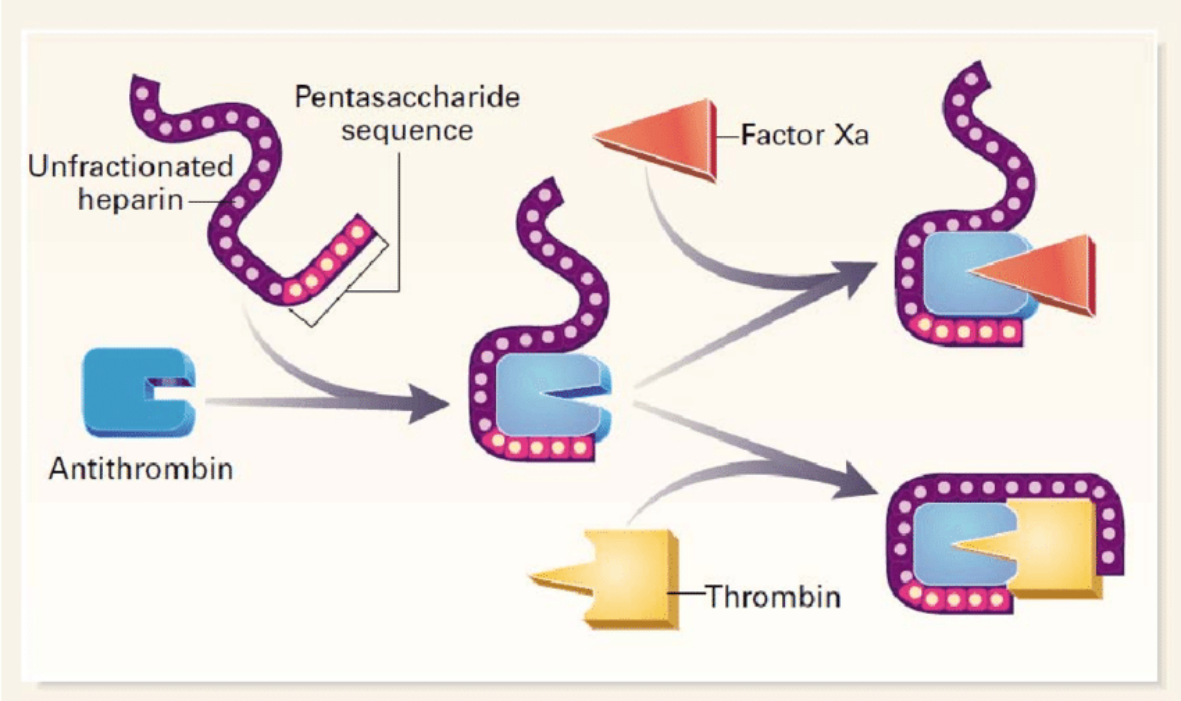


FIGURE 4. Catalysis of ATIII-mediated inactivation of thrombin by heparin (Weitz 1997, with permission from *New England Journal of Medicine)*.

Heparin is primarily eliminated by the reticuloendothelial system (RES), a component of the immune system, in a rapid dose-dependent way. When endothelial cell binding of heparin gets saturated, some heparin is cleared by the renal system but with a slower elimination rate.[35] Hypothermia is known to delay elimination of heparin due to induced decrease in enzyme activity. On the contrary, CPB-rewarming will increase heparin consumption and

lower ACT values and thus increase the risk thromboembolic events if not adequate doses of additional heparin are given. High heparin doses are used in connection with the CPB, and the elimination rate is slower when high doses are used. The mean half-life for a bolus dose of 400 International Units (IU)/kg is 152 minutes, while the mean half-life for 100 IU/kg is approximately 60 minutes,[36] To complicate matters further, there is substantial variability of heparin anticoagulant responsiveness among patients undergoing cardiac surgery, thus making dosing sometimes difficult.

1.2.2 Protamine

After end of CPB, normalization of coagulation is facilitated by neutralization of heparin with protamine, a polycationic protein derived from salmon sperm. Heparin, with its strong negative charge, binds ionically to the positively charged protamine. Protamine both neutralizes heparin and exerts a mild anticoagulant effect, which is independent of heparin.[7] The half-life of protamine is approximately five minutes and protamine is nearly completely eliminated from the circulation within 20 minutes.[37]

In contrast to the non-toxic heparin, protamine may induce severe adverse anaphylactic reactions due to release of histamine from mast cells including severe hypotension, high pulmonary arterial pressure, and bronchospasm.[38] Administration of excess doses may also have a negative effect on coagulation, including platelet dysfunction, down regulation of thrombin generation by inhibition of factor V, and weakened clot structure.[39, 40] Kunz et al.[41] concluded in a retrospective study that a higher protamine/heparin weight ratio was associated with a larger hemoglobin drop, higher chest tube output and increased risk of blood transfusion. Commonly, a fixed dose of 1.0 to 1.3 mg of protamine is used to neutralize each mg (100 IU) of given heparin, although it can be based either on initial or total given dose of heparin.[21] Moreover, although 84% of international centers add heparin to the prime of the CPB circuit,[42] it is in most studies unclear if this dose really is added to the total given dose of heparin. The reversal dose with protamine does not account for heparin elimination during CPB and might result in an excess protamine dosage after CPB.[43] Protamine titration and a low dose protamine regime after termination of CPB have been suggested by The Society of Cardiovascular Anesthesiologists (SCA), the Society of Thoracic Surgeons (STS) and the American Society of ExtraCorporeal Technology (AmSECT)[44] and the European Association of CardioThoracic Surgery/European Association of CardioThoracic Anaethesiology (EACTS/EACTA) in their guidelines regarding blood conservation clinical practice.[44] The EACTS/EACTA guidelines specifically state that "*protamine should be administered in a protamine-to-heparin dosing ratio <1:1 to reduce bleeding*".[45]

1.2.3 Aprotinin

Extracted from bovine lung tissue, aprotinin is a naturally occurring serine protease inhibitor that forms reversible complexes with many enzymes and factors including kallikrein (promotes contact activation, **Figure 5**), trypsin, chymotrypsin, plasmin, elastase, thrombin, active protein C (cleaves and degrades factor V), and tissue plasmin activator. The most important proteases inhibited by aprotinin are plasmin and kallikrein, whereof plasmin is the final enzyme in the fibrinolytic pathway.

The latest Cochrane review of anti-fibrinolytic use for minimizing packed red blood cells (PRBC) transfusion and reoperation due to bleeding is from 2011.[46] It evaluated 252 randomized controlled trials (RCT) of anti-fibrinolytic drugs in adults scheduled for non-urgent surgery that recruited over 25,000 participants, whereof 69% of the patients underwent cardiac surgery. Compared with control aprotinin (RR 0.66; 95% CI 0.60 to 0.72), tranexamic acid (TXA, RR 0.61; 95% CI 0.53 to 0.70) as well as epsilon aminocaproic acid (EACA, RR 0.81; 95% CI 0.67 to 0.99) reduced number of PRBC transfusions. However, aprotinin alone when compared with TXA/EACA significantly reduced transfusion rate of PRBC (RR 0.82; 95%CI 0.71- 0.95) as well as re-operation rate due to bleeding (RR 0.67, 95% CI 0.46-0.98).

Aprotinin is considered to attenuate the inflammatory cascade, which also modulates fibrinolysis and coagulation. Furthermore, reducing inflammatory response will preserve platelet function and thereby decrease its effect on the coagulation system. Thus, aprotinin deems to have anti-inflammatory properties, at least when the high dose regimen, also known as the full Hammersmith regimen, is used. The enzymatic activity of aprotinin is expressed in Kallikrein Inhibiting Units (KIU), with 1 KIU equivalent to the amount of aprotinin that decreases the activity of 2 biological kallikrein units by 50%. The high dose regimen consists of an intravenous bolus of 2×10^6 KIU as a loading dose, followed by 0.5×10^6 KIU/h during surgery, and 2×10^6 KIU in the CPB circuit prime. Lower-dosing regimens are not considered to provide a full anti-inflammatory effect.

By inhibiting thrombin activation of protease-activated receptor-1 on platelet activation during CPB, aprotinin reduces the activation and depletion of platelets during CPB, which allows platelets to retain their function perioperatively. By interacting with kallikrein at higher doses, aprotinin inhibits the intrinsic pathway of coagulation, possibly decreasing over-consumption of coagulation products during CPB.

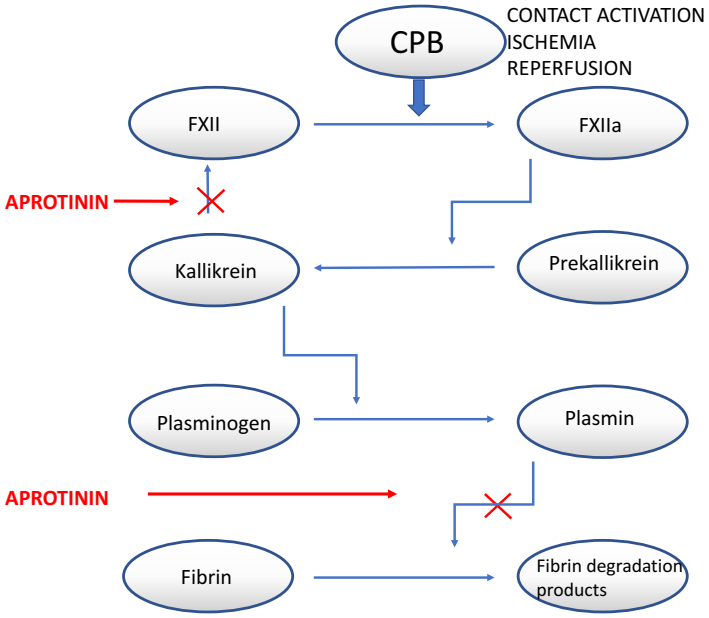


FIGURE 5. An illustration of some key coagulation and in inflammatory factors activated during cardio-pulmonary bypass (CPB) and the influence of aprotinin.

Contradicting many earlier randomized studies, including three meta-analyses,[47] two large propensity matched observational studies by Mangano et al. [48, 49] indicated that, when compared with lysine analogs or a control group, perioperative treatment with aprotinin significantly increased the risk of renal, cardiac or cerebral events, as well as mortality. However, the findings may be explained by inappropriate heparin dosing during CPB in a few participating centers that did not take in to account that aprotinin markedly prolongs celite based ACT results. The findings contradicted earlier observational studies by other investigators [50-52] except that the use of aprotinin in coronary artery bypass grafting (CABG) may be associated with slight transient creatinine increase.[53] In 2008, a large randomized trial, the Blood conservation using antifibrinolytics in randomized trial (BART) study,[54] was published, which evaluated aprotinin versus lysine analogs in patients categorized as "*high risk cardiac surgery patients*". In this study, a tendency to an increased mortality in those patients receiving aprotinin was observed in an interim analysis by an independent data and safety monitoring committee, whereby the study was terminated earlier than planned. After publication of the BART study, where aprotinin was found to significantly increase 30-day mortality after surgery compared with TXA/EACA, aprotinin was withdrawn from the world market and for a period only available for licensed use. However, in the BART study 52% of eligible patients were excluded from randomization indicating a possible selection bias. Furthermore, after a comprehensive review by Health Canada and European Medicines Agency (EMA),[55] some serious limitations of the BART study were identified. There was an

unexplained exclusion of 137 patients from analysis after randomization and the excluded group included several dead patients, none of whom received aprotinin. Moreover, there was an unusually large number of reclassifications of outcomes from the originally reported data, with a large (~75%) change rate in primary outcome (massive postoperative bleeding). The reclassifications were in opposite directions for aprotinin versus TXA and EACA, favoring the latter, and these changes increased with the duration of the study. As a result, an editorial entitled "*Aprotinin and cardiac surgery: a sorry tale of evidence misused*" was published in the in the British Journal of Anaesthesia.[56]

A re-analysis including originally excluded patients showed no statistical significance of the mortality signal for aprotinin. Because of the serious doubts regarding the correctness of the analysis, Health Canada and the EMA have rejected the BART study. As a consequence, aprotinin has gradually been reregistered and is currently available for use in cardiac surgery.[57] However, although EMA now approves the use of aprotinin in cardiac surgery, EMA requires on-line registration of patients receiving aprotinin to monitor its on- and off-label use.

Aprotinin augments ADP-induced platelet aggregation in patients on clopidogrel.[58] Furthermore, two randomized controlled trials showed that aprotinin reduced postoperative bleeding and transfusion rate in patients on DAPT requiring urgent or acute CABG.[59, 60] To our knowledge, no studies have presented the clinical use of aprotinin during the above mentioned, rather long suspension period, during which alterations in medical practice are likely to have occurred. Consequently, the EMA encouraged publishing of data about clinical use of aprotinin during this period, 2008-2014. An additional argument is the change of aprotinin manufacturer from Bayer AG (Leverkusen, Germany) to Nordic Group B.V. (Hoofddorp, the Netherlands), whereby Sweden received aprotinin from Nordic group since 2008. Furthermore, any European center using aprotinin since its relicensing must report clinical patient outcome to the Nordic Aprotinin Patient Registry (NAPaR), which is sponsored by Nordic Group, and NAPaR subsequently reports to the EMA.

Between 2008 and 2012, the use of aprotinin was approved by the national health authorities with a license from the Swedish Medical Agency. At Karolinska University Hospital, use of aprotinin continued during this period resulting in a database including several hundred patients who before and after 2008 underwent CABG and were given aprotinin.

1.3 COAGULATION MONITORING

1.3.1 Activated Clotting Time

ACT measures the anticoagulant effect of heparin. This test was introduced by Hattersley and Blakely in the 1960´s and is the gold standard for analyzing the heparin anti-coagulation effect when high doses are used, e.g. during CPB. This point of care test measures time to clot formation in seconds and is based on coagulation activation of whole blood with either kaolin or celite. Low ACT levels indicate an increased risk of thrombus formation in the CPB circuit. Many studies have been made to define the optimal ACT level for CPB. Even though there is no universal agreement, a minimum of 400 seconds is often referred to as a standard level regarding safety concerns[7] and current clinical practice guidelines recommend an ACT >480 seconds.[44] Besides heparin, other factors affecting ACT include hemodilution, hypothermia, low platelet count, coagulation deficiencies, and medications such as warfarin, platelet inhibitors, and thrombin inhibitors.[61]

To ensure that no remaining heparin is present after protamine reversal, a High Range Heparinase Cartridges (HTC, Medtronic, Minneapolis, MN, USA) analysis along with a standard ACT test, both analyzed with the Medtronic ACT Plus® System (Medtronic, Minneapolis, MN, USA) may be performed. One channel of the test includes heparinase and will present an ACT value reflecting unheparinized blood, whereas the standard ACT channel will identify the eventual presence of heparin.

1.3.2 Rotational Thromboelastometry (ROTEM)®

The rotational thromboelastometry, ROTEM® (TEM International, Munich, Germany), is a whole blood viscoelastic test measuring coagulation in real-time. The indication for using this test is mainly excessive or uncontrolled bleeding. This point-of-care test includes analysis of the external coagulation pathway, EXTEM®, the internal coagulation pathway, INTEM®, fibrinogen concentration, FIBTEM® and heparin presence, HEPTEM®, and gives the first results within 10 minutes. The use of ROTEM testing has been shown to decrease cost and transfusion requirements when used in cardiac surgery.[62]

1.3.3 ROTEM®*platelet*

It would be valuable if platelet function after cardiac surgery could be appropriately evaluated, to understand when and when not, platelet transfusions are indicated. Assessment of coagulation with ROTEM® has been reported to provide valuable information in deciding

therapy to optimize coagulation in patients bleeding after cardiac surgery.[63] Yet, the ROTEM® method is of limited value for assessment of platelet function.

Testing with ROTEM®*platelet* (TEM International, Munich, Germany) is quick POC test for possible 24/7 use. It is based on impedance aggregometry of whole blood, similar to Multiple Electrode Aggregometry (MEA; Multiplate Verum Diagnostica, Roche, Munich, Germany),[15] and has primarily been used for detection of platelet inhibition by anti-platelet drugs. The gold standard method for platelet function, Light Transmission Aggregometry (LTA), is not a POC-test and thus of limited value in acute situations. Both ROTEM® and ROTEM®*platelet* can be analyzed with the same instrument. Three different agonists are used; with ARATEM, activation is achieved with arachidonic acid (AA), with ADPTEM activation is achieved with ADP and with TRAPTEM platelets are activated with TRAP. Testing with ROTEM®*platelet* has shown that cardiac surgery with CPB impairs platelet function in all assays. [64]

1.3.4 Plateletworks®

Several platelet function tests are available for evaluation of platelet function. The Plateletworks® (Helena laboratories, Beaumont, TX, USA) assay is dependent on single platelet counting with a conventional cell counter before and after agonist stimulation in vitro. Test results are available within minutes, but a requirement is that the testing needs to be performed within 10 minutes after blood sampling.

1.3.5 HeProCalc guided perfusion system

The computer algorithm HeProCalc (HeProCalc AB, Huddinge, Sweden) is designed to optimize dosage of heparin and protamine with respect to patient's body surface area and the baseline ACT value. During CPB, all ACT values are measured and continuously inserted in the computer program along with data regarding additional heparin doses and temperature of the arterial line of the CPB system, to calculate the need for additional heparin during CPB and ideal protamine dose after termination of CPB. During CPB, the computer algorithm presents the calculated ACT value, the heparin consumption/minute and the calculated total heparin amount existing in the circulation expressed as IU/kg.

2 AIMS

The specific aims were to:

- Analyze HeProCalc-based dosage of protamine compared with traditional calculation of protamine dosage based on body weight.

- Investigate whether postoperative blood loss and transfusion requirement differed between HeProCalc-based dosage of protamine and traditional calculations of protamine dosage based on body weight.

- Investigate whether analysis with ROTEM®*platelet* may provide additional information to conventional ROTEM® analysis regarding platelet function after CPB and after platelet transfusions.

- Compare demographic and procedural characteristics as well as clinical outcomes of patients who underwent CABG surgery and received aprotinin during two time periods, 2006–2007 and 2008–2014, before and during aprotinin´s European suspensio

3 METHODS

3.1 STUDY DESIGN AND PATIENT SELECTION

Study I and **Study II** (NCT02785575) were prospective, double-blinded (only **Study II**), randomized, single-center trials. Patient inclusion was performed between October and November 2013 (**Study I**) and during the period April through September 2016 (**Study II**). All included patients were scheduled for elective, and urgent cardiac surgery on CPB due to cardiovascular and/or valve diseases. Patients undergoing surgery involving planned hypothermia <34°C (**Study I**) or ≤32.0°C (**Study II**) were also excluded, as a severe hypothermia might affect coagulation and thus interpretation of results. Patients unable to give informed consent and those with known coagulation defects were not included (**Study I-III**). Warfarin therapy was discontinued at least three days before surgery, but aspirin was continued until day of surgery (**Study I-IV**). ADP-receptor inhibitors and new oral anticoagulant drugs were discontinued five respectively three days before surgery (**Study I-III**). None of the patients received fondaparinux less than 24 hours prior to surgery (**Study I-III**).

In **Study III** we prospectively included patients over 18 years of age, electively scheduled for extensive cardiac surgery with CPB for example combined valve replacement, redo´s, or complex aortic arch surgery between January and October 2016. The selected patients were planned to undergo complex surgery with a presumed high risk of bleeding and thereby transfusion of platelets.

Study IV was an observational, retrospective, single tertiary center study where we reviewed the medical records of 268 consecutive patients, who underwent isolated first-time on-pump CABG and received aprotinin between 2006 and 2014. During this period, aprotinin was given to patients undergoing cardiac procedures if they were at high risk for excessive bleeding or had been receiving clopidogrel within 5 days before surgery.

Study I-IV were performed at the Department of Cardiothoracic Surgery and Anesthesiology, Karolinska University Hospital, Stockholm, Sweden.

3.2 CLINICAL PRACTICE

3.2.1 Cardio pulmonary bypass system

In **Study I-III**, Sorin's open CPB system (Sorin Group, Milan, Italy) was applied for CPB, and includes a single chamber hard shell venous reservoir, the Revolution centrifugal pump, and a phosphorylcholine coated oxygenator with an integrated arterial filter. Priming consisted of 1,100 to 1,500 mL of Ringer's acetate (Baxter, Deerfield, IL USA); 250 mL of mannitol 15% (Baxter); and 5,000 (**Study I**) or 7,500 IU (**Study II-III**) of heparin (heparin and protamine, Leo Pharmaceutical, Copenhagen, Denmark). The dose of heparin added to the CPB-prime was not included in the calculation of total given dose of heparin. All patients in **Study I-III** received tranexamic acid (Pfizer Inc, New York, NY) with a bolus of 10-20 mg/kg before the start of the operation, followed by an infusion of 5-10 mg/kg/h during the operation, depending on the patient's renal function.

3.2.2 Activated clotting time measurements

Study I-II: For ACT measurements, we used kaolin reagent-based Medtronic high-range ACT cartridges with the Medtronic ACT Plus System and the Medtronic high-range heparinase test cartridges were used to detect any remaining heparin after administration of calculated protamine dose. In this test, the cartridge consists of two channels of which one contains purified bacterial heparinase that rapidly destroys any heparin left in the blood by enzymatic cleavage of linkages at the ATIII binding site. The amount of heparinase present in the channel will neutralize 6 units of heparin /ml of blood. The other channel in the cartridge is a standard high-range ACT. The heparinase channel will show a clotting time reflecting the ACT value of unheparinized blood while the standard ACT channel will identify the eventual presence of heparin in whole blood. If a difference between the two channels is detected this would imply that the sample could contain heparin. A value out of range in the heparinase channel indicates that other factors than heparin, such as low levels of fibrinogen, platelet dysfunction, or dilution of the sample, is causing the high ACT-value. Prior to induction of anesthesia, a radial artery blood sample was taken for baseline ACT. Samples for identifying any remaining heparin effect were drawn at three minutes after administration of protamine and one hour after surgery. The ACT limit for starting CPB was 480 seconds. We analyzed ACT every 30 minutes during CPB or more frequent if the ACT value was volatile.

3.2.3 Assessment of platelet inhibition

In **Study IV**, we assessed Adenosine DiphosPhate (ADP)-induced platelet aggregation with the Plateletworks® assay. The test must be performed <10 minutes after blood sampling. Baseline platelet count was measured after addition of 1 ml whole blood to the 1st Plateletworks® tube, which was primed with synthetic anticoagulant ethylenediaminetetraacetic acid (EDTA). Thereafter, one milliliter of whole blood was added to the 2nd Plateletworks® tube, which contained citrate and 20 µmol of ADP inducing platelet aggregation (**Figure 6**). Platelet count was thereafter measured for each tube with a cell counter (ABX Micros 60; Horiba ABX Diagnostics, Holliston, Massachusetts). As platelet aggregates exceed normal platelet size, the cell counter discriminates between aggregated and non-aggregated platelets based on size. The difference in platelet count between the two samples was used as an assessment of platelet aggregation, resulting in a percentage platelet inhibition from 0 to 100%.

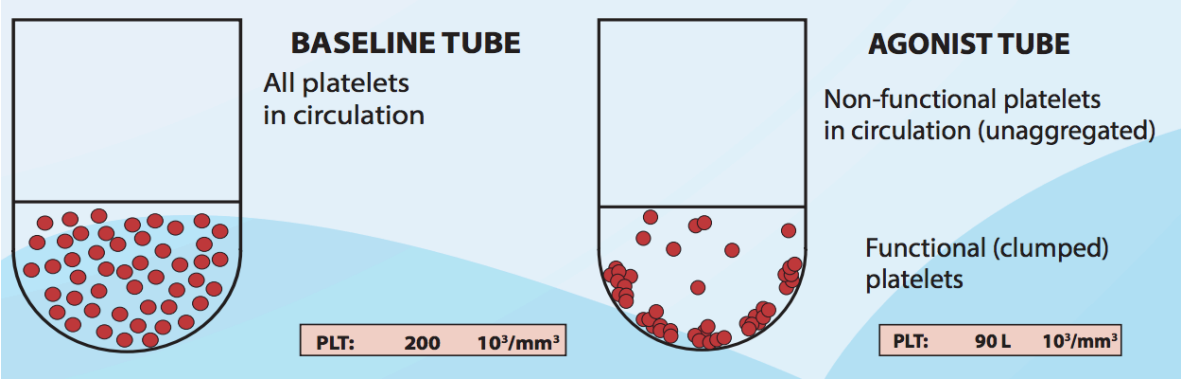


FIGURE 6. Agonist-induced platelet aggregation with examples of platelet count (reprinted with courtesy of Helena Laboratories)

3.2.4 Change in clinical use of aprotinin

Study IV: Before 2008, aprotinin was used in patients undergoing cardiac surgery if they were at high risk for excessive bleeding or had been taking clopidogrel within 5 days before surgery. In 2008, the clinical management was changed at our hospital: 1) platelet function testing (Plateletworks®) was used to assess platelet aggregation in all patients receiving clopidogrel within 5 days before surgery; 2) as a consequence of the BART study[54] and the suspension of aprotinin's market authorization, aprotinin was restricted to patients on clopidogrel and with platelet aggregation <85% on day of surgery; and 3) when possible, surgery was delayed until platelet aggregation was >85% after suspension of clopidogrel. Thus, these factors restricted the administration of aprotinin in our department after 2007.

3.2.5 HeProCalc guided perfusion system

The HeProCalc computer program has been developed to optimize heparin and protamine dosing in cardiac surgery. By using an empirically developed algorithm based on patients´ age, sex, height, weight, and baseline ACT, the HeProCalc calculates the initial dose of heparin. The program tracks and continually calculates the heparin concentration, consumption rate, as well as timing and requirement for supplemental heparin to the individual patient during CPB, by repeated input of measured ACT values, temperature, and additional doses of heparin and time points for these events. Furthermore, the algorithm calculates and continually updates the heparin concentration, and suggests additional ATIII when the calculated heparin concentration surpasses 600 IU/kg. After weaning from CPB, the algorithm estimates the protamine dose based on all the patient´s ACT values, and the quantity of heparin given over time. The target ACT was set to 550 seconds to give a border of safety if the onset of CPB would be postponed.

3.2.6 ROTEM® and ROTEM®*platelet*

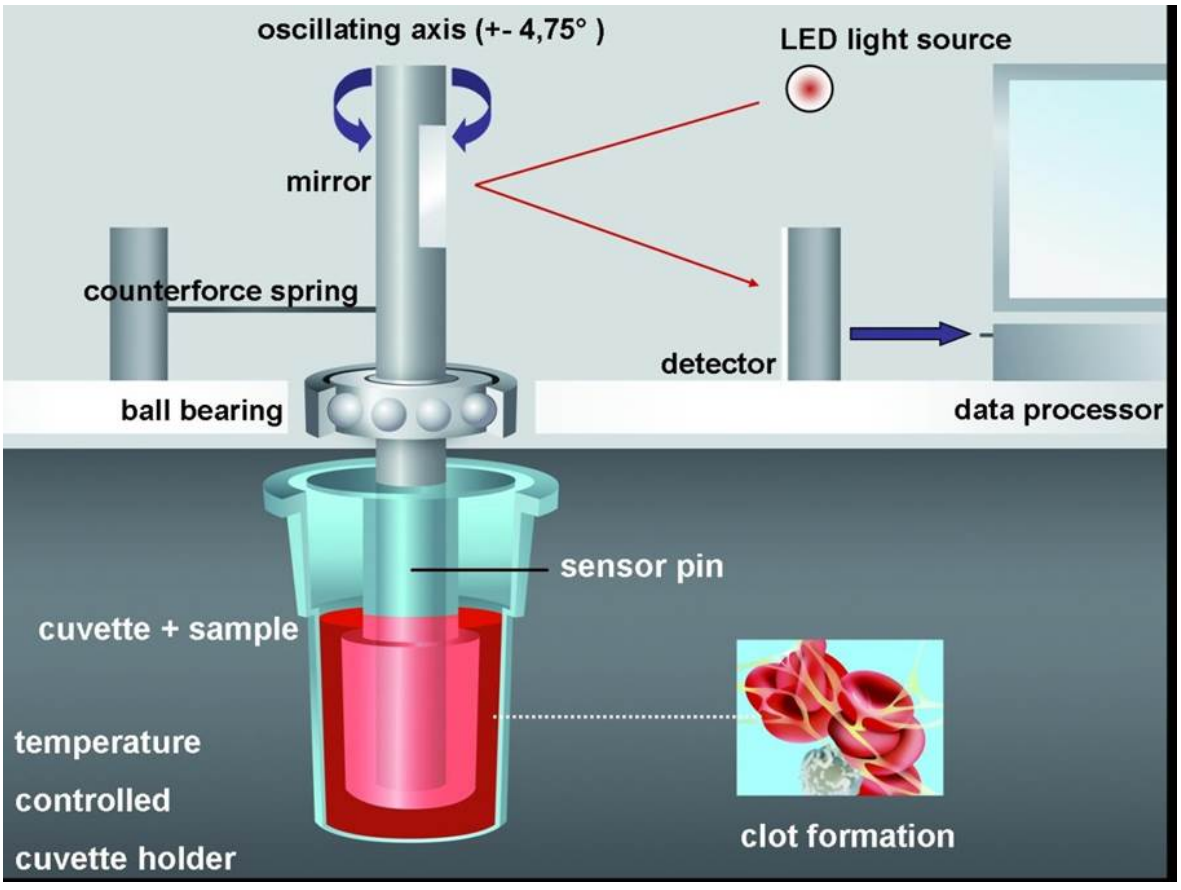


FIGURE 7. Principle functional outline of the ROTEM®, which is a whole blood viscoelastic test measuring coagulation in real time.

ROTEM®, a viscoelastographic whole blood test, is used to evaluate coagulation status. The clot formation is presented as four graphs, representing plasma coagulation, fibrinogen and platelet function, and fibrinolysis. To perform the test, a citrated whole blood sample is added

to a heated cup, in which calcium and tissue factor are inserted to induce clot formation (**Figure 7**). A rotating pin is submersed into the cup and as clotting initiates resistance increases. The changes in mechanical resistance are measured, calculated and transformed into graphs and numbers. In **Study III**, three tests were carried out; INTEM, EXTEM and FIBTEM and variables examined were clotting time (CT: time to initiation of clot formation, measured in seconds) clot formation time (CFT: time to reach a certain clot strength, measured in seconds), and maximum clot firmness (MCF measured in mm). CT reflects plasma coagulation, while CFT and MCF mirror fibrinogen levels and platelet function (**Figure 8**). FIBTEM is used to distinguish between the platelet and fibrinogen contribution to clotting.

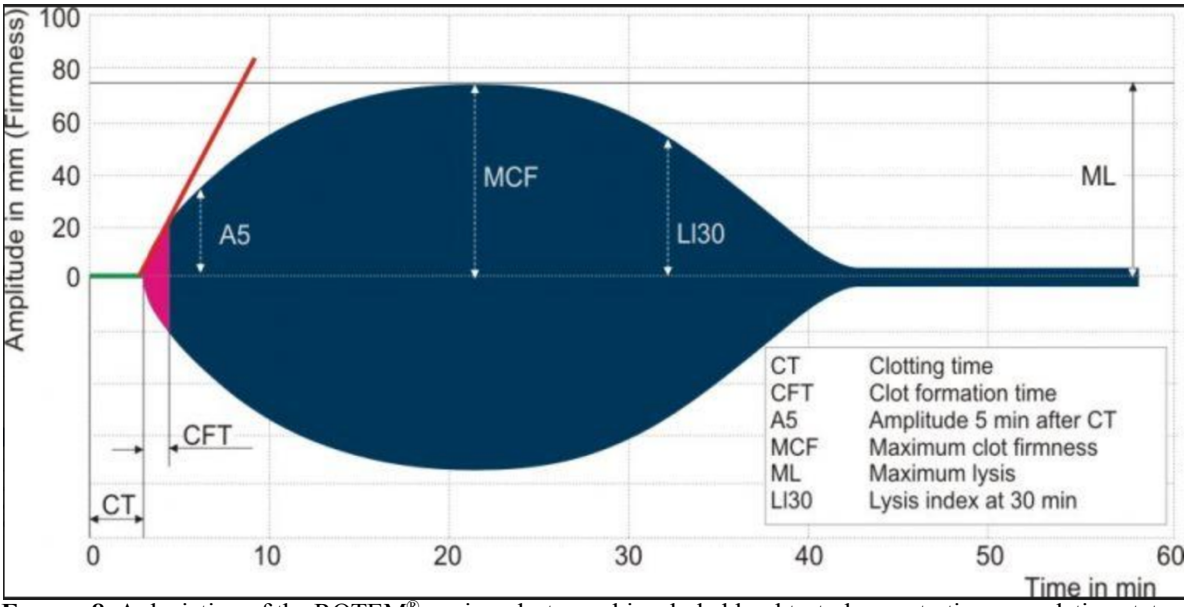


FIGURE 8. A depiction of the ROTEM®, a viscoelastographic whole blood test, demonstrating coagulation status in real time as clot initiation, propagation, stabilization, and lysis. See text for description of parameters.

3.2.7 ROTEM®*platelet* assay

The ROTEM®*platelet* assay uses impedance aggregometry. A sample of 200 µL whole blood is positioned in a cuvette containing an electrode. When a reagent is supplemented to the cuvette the platelets activation and aggregation is induced, intensifying impedance (**Figure 9**). The changes are measured and presented numerically and graphically as a curve with the results displayed as Area Under the Curve in Ohm*min (AUC). Three different reagents were utilized; ARATEM where activation is accomplished with arachidonic acid, ADPTEM where activation is prompted with ADP, and TRAPTEM where activation is stimulated with TRAP.[64]

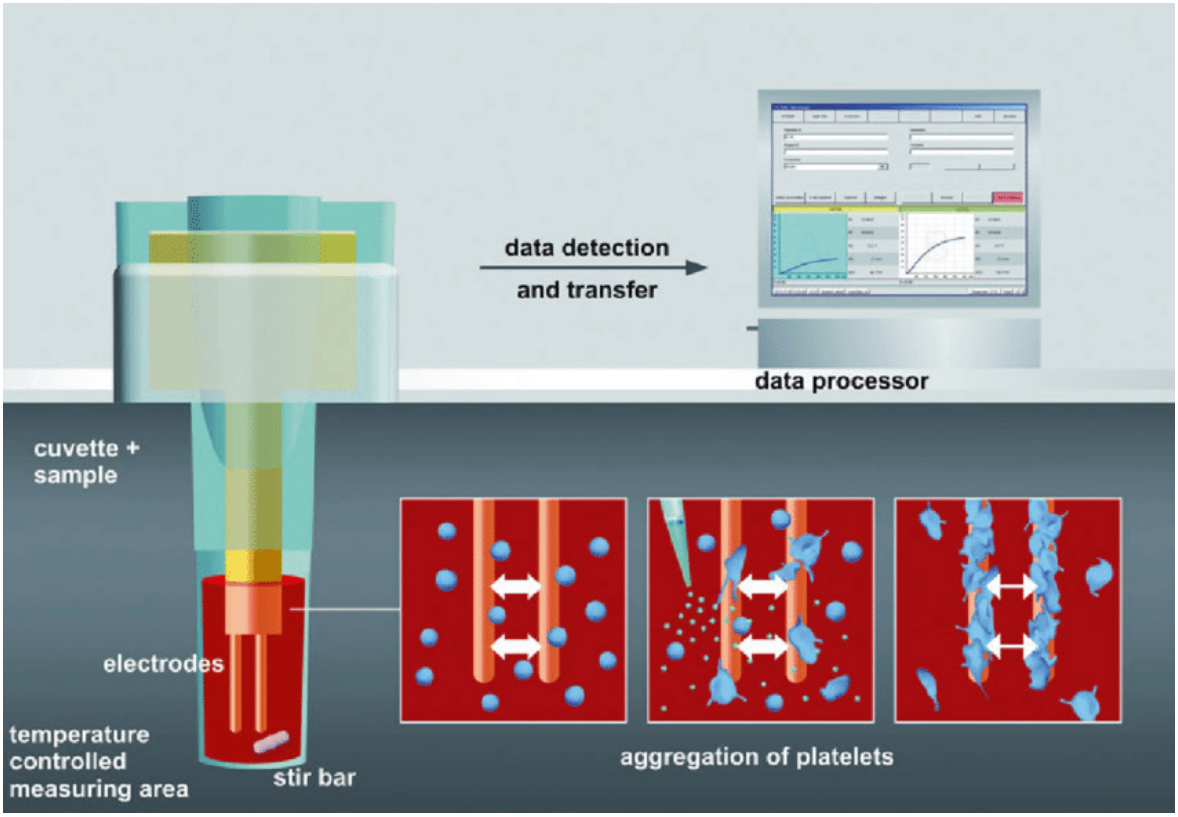


FIGURE 9. The ROTEM®*platelet* assay uses impedance aggregometry to evaluate platelet activation and aggregation, which intensifies impedance. The changes are measured and presented numerically and graphically as a curve with the results displayed as Area Under the Curve in Ohm*min (AUC).

In parallel with ROTEM® and ROTEM®*platelet* blood counts were analyzed in a hematology instrument pocH-100i (Sysmex Corp., Norderstedt, Germany).

3.2.8 Transfusions

Platelet units were leucocyte reduced and irradiated and each unit consisted of 240-300 x 10^9/L platelets. The platelet units were kept in platelet additive solution up to seven days, at 22°C in a platelet incubator (Helmer Agitator; Fenwal Europe, Mont Saint Guibert, Belgium).

Study I-II: Packed Red Blood Cells (PRBC) were transfused at an arterial hemoglobin of 70 g/L during CPB and <80 g/L after CPB, except in patients with major ongoing hemorrhage; plasma was administered if >2 U of PRBC was given; and platelets were administered if bleeding was excessive and clots were absent after the reversal of heparin with protamine and directed by thromboelastographic tests.

Study III: Decision regarding platelet transfusion was made by the attending surgeon and anesthesiologists and were based on the clinical condition without knowledge of ROTEM® or ROTEM®*platelet* results.

3.3 STUDY INTERVENTIONS

Study I-II: After informed consent, consecutive adult patients (n=40, **Study I**; n=210, **Study II**) were randomized into two groups: control group and treatment group (HeProCalc group). Random assignment was managed using unmarked envelopes, each holding a protocol indicating HeProCalc or control group that the perfusionist in charge of the patient drew during preparations for the operation. Neither surgeons or anesthetists were blinded to group assignment in **Study I**, whereas they both were blinded in **Study II**. Patients randomized to the control group obtained the traditional dose of 400 International Units (IU) of heparin/kg body weight, where after an ACT analysis was performed 3 minutes later. An additional dose of heparin (2,500 to 10,000 IU) was given at the discretion of the perfusionist if the ACT was <480 seconds. Thereafter, ACT was analyzed every 30 minutes or more often if the ACT value was unstable. Additional doses of heparin were given at the discretion of the perfusionist. After CPB, protamine was administered according to the fixed protamine dose regimen, at a ratio of 1.0 to 1.3 mg/100 IU of the first bolus dose of heparin, thereby allowing for additional protamine for reversal of additional heparin given during CPB. Patients randomized to the HeProCalc group, received heparin and protamine as suggested by the program as described above. In both groups, the last 50 mg of protamine was given after the residual heparin-containing blood from the CPB circuit had been administered.

3.4 DATA COLLECTION

Study I: During surgery, the lowest bladder temperature was registered and the number of transfused units of PRBC, plasma, or platelets per- and postoperatively were recorded. Intraoperative bleeding and postoperative blood loss from the surgical drains until the morning of postoperative day 1 were registered. Baseline ACT was collected before induction of anesthesia. HTC and ACT analyses were conducted at three time points: 3 minutes after protamine administration in the OR, 1, and 3 hours following arrival to the intensive care unit (ICU).

Study II: Standard perioperative clinical data were registered including 12-hour postoperative bleeding volume and units of postoperative transfusions, heparin and protamine dosages, baseline ACT, ACT after initial heparin dose, nadir ACT during CPB, as well as ACT and HTC after protamine and one hour after surgery, respectively. Transfusions of any blood products were recorded during the ICU stay up to 48 hours postoperatively.

Study III: The intra- and postoperative bleeding volume during the first 12 hours after surgery and number of transfusions during stay in OR and the ICU were recorded. Arterial blood

samples for ROTEM® and ROTEM®*platelet* were drawn in 2 four-mL citrate tubes BD Vacutainer™ (Becton, Dickinson and Company, Franklin Lake, NJ, USA) at 2 or 3 time points, before induction of anesthesia (T0), after protamine reversal (T1), and a 3rd sample was collected after CPB if platelets had been transfused (T2). All samples were analyzed within 2 hours.

3.5 ETHICS

The studies of this thesis conform to the principles of the Helsinki Declaration and ethics approval has been obtained from the Regional Ethical Review Board in Stockholm (**Study I**: 2013/849-31/2; **Study II**: 2015/2210; **Study III**: 2015/1788-31/2; **Study IV**: 2015/2151-32). Individual patient consent was obtained in **Study I-III**.

3.6 OUTCOME DEFINITIONS

3.6.1 Primary outcome

The primary outcome in **Study I** was the administered total dose of protamine. In **Study II** the primary outcome was postoperative bleeding within 12 hours after end of surgery. In **Study III** the primary outcome was to analyze if ROTEM®*platelet* could provide additional information to conventional ROTEM® analysis concerning platelet function. In **Study IV** the primary outcome included changes in demographic, procedural and clinical characteristics between the two time periods, 2006-2007 and 2008-2014.

3.6.2 Secondary outcomes

In **Study I** secondary outcomes were postoperative blood loss and heparin requirements. The secondary outcomes of **Study II** included transfusion rate, and heparin/protamine dosing. In **Study III** the secondary outcome was risk factors for platelet transfusion.

3.6.3 Statistics

Continuous variables were presented using means and standard deviations, and medians and 25th and 75th percentiles, and frequencies and percentages were used for categorical variables. In **Study I-III,** as most variables were not normally distributed, continuous variables were compared using the Mann–Whitney U-test. The Wilcoxon rank test was used to test related samples. Student t-test was only used for parametric data in **Study IV**, after testing for normality with the Shapiro-Wilk test. If data were not normally distributed, the Mann–Whitney test was applied. Categorical variables were compared with Fisher's exact test. All

presented p-values were two-sided, and $p<0.05$ was set as the threshold for statistically significant findings.

The following assumptions were used for estimation of power in

Study I: From previous clinical experience, we anticipated a 150 mg difference in protamine doses between the control and the HeProCalc group. Assuming a power of 0.80 and a significance level of 0.001, a study population of 20 patients in each group was necessary. Stata version 13.1 (StataCorp LP, College Station, Texas, United States) and R version 3.0.1 (R Foundation for Statistical Computing, Vienna, Austria) were applied for statistical analysis.

Study II: Based on the results from **Study I**, we estimated a significant difference in postoperative bleeding between the control and the treatment group. Assuming a power of 0.80 and a significance level of 0.05, a population sample of 43 patients in each group was required.

Study III: Founded on retrospective data we anticipated a need for platelet transfusion in 50 % of the cases. Since platelet function was tested before (T0) and after initiation of CPB (T1), and after platelet administration (T2), if given, the patients functioned as their own controls with appropriate paired testing. Thus, a number >20 was judged sufficient to avoid type I ($p<0.05$) and type II error (>80% power).

Study II-IV: All statistical analyses were performed with SPSS version 23 for Mac OS (IBM SPSS Statistics, NY, US) or with SPSS version 25 for Windows (IBM SPSS Statistics, NY, US).

4 RESULTS

4.1 STUDY I

Of 40 randomized patients, 3 patients were excluded from the final analysis; 2 patients were cooled below 34°C (exclusion criteria); and 1 patient because of initiation of extracorporeal membrane oxygenation after CPB (violence of protocol). Of the remaining 37 patients, 1 patient in the control group was reoperated <24 hours postoperatively due to an arterial bleeding (leaking aortotomy suture). All ACT values were above 400 seconds during CPB. As depicted in **Table 1**, no significant demographic baseline differences could be detected between the two groups.

TABLE 1. Baseline characteristics

	Control group (n = 19)	**HeProCalc (n = 18)**	***P* value[a]**
Age, y, mean (SD)	63.9 (10.4)	69.0 (10.7)	0.132
Median (Q1–Q3)	67 (52–73)	73 (59–78)	
Female sex	3 (16%)	5 (28%)	0.447
Weight, kg, mean (SD)	80.0 (13.5)	77.7 (12.6)	0.927
Median (Q1–Q3)	78 (71–85)	79.5 (70–87)	
Height, cm, mean (SD)	174 (8.0)	173 (8.5)	0.615
Median (Q1–Q3)	174 (170–177)	173 (170–178)	
Body surface area, m^2, mean (SD)	1.9 (0.2)	1.9 (0.2)	0.770
Median (Q1–Q3)	1.9 (1.8–2.0)	1.9 (1.8–2.0)	
Estimated GFR, mL/min/1.73 m^2, mean (SD)	86 (33)	76 (27)	0.315
Median (Q1–Q3)	78 (69–106)	76.5 (55–92)	
Hemoglobin, g/L, mean (SD)	135 (16)	131 (13)	0.513
Median (Q1–Q3)	137 (121–148)	134 (127–139)	
Platelets, 109/L, mean (SD)	206 (58)	218 (56)	0.475
Median (Q1–Q3)	201 (166–240)	214 (182–260)	
Prothrombin time, INR, mean (SD)	1.1 (0.2)	1.0 (0.1)	0.318
Median (Q1–Q3)	1.0 (1.0–1.1)	1.0 (0.9–1.1)	
Baseline ACT, s, mean (SD)	143 (22)	138 (18)	0.378
Median (Q1–Q3)	137 (133–153)	135 (129–144)	
Aspirin	12 (63%)	14 (78%)	0.476
Warfarin	4 (21%)	0	0.105
Procedure			0.769
CABG	6 (32%)	5 (28%)	
Aortic valve replacement	4 (21%)	6 (33%)	
Other	9 (47%)	7 (39%)	

Mean (standard deviation) and median (interquartile range) or number of patients and percentage, [a]compared with Mann-Whitney U-test or Fisher's exact test. Abbreviations: ACT, activated clotting time; CABG, coronary artery bypass grafting; GFR, glomerular filtration rate; INR, international normalized ratio; SD, standard deviation; Q1, 25th percentile; Q3, 75th percentile

Moreover, we did not find any significant differences in duration of surgery, duration of CPB, perioperative blood loss, and fluid balance, **Table 2.**

TABLE 2. Perioperative data

	Control group (n = 19)		**HeProCalc (n = 18)**		***P* value[a]**
	Mean (SD)	Median (Q1–Q3)	Mean (SD)	Median (Q1–Q3)	
Duration of surgery, min	212 (82)	201 (145–277)	213 (61)	207 (170–264)	0.988
Cardiopulmonary bypass time, min	104 (49)	99 (72–119)	103 (35)	102 (81–112)	0.915
Perioperative fluid balance, mL	1,893 (957)	1,650 (1,325–2,275)	1,893 (1,127)	1,925 (1,600–2,100)	0.704
Bleeding during surgery, mL	556 (426)	450 (300–750)	485 (241)	500 (300–700)	0.927

Mean (standard deviation) and median (interquartile range), [a]Compared with Mann-Whitney U-test. SD, standard deviation; Q1, 25th percentile; Q3, 75th percentile

4.1.1 Heparin

We did not find significant differences in mean total dosage of heparin, including bolus- and iterated doses, between the HeProCalc group and the control group (43,300 ± 13,000 IU vs. 40,500 ± 10,500 IU, p=0.359; **Table 3.**).

TABLE 3. Heparin, protamine, and postoperative bleeding in **Study I**

	Control group (n = 19)		**HeProCalc (n = 18)**		***P* value[a]**
	Mean (SD)	Median (Q1–Q3)	Mean (SD)	Median (Q1–Q3)	
Heparin before CPB, IU x10^3	33 (7.2)	31.2 (30–35)	31.2 (6.4)	30 (25–35)	0.436
Heparin total, IU x10^3	40 (10.6)	37.5 (32.5–47.5)	43.3 (12.9)	41.2 (37.5–47.5)	0.359
Protamine, mg	330 (61)	300 (300–350)	211 (56)	200 (200–250)	<0.001
Postoperative bleeding[b], mL	649 (279)	595 (400–870)	480 (229)	440 (340–530)	0.074

Mean (standard deviation) and median (interquartile range), [a]Compared with Mann-Whitney U-test. CPB, cardiopulmonary bypass; IU, international units; [b]One patient in the control group was excluded from the analysis of postoperative blood loss because of reoperation for surgical bleeding. SD, standard deviation; Q1, 25th percentile; Q3, 75th percentile

4.1.2 Protamine

The mean total amount of protamine was significantly lower in the HeProCalc group (211 ± 56 mg vs. 330 ± 61 mg, p <0.001; **Table 3.**). No significant differences were detected in ACT values before heparin administration or after protamine administration between the two groups (**Figure 10**).

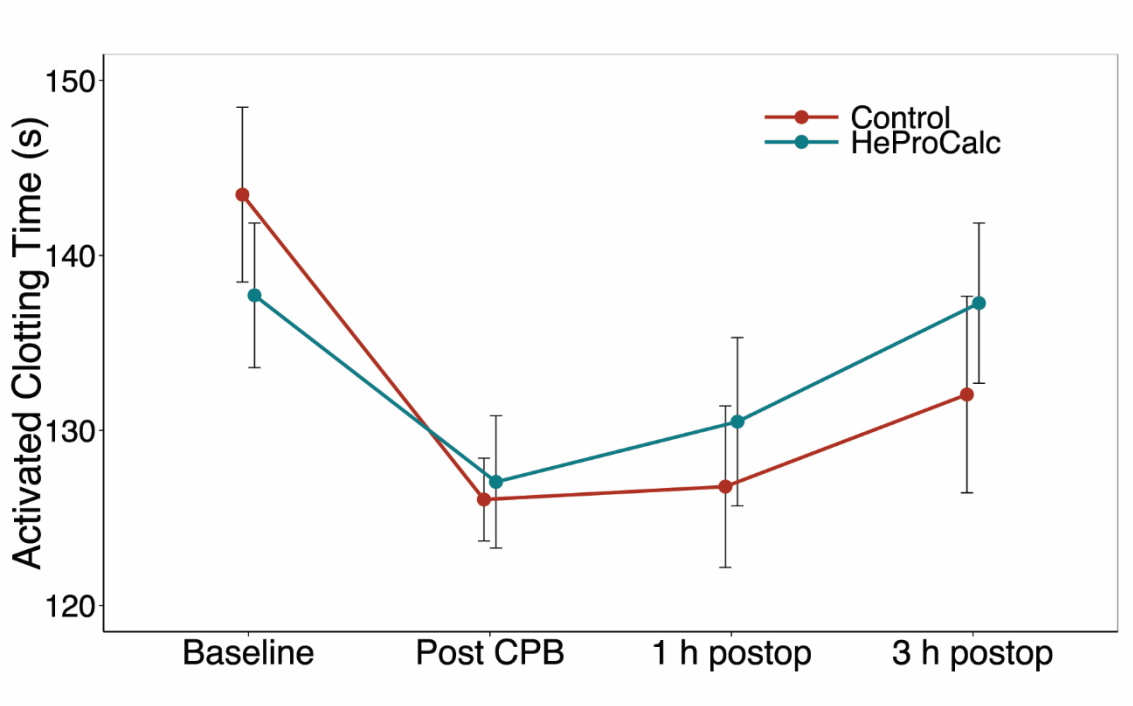


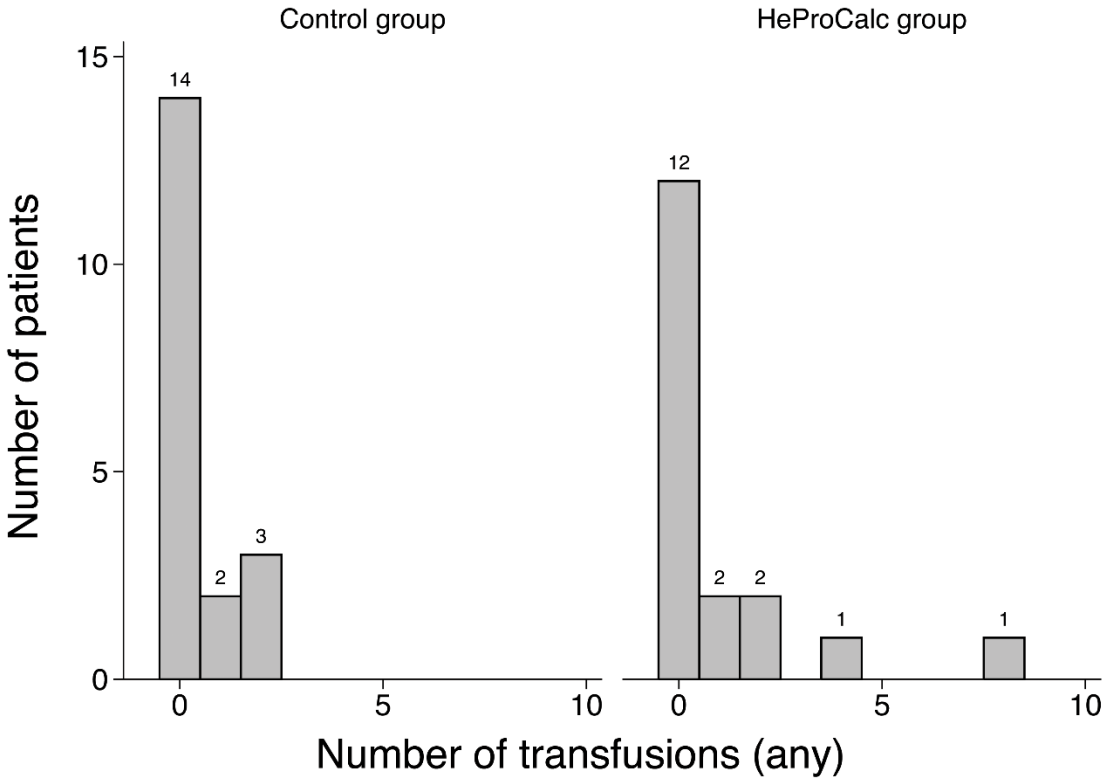
FIGURE 10. Activated clotting time (ACT) as measured at 4 different time points in unheparinized blood in the HeProCalc group versus the control group in **Study I**. Baseline ACT was measured before the induction of anesthesia. Values depicted are mean (SEM). No statistically significant differences between the groups were detected at any of the time points ($p > 0.05$).

4.1.3 Additional Heparin, Activated Clotting Time, Bleeding, and Transfusion Requirements

Mean ACT after the initial bolus of heparin was 514 ± 138 seconds in the HeProCalc group against 621 ± 141 seconds in the control group ($p=0.013$). In the HeProCalc group, 4 patients did not reach an ACT value exceeding 400 seconds after the initial bolus of heparin, while in the control group, all patients achieved an ACT value above 400 seconds. To accomplish ACT >480 seconds before initiation of CPB, additional heparin was administered in 8 of the 18 (44.4%) patients in the HeProCalc group versus in 2 of 19 (10.5%) in the control group. Mean postoperative blood loss presented no significant difference between the groups (480 ± 229 mL vs. 649 ± 279 mL, $p=0.074$), after 1 patient in the control group had been excluded due to reoperation for surgical bleeding (**Table 3**). **Table 4** and **Figure 11** depict the number of blood products transfused intra- and postoperatively.

TABLE 4 Transfusion of blood products in **Study I**

	Control group (n =19)	HeProCalc (n = 18)
Intraoperatively (total number of units)	2	8
Packed red blood cells	0	4
Plasma	0	2
Platelet concentrate	2	2
Postoperatively (total number of units)	6	10
Packed red blood cells	4	3
Plasma	0	5
Platelet concentrate	2	2



Transfusion of blood products			
	Control group (n=19)	HeProCalc (n=18)	p-value
Number of transfusions (any), median, (25^{th}/75^{th} percentile)	0 (0/1)	0 (0/1)	0.546
Number of patients receiving any transfusion	5 (26%)	6 (33%)	0.641

FIGURE 11. Transfusion of blood products in the HeProCalc group and the control group in **Study I**.

4.2 STUDY II

Twenty of the 210 randomly allocated patients were excluded from the analysis due to re-exploration for localized surgical bleeding (n=9), violation of protocol (n=2), administration of aprotinin (n=3), or nadir body temperature <32℃ during CPB (n=6). Hence, the final analysis included 190 patients, with 93 patients in the HeProCalc group (49%) and 97 patients in the control group (51%). **Figure 12** illustrates the patient flow chart matching with the CONSORT (Consolidated Standards of Reporting Trials) statement.

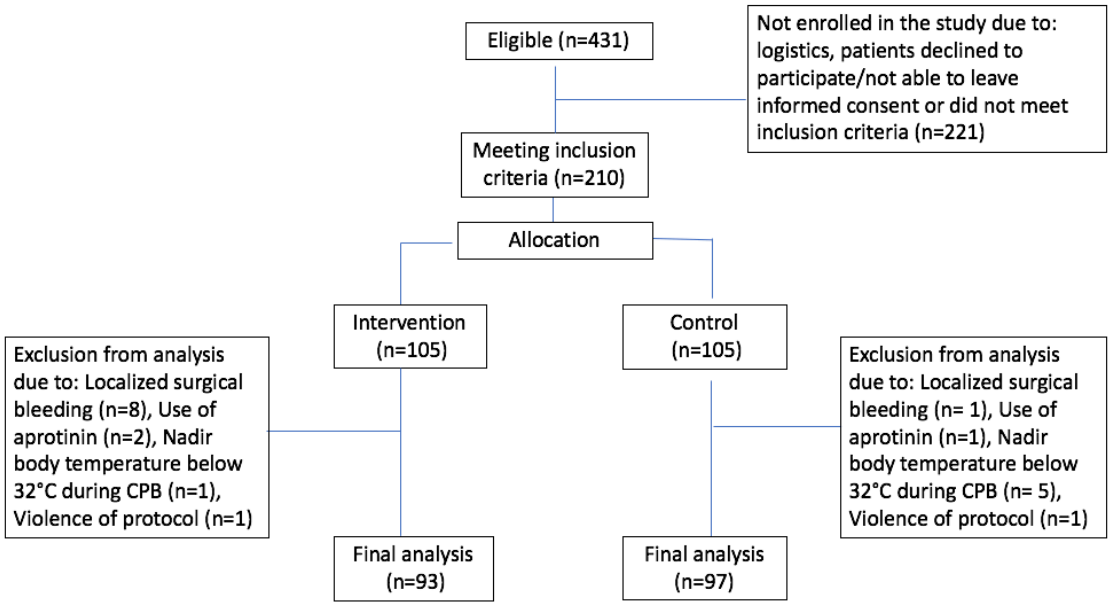


FIGURE 12. Patient flow chart according to the CONSORT statement. CPB, cardiopulmonary bypass in **Study II**.

As presented in **Table 5**, no significant differences were detected in baseline characteristics, including age, body mass index, EuroSCORE (European System for Cardiac Operative Risk Evaluation) II, preoperative hemoglobin, and preoperative anticoagulation therapy between the two groups. All except two patients stopped taking new oral anticoagulants >4 days prior to surgery, and of the two patients, one stopped intake of new oral anticoagulants 3 days prior to surgery and the other patient 2 days before surgery.

TABLE 5. Baseline characteristics in **Study II**

	Intervention group n = 93 (49)	**Control group n = 97 (51)**	***P* value[a]**
Age, years	68 (60-72) 65 ± 10.1	68 (64-74) 67 ± 9.7	0.279
Female gender	16 (17)	21 (22)	0.469
Height, cm	175 (170-180) 176 ± 8.0	175 (169-181) 175 ± 9.3)	0.623
Weight, kg	83 (75-92) 84.3 ± 14	85 (74-95) 85.3 ± 16	0.629
BMI, kg/m^2	27.0 (24.2-29.8) 27.3 ± 4.3	27.4 (24.6-30.1) 28.0 ± 4.6	0.486
EuroSCORE II score	1.25 (0.91-1.96) 1.89 ± 2.6	1.44 (0.95-2.64) 2.21 ± 2.1	0.061
Creatinine, mmol/L	86 (73-95) 86.2 ± 23.4	83 (73-95) 85.5 ± 21	0.744
INR	1.0 (1.0-1.1) 1.05 ± 0.17	1.0 (1.0-1.1) 1.05 ± 0.14	0.541
Aspirin	51 (54.8)	51 (53.1)	0.884

Categorical variables are presented as n (%) and compared with the Fischer's exact test. Continuous variables are presented as mean (standard deviation), median (25th/75th percentile), and [a]compared with the Mann-Whitney U-test. BMI body mass index; INR international normalized ratio, EuroSCORE, European System for Cardiac Operative Risk Evaluation

Perioperative data are presented in **Table 6**. There were no significant differences in any of the variables, including type of surgical procedure, duration of CPB or surgery, nadir body temperature, and intraoperative bleeding between the 2 groups.

TABLE 6. Perioperative data in **Study II**

	Intervention group n = 93 (49)	**Control group n = 97 (51)**	***P* value[a]**
Body temperature nadir, °C	35.8 (35.5-36.0) 35.6 ± 0.7	35.8 (35.0-36.0) 35.5 ± 0.8	0.381
Duration of surgery, minutes	187 (167-218) 195 ± 45.6	205 (167-242) 212 ± 64.1	0.083
CPB, minutes	96 (71-117) 98 ± 33.7	94 (75-128) 105 ± 45.7	0.576
Aortic cross clamp, minutes	72 (53-89) 73 ± 27.6	70 (54-93) 79 ± 36.3	0.563
Type of Surgical Procedure			0.311
CABG	37 (40)	35 (36)	
Minimal invasive MV-repair	8 (8.6)	8 (8.2)	
Minimal invasive AVR	5 (5.4)	6 (6.2)	
MVR	4 (4.3)	0	
AVR	22 (24)	21 (22)	
CABG + AVR	4 (4.3)	12 (12)	
Replacement of ascending aorta + AVR	5 (5.4)	3 (3.1)	
Replacement of ascending aorta	1 (1.1)	2 (2.1)	
Other surgical procedures	7 (7.5)	10 (10.3)	

Categorical variables are presented as n (%) and compared with the Fischer's exact test. Continuous variables are presented as mean (standard deviation), median (25th/75th percentile) and [a]compared with the Mann-Whitney U-test. AVR, aortic valve replacement; CABG, coronary artery bypass grafting; CPB, cardiopulmonary bypass; MV, mitral valve; MVR, mitral valve replacement

According to **Table 7**, the initial median dose of heparin was significantly lower in the HeProCalc group (32,500 IU; IQR 30,000-35,000) compared with the control group (35,000 IU; IQR 30,000-37,500; p=0.025), while the median total heparin dose did not vary significantly between the HeProCalc group (40,000 IU; IQR 32,500-47,500) and the control group (42,500 IU; IQR 35,000-50,000; p=0.685). After the initial heparin dose no patient in either group had an ACT <350 seconds. None of the patients in the HeProCalc group had a nadir ACT value <400 seconds during CPB compared with 2 patients in the control group (320 and 398 s). Antithrombin was administered at a dose of 500 to 1,000 IU to four patients in the HeProCalc group during CPB when the anticipated heparin concentration, according to the HeProCalc program, surpassed 600 IU/kg, while antithrombin was not administered to any patient in the control group.

TABLE 7. Heparin, Protamine and ACT measurement in **Study II**

	Intervention group n = 93 (49)	**Control group n = 97 (51)**	***P* value[a]**
Heparin initial dose, $x10^3$, IU	32.5 (30-35) 32.4 ± 4.5	35 (30-37.5) 34.3 ± 6.3	0.025
Number of extra heparin doses pre-CPB	0 (0-0) 2.5 ± 5.9	0 (0-0) 1.2 ± 2.7	0.248
Heparin total dose, $x10^3$ IU	40 (32.5-47.5) 41.7 ± 11	42.5 (35-50) 42.6 ± 10	0.685
Number of extra heparin doses during CPB	1 (0.0-2.0) 1.44 ± 1.2	1 (0.0-2.0) 0.99 ± 1.1	0.007
Protamine, mg	210 (190-240) 216 ± 48	350 (300-380) 352 ± 64	<0.001
Ratio Protamine/initial heparin dose (mg/100 IU)	0.62 (0.54-0.68) 0.62 ± 0.1	1.00 (1.00-1.00) 0.99 ± 0.1	<0.001
Ratio Protamine/Total heparin dose (mg/100 IU)	0.53 (0.47-0.57) 0.52 ± 0.1	0.86 (0.75-1.00) 0.85 ± 0.1	<0.001
ACT pre-heparin, seconds	139 (132-151) 140 ± 16	144 (129-153) 141 ± 19	0.636
ACT pre-CPB, seconds	549 (493-653) 579 ± 129	594 (493-712) 630 ± 170	0.095
Number of patients with a nadir ACT <400 seconds during CPB	0 (0)	2 (2.1)	0.498
Each patient's average ACT during CPB, seconds	557 (523-630) 589 ± 97.6	566 (524-660) 611 ± 122	0.431
Each patient's median ACT during CPB, seconds	554 (520-596) 575 ± 99	549 (518-635) 600 ± 124	0.405
ACT post protamine, seconds	127 (121-133) 126 ± 12	126 (121-133) 128 ± 12	0.538
HTC post protamine, seconds	125 (117-133) 126 ± 12	128 (121-133) 128 ± 11	0.148
ACT 1 hour after end of surgery, seconds	124 (111-134) 122 ± 19	123 (110-133) 122 ± 15	0.575
HTC 1 hour after end of surgery, seconds	123 (109-130) 119 ± 17	123 (110-130) 121 ± 16	0.578

Categorical variables are given as n (%) and compared with the Fischer's exact test. Continuous variables are displayed as mean (standard deviation), and median (25^{th}/75^{th} percentile) and [a]compared with the Mann-Whitney U-test. ACT, activated clotting time; CPB, cardiopulmonary bypass; HTC, heparinase test cartridge; IU, international units.

The median protamine doses were 210 mg (IQR 190-240) and 350 mg (IQR 300-380) in the HeProCalc and control groups, respectively (p <0.001), giving a ratio of protamine/initial heparin dose of 0.62:1 (range 0.40-0.93) for the HeProCalc group and 1.0:1 for the control group (p <0.001). The ACT and HTC values did not disagree significantly between the two groups.

TABLE 8. Hemoglobin, bleeding and transfusions in **Study II**

	Intervention group n = 93 (49)	**Control group N = 97 (51)**	***P* value[a]**
Preoperative hemoglobin, g/L	142 (129-148) 139 ± 14.4	141 (130-147) 138 ± 14	0.771
Nadir postoperative hemoglobin, g/L	100 (88.5-111.5)100 ± 14	92 (87-103) 95 ± 14	0.015
Preoperative hemoglobin – nadir postoperative hemoglobin, g/L	40 (29-47.5) 39.1 ± 15	44 (34-53) 43.3 ± 14	0.058
Intraoperative bleeding, mL	300 (200-500) 385 ± 244	400 (250-500) 510 ± 713	0.219
Postoperative bleeding 12h, mL	320 (250-460) 355 ± 157	350 (250-450) 362 ± 156	0.754
PRBC U, Intraoperatively	0 (0-0) 0.08 ± 0.5	0 (0-0) 0.25 ± 1.1	0.072
PRBC U, Postoperatively	0 (0-0) 0.49 ± 1.7	0 (0-0) 0.35 ± 0.9	0.909
Plasma U, Intraoperatively	0 (0-0) 0.00 ± 0.0	0 (0-0) 0.04 ± 0.3	0.498
Plasma U, Postoperatively	0 (0-0) 0.00 ± 0.0	0 (0-0) 0.05 ± 0.3	0.121
Platelets U, Intraoperatively	0 (0-0) 0.05 ± 0.2	0 (0-0) 0.14 ± 0.5	0.283
Platelets U, Postoperatively	0 (0-0) 0.05 ± 0.3	0 (0-0) 0.02 ± 0.1	0.362
Intraoperative number of transfused allogeneic blood products	0 (0-0) 0.13 ± 0.6	0 (0-0) 0.43 ±1.7	0.044
Postoperative number of transfused allogeneic blood products	0 (0-0) 0.55 ± 1.7	0 (0-0) 0.42 ±1.0	0.901
Total number of transfused allogeneic blood products	0 (0-0) 0.68 (2.0)	0 (0-1.00) 0.86 (2.2)	0.181
Any transfusion, number of patients	17 (18)	24 (25)	0.295
UDPB			
Class 0	77 (83)	79 (81)	0.851
Class 1	6 (6.5)	8 (8.2)	0.783
Class 2	7 (7.5)	10 (10)	0.614
Class 3	3 (3.2)	0	0.115
Class 4	0	0	-
BARC CABG-related bleeding class 4	0	0	-
BART	0	0	-
PLATO major bleeding	20 (22)	30 (31)	0.102

Categorical variables are presented as n (%) and compared with the Fischer's exact test. Continuous variables are presented as mean, standard deviation, and median (25^{th}/75^{th} percentile) and [a]compared with the Mann-Whitney U-test ACT, activated clotting time; BARC, bleeding academic research consortium; BART, the blood conservation using antifibrinolytics in a randomized trial; CPB, cardiopulmonary bypass; ICU, intensive care unit; HTC, heparinase test cartridges; PRBC, packed red blood cells; PLATO, platelet inhibition and patient outcomes; UDPB, universal definition for perioperative bleeding; U, units.

Median postoperative blood loss during the first 12 hours was 320 mL (IQR 250-460) and 350 mL (IQR 250-450) in the HeProCalc and the control groups, respectively (p=0.754, **Table 8**). Postoperative transfusion rate of PRBC units during the ICU stay was comparable in both groups, with 12.9% in the HeProCalc group and 12.4% in the control group (p=1.00). There were no significant differences in number of transfused blood products during the 48-hour study period, aside from fewer transfused units intraoperatively in the HeProCalc group (p=0.044). The difference in median preoperative hemoglobin and nadir post-CPB hemoglobin values did not differ significantly between the two groups (p=0.058), with a

decrease of 40 g/L in the HeProCalc group and 44 g/L in the control group. However, the median nadir post-CPB hemoglobin concentration differed significantly between the two groups (p=0.015), with 100 g/L in the HeProCalc group and 92 g/L in the control group. Moreover, postoperative bleeding and transfusion rate did not differ significantly between the groups when classified concurring with the adapted definitions of Bleeding Academic Research Consortium (BARC), Blood Conservation Using Antifibrinolytics in Randomized Trial (BART), Platelet Inhibition and Patient Outcomes (PLATO), and Universal Definition of Perioperative Bleeding (UDPB (**Table 8**).

4.3 STUDY III

The included patients underwent combined surgical procedures (n=10), redo surgery (n=14), surgery requiring circulatory arrest (n=2) and other complicated cardiac surgery (n=2).

TABLE 9. Baseline characteristics in **Study III**

	Whole cohort (n = 23)	**Transfused patients (n = 10)**	**Non-transfused Patients (n = 13)**	***P* value**
Clinical Parameters				
Age, years	62 (52/74)	60.5 (50/73)	63 (57/74)	0.436
Female gender, %	7 (30)	4 (40)	3 (23)	0.650
BMI, kg/m^2	24 (22/28)	23 (22/26)	25 (23/28)	0.533
EuroSCORE II	3.33 (2.30/7.47)	7 (3.34/10.4)	3 (1.85/3.33)	0.012
Preoperative laboratory data				
Hemoglobin, g/L	123 (111/133)	124 (104/133)	123 (115/128)	0.964
Hematocrit, %	36.4 (33.4/38.3)	36.6 (33.0/39.0)	36.4 (34.0/38.0)	0.867
Creatinine, µmol/L	84 (68/109)	87 (73/129)	84 (68/105)	0.532
eGFR	77 (57/116)	69 (61/108)	95 (57/116)	0.522
Platelet count x 10^9/L	156 (140/186)	154 (148/177)	156 (140/186)	0.892
INTEM CT, seconds (100-240)	157 (135/183)	150.5 (131/186)	164 (153/180)	0.573
INTEM CFT, seconds (30-110)	70 (61/81)	63.5 (50/82)	72 (66/81)	0.256
EXTEM CT, seconds (38-79)	61 (52/66)	62 (50/65)	57 (53/66)	0.681
EXTEM CFT, seconds (34-159)	75 (64/84)	74.5 (55/83)	75 (66/85)	0.512
EXTEM MCF, mm (50-72)	64 (62/67)	65.5 (63/69)	63 (62/64)	0.189
FIBTEM MCF, mm (9-25)	16 (11/21)	20 (15/23)	15 (10/16)	0.050
ARATEM, AUC (70-153)	62 (33/73)	55 (28/87)	65 (45/71)	0.843
ADPTEM, AUC (56-139)	48 (39/68)	44 (37/68)	59 (48/64)	0.263
TRAPTEM, AUC (61-156)	89 (76.8/111)	86.5 (75/102)	89 (81/114)	0.446

Data are presented as median (25th/75th percentile) for continuous variables, and as frequency (percentage) for categorical variables. Continuous variables were tested with the Mann-Whitney Test and categorical variables with the Chi-Square test or Fisher's exact test. ROTEM and ROTEM®*platelet* normal values are presented in brackets (as presented by manufacturer TEM Innovations, GmbH, Munich, Deutschland). BMI, body mass index; eGFR, estimated glomerular filtration rate; AUC, area under curve; CFT, clot formation time; CT, clotting time; MCF, maximum clot firmness.

After exclusion of 5 patients (use of aprotinin, n=3; clopidogrel not stopped according to the study protocol, n=1; laboratory sample was by mistake not analyzed, n=1), results from 23

patients were comprised in the final analysis. Ten out of 23 (43%) patients were given platelet transfusion during surgery after end of CPB. We did not find significant differences between transfused and non-transfused patients in respect to age, body mass index (BMI), or laboratory findings, including ROTEM® and ROTEM®*platelet* prior to surgery, with the exception that the transfused group had a higher EuroSCORE II, **Table 9**.

4.3.1 ROTEM® and ROTEM®*platelet* analyses after CPB and after platelet transfusion

For all patients, results from ROTEM®, ROTEM®*platelet*, hemoglobin, hematocrit, and platelet count differed significantly between T0 and T1, **Table 10**.

TABLE 10. ROTEM® and ROTEM®*platelet* analyses after CPB and after platelet transfusion in **Study III.** Whole cohort (n = 23).

	Before CPB (T0)	**After CPB (T1)**	***P* value[a]**
Platelet count ($x10^9$)	156(140/186)	101(81/135)	<0.001
ROTEM® analyses			
INTEM CT, seconds (100-240)	157 (135/183)	197 (170/217)	<0.001
INTEM CFT, seconds (30-110)	70 (61/81)	108 (81/128)	<0.001
EXTEM CT, seconds (38-79)	61 (52/66)	85 (75/88)	<0.001
EXTEM CFT, seconds (34-159)	75 (64/84)	97 (77/121)	<0.001
EXTEM MCF, mm (50-72)	64 (62/67)	57 (54/61)	<0.001
FIBTEM MCF, mm (9-25)	16 (11/21)	11 (10/15)	<0.001
ROTEM®*platelet* analyses			
ARATEM, AUC (70-153)	62 (33/73)	15 (8/26)	<0.001
ADPTEM, AUC (56-139)	48 (39/68)	32 (20/42)	<0.001
TRAPTEM, AUC (61-156)	89 (77/111)	65 (49/86)	<0.001

Data are presented as median (25^{th}/75^{th} percentile). [a]The variables were tested with the Wilcoxon test. ROTEM and ROTEM®*platelet* normal values are presented in brackets (as presented by manufacturer TEM Innovations, GmbH, Munich, Deutschland). AUC, area under curve; CPB, cardiopulmonary bypass; CFT, clot formation time; CT, clotting time; MCF, maximum clot firmness.

Patients receiving platelets had a significantly augmented EXTEM CT compared with the non-transfused group after end of CPB and prior to platelet transfusion (T1, 88 and 76 seconds, respectively, p=0.008). At T1, other ROTEM® parameters or platelet counts were not significantly different between the groups, and with the ROTEM®*platelet* assay a significant difference between the two groups was only detected with TRAP activation (51 vs. 80 AUC, p=0.004), **Figure 13** and **Table 11**.

TABLE 11. Hematological variables at T1 (after heparin reversal with protamine and before platelet transfusion) in **Study III**

	Patients receiving platelet transfusions (n=10)	**Patients not receiving platelet transfusions (n=13)**	***P* value[a]**
Hemoglobin, g/L	83·5 (76/91·8)	90 (88/98)	0·170
Erythrocyte Volume Fraction	0·25 (0·22/0·27)	0·27 (0·29/0·3	0·335
Platelet count (x10^9/L)	87·5 (77/121)	106 (81/137)	0·250
ROTEM® analyses and normal values			
INTEM CFT, seconds (30-110)	101·5 (86/157)	108 (80/126)	0·726
INTEM CT, seconds (100-240)	217 (169/247)	194 (173/209)	0·119
EXTEM CT, seconds (38-79)	88 (80/94)	76 (71/85)	0·008
EXTEM CFT, seconds (34-159)	101 (78·3/123)	89 (77/121)	0·704
EXTEM MCF, mm (50-72)	58 (53·3/62·3)	55 (54/59)	0·473
FIBTEM MCF, mm (9-25)	14 (10·8/17·8)	11 (9/12)	0·042
ROTEM®platelet analyses and normal values			
ARATEM, AUC (70-153)	13·5 (8·8/26)	20 (6/26)	0·659
ADPTEM, AUC (56-139)	22 (15/35·8)	39 (24/43)	0·097
TRAPTEM, AUC (61-156)	50·5 (47/67)	80 (65/90)	0·004

Data are presented as median (25^{th} / 75^{th} percentile) and compared with [a]the Mann-Whitney test. ROTEM and ROTEMplatelet normal values are presented in brackets (as presented by manufacturer TEM Innovations, GmbH, Munich, Deutschland). AUC**,** Area Under Curve; CFT**,** Clot Formation Time; CT**,** Clotting Time; MCF**,** Maximum Clot Firmness

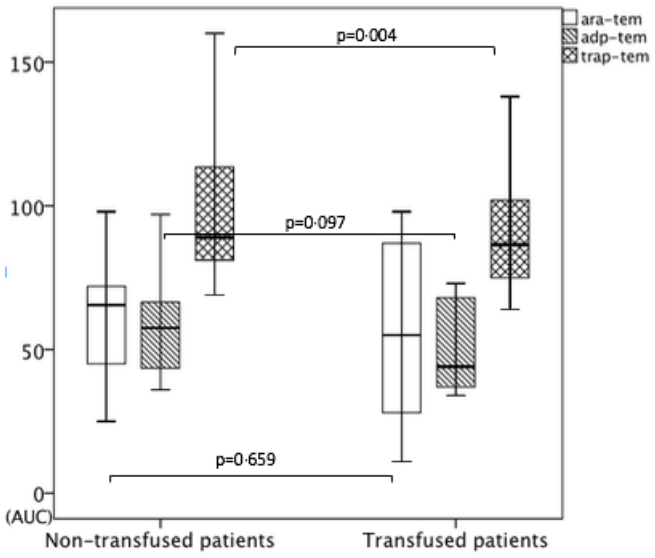


FIGURE 13. In the ROTEM®*platelet* assay (**Study III**), a significant difference between the transfused and non-transfused patients was only seen at T1 with TRAPTEM activation (51 vs. 80 AUC, p=0.004).

In patients receiving transfusion of platelets, ROTEM®*platelet* showed significantly superior results after transfusion (T2) versus before (T1) regarding TRAPTEM (p=0.008), but without significant changes concerning ADPTEM or ARATEM, **Figure 14**.

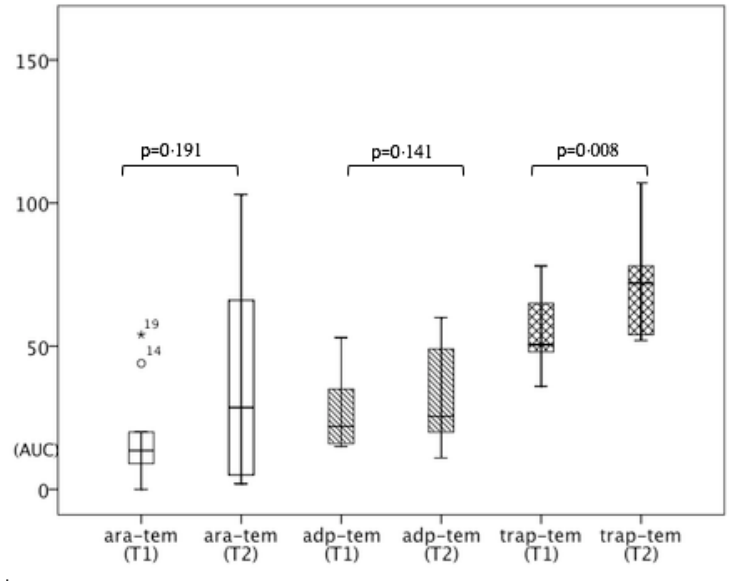


FIGURE 14. In patients receiving transfusion of platelets (**Study III**), ROTEM®*platelet* showed significantly superior results after transfusion (T2) versus before (T1) regarding TRAPTEM (p=0.008), but without significant changes concerning ADPTEM or ARATEM.

As depicted in **Table 12**, ROTEM® results improved and platelet count augmented significantly after platelet transfusion (87.5 x 10^9/L compared with 139 x 10^9/L following transfusion, p=0.002). After heparin reversal, ACT returned to baseline both for transfused and non-transfused patients (130 vs. 123 seconds, p=0.213).

TABLE 12. Platelet transfused patients in **Study III** (n = 10)

	After CPB (T1)	**After transfusion of platelets (T2)**	***P* value[a]**
Laboratory data			
Hemoglobin, g/L	83.5 (76/91.8)	84 (73.8/90.8)	0.236
Erythrocyte Volume Fraction	0.25 (0.22/0.27)	0.25 (0.22/0.27)	0.221
Platelet count (x10^9/L)	87.5 (77.2/121)	139 (124/173)	0.002
ROTEM® analyses			
INTEM CFT, seconds (30-110)	101.5 (86/157)	73 (63.3/85.5)	0.002
INTEM CT, seconds (100-240)	217 (169/247)	199.5 (161/207)	0.127
EXTEM CT, seconds (38-79)	88 (80.5/94)	64 (59/66.5)	0.002
EXTEM CFT, seconds (34-159)	100.5 (78.3/123)	71.5 (61.8/82)	0.002
EXTEM MCF, mm (50-72)	58 (53.3/62.3)	66.5 (61.8/68.8)	0.004
FIBTEM MCF, mm (9-25)	14 (10.8/17.8)	19.5 (14.3/24.8)	0.004
ROTEM®*platelet* analyses			
ARATEM, AUC (70-153)	13.5 (8.8/26)	28.5 (4.5/70.8)	0.191
ADPTEM, AUC (56-139)	22 (15.8/35.8)	25.5(18.5/50)	0.141
TRAPTEM, AUC (61-156)	50.5 (47/67)	72 (53.8/79)	0.008

Data are presented as median (25^{th}/75^{th} percentile). [a]The variables were tested with the Wilcoxon test. ROTEM and ROTEM®*platelet* normal values are presented in brackets (as presented by manufacturer TEM Innovations, GmbH, Munich, Deutschland). AUC, area under curve; CPB, cardiopulmonary bypass; CFT, clot formation time; CT, clotting time; MCF, maximum clot firmness

4.3.2 Risk factors for transfusion

Patients given platelet transfusion had, in addition to a preoperatively elevated EuroSCORE II (7 vs. 3, p=0.012), significantly prolonged duration of surgery (290 vs. 254 minutes, p=0.034) and significantly lengthier time on CPB than patients in the non-transfused group (192.5 vs. 117 minutes, p=0.005). Also, intraoperative blood loss was significantly elevated in patients receiving platelet transfusions (850 vs. 600 ml, p=0.002). Concerning the 12-hour postoperative bleeding volume, no significant difference was found between transfused and non-transfused patients, **Table 13.** Five of the transfused patients received 1 unit of platelets and 5 patients received 2 units. No adverse outcomes related to the transfusions were detected. Of the 10 patients receiving platelet transfusion, only 4 received PRBC (range 1-4 units) and/or plasma (range 1-2 units), and all 10 were administered fibrinogen (range 1-5 g) during the perioperative procedure.

TABLE 13. Peri- and postoperative parameters in **Study III**

	Whole cohort (n = 23)	**Transfused patients (n = 10)**	**Non-transfused patients (n = 13)**	***P* value[a]**
Duration of surgery, minutes	276 (230/333)	290 (270/436)	254 (214/286)	0.034
CPB, minutes	135 (110/203)	192.5 (147/284)	117 (110/131)	0.005
Intraoperative bleeding, mL	700 (450/900)	850 (800/1800)	600 (400/700)	0.002
12 h postoperative bleeding, mL	410 (270/710)	510 (270/860)	410 (320/620)	0.594
PRBC, Units intraoperatively	0 (0/2)	0 (0/2)	0 (0/0)	0·256
Plasma, Units intraoperatively	0 (0/0)	0 (0/0)	0 (0/0)	0·435
Fibrinogen, grams intraoperatively	2 (2/4)	3 (2/4)	2 (0/4)	0·085
PCC, IU intraoperatively	0 (0/125)	0 (0/0)	0 (0/0)	1·000
Platelets, Units intraoperatively	0 (0/1)	1 (1/2)	0 (0/0)	<0·001

Data are presented as median (25^{th}/75^{th} percentile) and compared with [a]the Mann-Whitney test. CPB, cardio pulmonary bypass. U**,** International Units**;** PCC**,** Prothrombin complex concentrate.

4.4 STUDY IV

Between 2006 and 2014, 268 patients underwent isolated CABG surgery (not including redo and combined procedures) and obtained aprotinin. Administration of aprotinin diminished considerably after 2007: 226 patients received the drug in 2006–2007 (mean 113 patients/year) versus 42 in 2008–2014 (mean 6 patients/year). Demographics and procedural characteristics are described in **Table 14**.

TABLE 14. Demographics in **Study IV**

	CABG patients receiving aprotinin, 2006-2014 (n = 268)	**CABG patients receiving aprotinin, 2006-2007 (n = 226)**	**CABG patients receiving aprotinin, 2008–2014 (n = 42)**	***P* value**
Age	65.5 ± 9.8	65.2 ± 9.7	66.9 ± 10.1	0.32
Male gender	212 (79%)	181 (80%)	31 (74%)	
Size (cm)	173.5 ± 10.3	173.5 ± 10.5	173.9 ± 9.6	0.83
Weight (kg)	82.7 ± 15.9	82.4 ± 15.9	84.2 ± 16.0	0.52
Body surface area (m^2)	2.0 ± 0.2	2.0 ± 0.2	2.0 ± 0.2	0.49
Higgins risk score	0.026 ± 0.058	0.022 ± 0.046	0.046 ± 0.10	0.134*
EuroSCORE I (additive)	4.9 ± 3.4	4.6 ± 3.1	7.1 ± 4.8	0.009
EuroSCORE I (logistic)	6.0 ± 9.6	5.1 ± 6.6	13.4 ± 20.7	0.039*
EuroSCORE II (logistic)	5.5 ± 8.4	N/A	5.5 ± 8.4	N/A
Procedural characteristics				
CPB (min)	65.1 ± 26.9	63.6 ± 24.4	73.2 ± 37.1	0.032
Cross-clamp (min)	39.0 ± 18.1	38.8 ± 19.0	39.6 ± 11.7	0.8
Ventilator time (h)	10.1 ± 32.4	9.3 ± 27.8	13.8 ± 48.5	0.44
Laboratory data				
Albumin (g/L)	36.4 ± 3.7	36.5 ± 3.5	36.0 ± 4.7	0.48*
Creatinine (µmol/L)	94.0 ± 56.3	92.9 ± 49.9	100.0 ± 83.1	0.46
Creatinine clearance (mL/min)†	75.5 ± 24.3	75.8 ± 24.7	73.8 ± 22.5	0.63

Data are expressed as numbers (percentage) for categorical variables, or as mean ± SD, *Calculated with non-parametric test; †Estimated using the Cockcroft-Gault equation. CPB, cardio pulmonary bypass, CABG, coronary artery bypass grafting.

Generally, patient demographics were comparable for both time periods. Nevertheless, mean EuroSCORE I and average CPB duration were higher during the second period, reflecting both an enlarged operative risk and an increased risk of bleeding. Clinical outcomes and postoperative laboratory data are shown in **Table 15**.

TABLE 15. Clinical outcomes in **Study IV**

	CABG patients receiving aprotinin, 2006-2014 (n = 268)	**CABG patients receiving aprotinin, 2006-2007 (n = 226)**	**CABG patients receiving aprotinin, 2008-2014 (n = 42)**	***P* value**
Extracorporeal membrane oxygenation	4 (1%)	1 (0.4%)	3 (7.1%)	0.013
Intra-aortic balloon pump	10 (4%)	6 (2.7%)	4 (9.5%)	0.054
ICU stay (days)	1.95 ± 2.9	1.88 ± 2.86	2.31 ± 2.85	0.37
30-day mortality	17 (6.3%)	12 (5.3%)	5 (11.9%)	0.157
1-year mortality#	26 (9.7%)	21 (9.3%)	5 (11.9%)	0.574
Stroke	3 (1.1%)	2 (0.9%)	1 (2.4%)	0.40
Multi-organ failure	19 (7.1%)	13 (5.8%)	6 (14.3%)	0.092
Intraoperative blood loss (mL)	628 ± 819	591 ± 643	846 ± 1469	0.298*
Total postoperative blood loss (mL)	628 ± 455	631 ± 467	604 ± 380	0.741
Reoperation for bleeding	22 (8.2%)	18 (8.0%)	4 (9.5%)	0.760
PRBC within 48 h (U)	1.7 ± 3.9	1.44 ± 2.92	3.14 ± 6.90	0.123*
Plasma within 48 h (U)	1.3 ± 4.4	0.92 ± 2.67	3.33 ± 8.98	0.092*
Platelets within 48 h (U)	0.36 ± 0.79	0.31 ± 0.72	0.62 ± 1.08	0.081*
PRBC >48 h to discharge (U)	1.3 ± 4.0	1.27 ± 3.69	1.17 ± 5.43	0.88
Plasma >48 h to discharge (U)	0.62 ± 3.5	0.52 ± 2.62	1.17 ± 6.42	0.52*
Platelets >48 h to discharge (U)	0.04 ± 0.48	0.02 ± 0.33	0.17 ± 0.94	0.33*
Postoperative laboratory data				
ASAT on day 1 (IU/L)	2.0 ± 8.9	2.07 ± 9.65	1.68 ± 2.89	0.80
ALAT on day 1 (IU/L)	0.87 ± 1.0	0.93 ± 1.08	0.58 ± 0.61	0.052
CK-MB on day 1 (IU/L)	32.1 ± 82.8	29.9 ± 55.9	55.2 ± 159	0.33*
Creatinine on day 1 (µmol/L)	91.4 ± 44.3	90.6 ± 46.5	95.5 ± 29.7	0.52
Creatinine clearance on day 1 (mL/min)†	92.9 ± 33.1	94.1 ± 33.4	85.8 ± 30.9	0.14
Δ creatinine clearance (day 0 – day 1 (mL/min)†	17.2 ± 18.6	18.4 ± 18.0	10.5 ± 20.6	0.012

Data are expressed as numbers (percentage) for categorical variables, or as mean ± SD.*Calculated with non-parametric test; †Estimated using the Cockcroft-Gault equation; #Available for all but 1 patient. Abbreviations: ALAT, alanine transaminase; ASAT, aspartate aminotransferase; CK-MB, creatine phosphokinase-MB; PRBC, pack red blood cell transfusion, CABG, coronary artery bypass grafting.

There were no significant differences between the 2 periods regarding postoperative mortality, postoperative complications, intra- and postoperative bleeding, and transfusion rates. More patients needed extracorporeal membrane oxygenation (ECMO) during the 2nd period (7.1% versus 0.4%; p=0.013), which may be related to the increased operative risk in the 2nd group and to the more frequent use of ECMO during this period. The increase in creatinine clearance from day 0 to day 1 was lesser during the 2nd period (10.5 ± 20.6 versus 18.4 ± 18.0; p=0.012

5 DISCUSSION

The main findings of this thesis were as follows. A small pilot RCT using the HeProCalc computer program indicated a significant reduction in protamine used for heparin reversal after CPB, without prolongations of ACT, compared with a control group, where the protamine dose was administered according to body weight. Furthermore, the HeProCalc-based algorithm was associated with less postoperative bleeding. In a subsequent large RCT the HeProCalc guiding reduced protamine dosage and protamine/heparin ratio after CPB compared with traditional dosage by weight, without significantly reducing postoperative blood loss or transfusion rate. Moreover, in a small observational study, platelet transfusion after CPB significantly improved response to thrombin activation, measured with ROTEM®*platelet* TRAPTEM, indicating that CPB induced platelet dysfunction may be reversed by platelet transfusion. Finally, a retrospective clinical study showed that CABG patients on DAPT with platelet aggregation <85% on day of surgery receiving aprotinin between 2008 and 2014 did not have significantly different clinical outcomes or transfusion rates compared to those patients receiving aprotinin during the prior two years, despite a significantly higher operative risk and a longer mean CPB time.

5.1 HEPROCALC GUIDANCE VS. STANDARD HEPARIN/PROTAMINE DOSAGE

The use of heparin influences hemostasis after CPB and the specific antidote protamine reverses the anticoagulant effect of heparin. Different methods are used to calculate the appropriate heparin dosing, including heparin dose response (HMS Plus Hemostasis Management System, Medtronic, Minneapolis, MN, USA) or dosage based on body weight, gender and pump priming volume.[65] HMS Plus System uses heparin titration assays to estimate the initial heparin dose, dosing during CPB, and needed dose of protamine for reversal of the heparin after CPB.[66, 67] The system uses channels with different amount of protamine and the channel containing the dose of protamine resulting in the shortest ACT decides the protamine dose for reversal of heparin. However, the HMS Plus system has disadvantages including high costs and a tendency to underestimate the initial dose response of heparin.[68] Aiming for a specific heparin concentration is difficult as blood volume during CPB tends to change during CPB and fluid may be redistributed. Furthermore, the response to heparin varies considerably between patients and is also influenced by ATIII levels.

5.1.1 Heparin dosing

Traditionally and the most commonly (75%) used method worldwide is to calculate heparin dosage for CPB based on body weight,[42] resulting in the majority of centers in initial doses of heparin between 300 and 400 IU/kg.[42] Likewise, most international centers (88%) maintain anticoagulation during CPB via additional heparin dosed to target ACT, and only 7% of centers administer additional heparin to maintain heparin level, e.g. HMS Plus System.[42] According to the STS/SCA/AmSECT guidelines "*use of heparin concentration in addition to ACT might be considered for the maintenance of CPB*" (Level IIb, Evidence B, **Table 15**).[44]

The heparin effect is usually reversed with a protamine dose of 1.0-1.3 mg per 100 IU (1 mg) of heparin[7] given and is assumed to neutralize heparin's anticoagulant effect. Excessive protamine possibly increases further bleeding by weakening platelet function, downregulating thrombin generation, and diminishing clot structure.[39, 40] Still, in cardiac surgery with CPB, protamine reversal is inevitable after full heparinization. Traditional dosing of heparin and protamine may be put in question, and several studies have attempted to define the ideal ACT level for CPB, where a minimum value of 400-480 seconds is mostly used.[42] However, latest guidelines recommend a minimal ACT level of 480 seconds during CPB (Level IIa, Evidence C, **Table 16**).[44] An ACT between 500 and 700 seconds during CPB is associated with less postoperative bleeding compared with when ACT levels < 500 or > 700 seconds are used.[69] The increased postoperative bleeding associated with an ACT < 500 seconds during CPB may be explained by augmented consumption of coagulation factors, while excessive heparin dosage with ACT > 700 seconds may amplify the risk of post-CPB bleeding after protamine reversal due to possible heparin rebound.[69]

In **Study I**, additional heparin doses were more frequently given in the HeProCalc group compared with the control group to achieve acceptable ACT values before start of CPB. This possible disadvantage must be balanced against the risk of excessive heparin dosing.

In contrast to the small **Study I**, the initial dose of heparin in the larger **Study II** given to HeProCalc group was slightly but significantly less than that administered to the control group (400 IU/kg) (p=0.025), although in both groups median ACT after initial heparin dosage was considerably above 500 seconds, without differing significantly (**Table 7)** p=0.095). However, none of the patients in the HeProCalc group and the control group had an ACT value <400 and <320 seconds, respectively. This is in distinct contrast to the study by Garvin et al.,[68] where

the 300 seconds ACT-level was missed in 7.4% of patients and the 350 seconds level was missed in 16.9% of patients using the HMS Plus System.

In **Study II**, the total heparin dose did not differ significantly between the two groups, but heparin was administered significantly more frequently in the HeProCalc group during CPB. This probably was a result of the perfusionist responding to the HeProCalc system, which displays when the estimated ACT is close to fall below 480 seconds. Also, the more repeated dosing of heparin may clarify why none of the patients in the HeProCalc group versus two patients (2.1%) in the control group had nadir ACT levels below 400 seconds during CPB. Furthermore, four patients (4.1%) in the HeProCalc group received additional ATIII because of the close monitoring with the HeProCalc system. A summary of the different guidelines regarding heparin dosing, ATIII dosing and ACT levels are presented in **Table 16** and **Table 17**, respectively.

TABLE 16. International guidelines for heparin dosing.

HEPARIN DOSING		**Class, Level of evidence**
2011 STS/SCS Blood conservation clinical practice guidelines[70]	In patients requiring longer CPB times (>2 to 3 hours), maintenance of higher and/or patient-specific heparin concentrations during CPB may be considered to reduce hemostatic system activation, reduce consumption of platelets and coagulation proteins, and to reduce blood transfusions.	IIb, B
2017 EACTS/EACTA Guidelines patient blood management[21]	Heparin level-guided heparin management should be considered over ACT-guided heparin management to reduce bleeding	IIa, B
2018 STS/SCS/ AmSECT Clinical Practice Guidelines- Anticoagulation During Cardiopulmonary Bypass[44]	Bolus administration of unfractionated heparin based on weight is reasonable for achieving adequate anticoagulation, but individual response to heparin is heterogeneous and requires a therapeutic functional test of clot inhibition before initiation of CPB, independent of the bolus dose used.	IIa, C
	Use of a heparin dose-response formula may identify reduced sensitivity to heparin but has not been shown to be more useful than weight-based heparin dosing in determining the heparin dose required to achieve an adequate ACT for initiation of CPB.	IIb, B
	Use of heparin concentration monitoring in addition to ACT might be considered for the maintenance of CPB, as this strategy has been associated with a significant reduction in thrombin generation, fibrinolysis, and neutrophil activation. However, its effects on postoperative bleeding and blood transfusion are inconsistent.	IIb, B
	During CPB, routine administration of unfractionated heparin at fixed intervals, with ACT monitoring, might be considered and offers a safe alternative to heparin concentration monitoring.	IIb, C

TABLE 17. International Guidelines regarding ATIII dosing and ACT levels.

ATIII MANAGEMENT		Class, Level of evidence	ACT LEVEL	Class, Level of evidence
2011 STS/SCS Blood conservation clinical practice guidelines[70]	Antithrombin III (AT) concentrates are indicated to reduce plasma transfusion in patients with AT mediated heparin resistance* immediately before cardiopulmonary bypass.	I, A	No Guidelines	
2017 EACTS/EACTA Guidelines patient blood management[21]	AT supplementation is indicated in patients with AT deficiency to improve heparin sensitivity.	I, B	No Guidelines	
2018 STS/SCS/ AmSECT Clinical Practice Guidelines- Anticoagulation During Cardiopulmonary Bypass[44]	No Guidelines		It is reasonable to maintain ACT above 480 seconds during CPB. For instruments using "maximal activation" of whole blood or microcuvette technology, values above 400 seconds. are frequently considered therapeutic A functional whole blood test of anticoagulation, in the form of a clotting time, should be measured and should demonstrate adequate anticoagulation before initiating and at regular intervals during CPB.	IIa, C I, C
*Heparin resistance is defined as the failure to achieve activated clotting time of at least 400 s to 480 s after a standard heparin dose, although there is a substantial variability in the definition of the "standard" heparin dose, ranging from less than 400 to 1,200 U/kg				

5.1.2 Protamine dosing

Earlier reports have indicated that a lower dose of protamine than the traditional 1:1 weight based ratio used may be sufficient to completely reverse the heparin effect.[65, 71-73] Even if protamine is an antidote to heparin, it is not a procoagulant and it may in fact have anticoagulant effects by itself.[7] Excessive amounts of protamine may according to reports increase ACT values.[39, 44] When reversal of heparin is based on titration, the protamine dose intends to match the circulating amount of heparin. Indeed, heparin/protamine titration with heparin concentration management may lower the protamine/heparin ratio without augmented postoperative bleeding.[65, 74, 75]

The HeProCalc algorithm suggests time and dosage of heparin during CPB as the program also considers the level of hemodilution (volume of prime), CPB duration, and current temperature when continuously displaying calculated ACT and calculated remaining amount of body heparin. Besides, after CPB weaning the HeProCalc program estimates the individual protamine dose required for heparin neutralization based on the calculated remaining amount of body heparin.

To our knowledge there are three other models for protamine dosage after CPB, although not for heparin. First, the statistical model, developed by Davidsson et al.[72] includes body surface area, preoperative platelet count and heparin clearance along with total amount of heparin to calculate protamine dosage for heparin reversal, resulting in a protamine/total heparin ratio of 0.51:1. Second, a pharmacokinetic model for protamine dosage, based on heparin elimination, resulted in a protamine/total heparin ratio of 0.5:1.[71] Third, Suarez et al.[76] developed a mathematical model for protamine dosage, which resulted in a protamine/heparin dosage ratio to the initial and to the total heparin dosage of 0.96:1 and 0.60:1, respectively.

The results of **Study I**, a small RCT, are consistent with earlier studies, and suggest that lower protamine doses than conventionally given, do not exacerbate postoperative blood loss. Conversely, in **Study I** there was a trend to less postoperative bleeding in the HeProCalc group compared with the control group, even if 36% less protamine was given in this group. Both groups in **Study I** received equal total amounts of heparin.

In **Study I**, a significantly lower dose of protamine was given in the HeProCalc group despite similar total heparin dosages in both groups. Postoperative ACT values did not vary much between the groups (**Figure 10**). Moreover, parallel HTC measuring with heparinase did not indicate that the slight postoperative ACT increase in both groups could be related to remaining heparin. **Study I** support previous studies[77-79] that recommend a low-dose protamine regime to avoid possible disadvantages with the drug. A ratio of >1.0 between total protamine and total intraoperative heparin has been associated with an increased risk (odds ratio 3.4, 95% confidence interval 2.4-4.9; $p < 0.001$) of transfusion and postoperative blood loss.[41] Furthermore, the STS/SCA/AmSECT guidelines state that one should consider protamine titration[44, 70] (**Table 18**) and to limit the administered heparin/protamine weight ratio (e.g. 50% of total heparin dose)[70] to <2.6[44] to avoid excessive postoperative bleeding. However, the 2017 EACTS/EACTA guidelines recommend a protamine/initial heparin ratio of <1:1 to reduce post-CPB bleeding (Level IIb, Level B) and to limit protamine dosage, preferably by using a titration method (Level IIb, Level B).[21]

TABLE 18. International guidelines regarding protamine dosing.

PROTAMIN DOSING		Class, Level of evidence
2011 STS/SCS Blood conservation clinical practice guidelines[70]	Use either protamine titration or empiric low dose regimens (e.g., 50% of total heparin dose) to lower the total protamine dose and lower the protamine/heparin ratio at the end of CPB may be considered to reduce bleeding and blood transfusion requirements.	IIb, B
2017 EACTS/EACTA Guidelines patient blood management[21]	The dose of protamine is usually based on the initial or total administered dose of heparin throughout the procedure.	IIb, B
	Heparin level-guided protamine dosing may be considered to reduce bleeding and transfusions.	IIa, B
	Protamine should be administered in a protamine-to heparin dosing ratio <1:1 to reduce bleeding.	IIa, B
2018 STS/SCS/ AmSECT Clinical Practice Guidelines- Anticoagulation During Cardiopulmonary Bypass[44]	It can be beneficial to calculate the protamine reversal dose based on a titration to existing heparin in the blood, since this technique has been associated with reduced bleeding and blood transfusion.	IIa, B
	It is reasonable to limit the ratio of protamine/heparin to less than 2.6 mg protamine /100 units heparin because total doses above this ratio inhibit platelet function, prolong ACT, and increase the risk of bleeding.	IIa, C

In **Study II** the initial heparin dosing according the HeProCalc algorithm was not apparently different from 400 IU/kg in the control group, and the protamine dose was about 0.6 times the initial heparin bolus. This raises the question why to use the HeProCalc algorithm when it can be calculated much easier? However, the median ratio protamine/initial heparin dose (mg/100 IU) was 0.62 (25^{th}/75^{th} percentile 0.54-0.68), according to **Table 7** and the range was 0.40–0.93 in the intervention group. Thus, the variation in ratio between patients was rather high (0.53). This would not have been possible with a fixed ratio.

5.1.3 Postoperative bleeding

In the small **Study I**, mean postoperative blood loss did not differ significantly between the HeProCalc and the placebo group, with 480 mL and 649 mL, respectively (p=0.074). First, it was assuring that **Study I** did not indicate increased postoperative bleeding in the HeProCalc group compared with the control. Second, the non-significant difference initiated us to conduct the larger **Study II** to better assess possible impact on postoperative bleeding. Although the mean difference of approximately 200 mL may seem small when deciding whether to transfuse or not, it may be the tipping point for some patients. Furthermore, any measures that decrease transfusion rate may be considered as vital, because transfusions have been associated with morbidity and mortality[,1-6]. Additionally, the number of patients in **Study I** was too low to study effects of transfusion rates. Again, this was another reason for us to conduct **Study II** to better clarify if the HeProCalc algorithm may significantly influence postoperative transfusion rates.

The cause of bleeding after CPB is multi-factorial and bleeding itself may promote further bleeding unless properly treated. A lack of protamine as a cause of bleeding may be ruled out, for example, with HTC testing. Improved treatment of postoperative bleeding may be achieved with additional POC analyses, e.g. thromboelastography and platelet function testing, as the HTC test is specific for heparin and thus will not indicate other explanations for a prolonged ACT.

The relatively high protamine reversal dose for heparin at a ratio to 1:1 has a negative impact on coagulation, including prolonged clotting time according to thromboelastography,[43, 71] platelet dysfunction, and inhibition of factor V activation with an resulting prolonged bleeding time.[40, 80] This has motivated several groups to investigate reduced ratios.[71-73, 76] In **Study II**, the reversal ratio in the HeProCalc group was 0.62:1 (median and mean) compared with 1:1 to the initial heparin dose in the control group. This corresponds, when using total instead of initial heparin dose, to a median and a mean ratio of 0.53 and 0.52, respectively, in the HeProCalc group compared with 0.86 and 0.85, respectively, in the control group. The total ratio in the HeProCalc group was equal or similar to those in other RCTs using the HMS Plus system, including Vonk et al. (total ratio 1:0.62),[67] Hofmann et al. (total ratio 1:0.67),[75] and Hoenicka et al. (total ratio 1:0.54).[66] Moreover, the total ratio was comparable to the statistical protamine reversal algorithm, tested by Davidsson et al.[72](total ratio 1:0.51), which is based on results with the HMS Plus system. In **Study II** the capability of the reduced protamine dose to sufficiently reverse blood heparin was proven by additional heparinase testing (HTC) 3 minutes after protamine reversal and 1 hour after end of surgery (**Table 7**). This coincides with the results of the earlier mentioned studies, which, however, using different criteria to verify sufficient reversal. Specifically, in **Study I-II** we used the HTC/ACT to show if heparin was completely reversed or not as this is standard technique at our center to detect possible residual heparin. This assay is a rather "rough" test. Within the context of a clinical study, the use of an anti-Xa test would have been preferable. In addition, the complete reversal of heparin is only one side of the medal. The other question is, whether the protamine dose calculated by the HeProCalc algorithm perfectly matched the real protamine demand or still was excessive. In this regard it would have been interesting to compare the protamine demand calculated by the device with the reference method of the HMS Plus system. There are number of other ways to verify complete reversal of heparin including delta ACT[71], ACT<140 sec after protamine administration[76], return to baseline ACT ± 10%,[66] ACT ratio was greater than 1.1[73] or heparin-protamine titration (HMS Plus system)[67], as well as the anti-Xa-test.

Motivated by the tendency of less postoperative blood loss (p=0.074) with the HeProCalc system in **Study I** (n=40), according to a power analysis, 86 patients were found to be needed for **Study II** regarding bleeding and possibly also transfusions. However, despite including 190 patients in **Study II**, we did not find that the HeProCalc system had a significant effect on postoperative bleeding volume or transfusion rate. Interestingly, we found that the nadir postoperative hemoglobin concentration was significantly lower in the control group (p=0.015), while the difference between preoperative and nadir postoperative hemoglobin concentrations was merely close to being significantly lower in the HeProCalc group (p=0.058). Besides, the total number of transfused units was lower in the intervention group (p=0.181). It may be argued whether these nonsignificant results regarding postoperative blood loss and transfusion rate are explained by a type II error or not. Nevertheless, the low median 12-hour postoperative blood loss of 320 ml and 350 mL in the HeProCalc and the control group, respectively, and the low rate of patients receiving transfusions, 18.3% and 24.7%, respectively, may hint in this direction. Other reports with low protamine ratios have presented conflicting results. The large RCT (n=200) by Koster et al.[81] using the HMS Plus system in their intervention group likewise founds a nonsignificant decrease in 12-hour postoperative bleeding volume, and Vonk et al.[67](n=38) reported significantly lower blood loss (<450 mL 24 h postoperatively) with the HMS Plus system. On the other hand, Slight et al.[82] (n=38) found increased 12-hour bleeding volume (p=0.06) with the HMS Plus system, comparable to Hoenicka et al.[66] (n=120), who reported that postoperative blood loss was significantly increased at 12 hours (p=0.004) but not at 24 hours (p=0.11). Additionally, the meta-analysis of 4 RCTs[81, 83-85] by Wang et al.[86] advocated "*that titrated protamine dosing is more effective than standard protamine dosing for alleviating postoperative bleeding after CPB*." Thus, **Study II** and previous similar reports may have included too few patients, and we support with the recent call from the Task Force on Patient Blood for Adult Cardiac Surgery of the EACTS/EACTA guidelines[21] that there is a "lack of perioperative bleeding and transfusion rates as primary end points in these studies", and that larger preferably multicenter "*RCTs are required to determine the added value of individual heparin management*".

5.2 PLATELET FUNCTION AFTER CPB AND PLATELET TRANSFUSION

In **Study III** we analysed platelet function with ROTEM and ROTEM®*platelet* before and after CPB, and after platelet transfusions. We found significantly impaired results in all parameters after CPB. After platelet transfusion, ROTEM parameters, except INTEM CT, improved significantly, but of the ROTEM®*platelet* parameters only TRAPTEM increased significantly. Ten out of 23 patients received platelet transfusions based on clinical indications.

Significant risk factors for platelet transfusion included higher EuroSCORE II values and a longer duration of surgery and CPB time, in accordance with previous studies.[87] EXTEM CT and TRAPTEM were significantly impaired in the transfused group before the platelet transfusions, compared with the group not receiving transfusion.

The decreases in ROTEM results and platelet count after CPB correlate well with previous findings after CPB.[11] However, the ROTEM®*platelet* results in our study all decreased significantly compared with pre-bypass values in contrast to findings where platelet function was tested with the Multiplate-instrument and only ADP and arachidonic acid-triggered aggregation was significantly decreased after CPB.[15]

Our findings regarding a significant increase of TRAPTEM after platelet transfusion may be interpreted with previous findings in mind, where an in-vitro study showed that platelet function decreases over storage time measured by Multiplate.[88] TRAP-induced aggregation in that study was better preserved (65%) at day 7 than both ADP (5%) and arachidonic acid (12%).

ROTEM parameters were close to within normal range after CPB and before platelet transfusion, even if significantly decreased compared with baseline. Regarding the ROTEM®*platelet* results, ARATEM and ADPTEM were below normal range and together with TRAPTEM they all significantly decreased at this time point after CPB and before platelet transfusion as compared to baseline indicating an impaired platelet function. ROTEM is commonly used to monitor the perioperative coagulation status. The main advantage of the method is a rapid assessment of the concentration of clotting factors, platelet contribution to clotting, fibrinogen levels, heparin effect and fibrinolysis. The platelet contribution is based on the platelet count, while the platelet function is less well reflected with tissue factor as agonist in the ex-tem reagent. "*The ROTEM® delta and sigma tests are not sensitive to the effects of the platelet inhibitors Aspirin®, clopidogrel and Reopro®*", and ROTEM®*platelet* is instead recommended according to the manufacturer (Guide to ROTEM® Analysis, www.rotem.de/en/products/rotem-platelet/). Moreover, they state that "*the effect of von Willebrand factor is not detected. Furthermore, a normal ROTEM® does not exclude the anticoagulants Orgaran®, pentasaccharide, low-molecular-weight heparin as well as oral anticoagulants such as Warfarin®. For analysis of these factors, other diagnostic tests have to be performed"*. To assess the platelet function in patients with on-going anti-platelet therapy other POC devices are considered more specific, including platelet function analyser (PFA-100) (Siemens Health Care Diagnostics Inc, Deerfield IL), Multiplate (Roche, Basel, Switzerland), Plateletworks® (Helena laboratories, Beaumont, TX, USA) or Verify Now (Accumetrics, San Diego, CA, USA). These

instruments are all based on platelet activation with specific platelet aggregation agonists but use slightly different techniques. It has not to our knowledge been shown that any of the instruments is superior to any of the others in predicting bleeding risk. ROTEM®*platelet* has been developed to measure platelet function, after agonist activation, similarly to the other POC instruments. We aimed to evaluate the potential use of ROTEM®*platelet* as a complement to ROTEM, which is routinely used in our centre.

We are aware of one earlier study investigating ROTEM®*platelet* and Multiplate before and after cardiac surgery with CPB. In the study by Petricevic et al.[64] when evaluating bleeding after CPB, platelet function was impaired 5–10 min after protamine administration in all ROTEM®*platelet* and Multiplate tests but ROTEM®*platelet* TRAPTEM. Results after protamine administration demonstrated the best correlation with 24-hour postoperative chest tube drainage. Both devices provided similar predictability for postoperative chest tube drainage and packed red blood cell transfusion requirements. The latter was associated with the degree of platelet inhibition and the number of pathways inhibited determined respective cut-off values. In contrast to our study, where surgery was delayed for at least five days after last intake of clopidogrel, approximately 10% of their patients were on clopidogrel and usually discontinued on admission, which in turn, resulted in different pre-operative clopidogrel-free intervals, although not presented. Additionally, they did not report if patients were on new oral anticoagulant drugs nor did they evaluate risk factors for platelet transfusion and impact of platelet transfusion on the tests. Moreover, the duration on CPB was considerably longer in our patients with a median duration of 117 (mean 128) and 193 (mean 202) minutes for non- and platelet transfused patients, respectively, compared with a mean of 103 and 121 minutes in their patients with normal 24-hour postoperative bleeding (mean 586 mL) and excessive bleeding (25th percentile, mean 1454 mL), respectively. Despite the large mean bleeding volume in their excessive bleeding group and significantly reduced ARATEM and ADPTEM tests none of the patients received platelet transfusions. Interestingly, mean TRAPTEM AUC was not significantly changed after CPB and protamine in their study, whereas all three ROTEM®*platelet* AUC values were significantly decreased in our study. These differences may be due to the longer duration of CPB in our study, although absolute ROTEM®*platelet* AUC values could not be compared, as we collected blood in citrated plastic tubes and they used heparin tubes.

Administration of platelets tends to be based on empiric data instead of coagulation testing and algorithms. According to Goodnough[89] 47% of transfusions of platelets were deemed inappropriate when reviewing 30 patients in 18 different centres undergoing CABG.

Transfusions of platelets, even in smaller quantities, may add risks to these already vulnerable patients. *"The American Association of Blood Banks (AABB) recommends against routine prophylactic platelet transfusion for patients who are non-thrombocytopenic and have cardiac surgery with CPB. The AABB suggests platelet transfusion for patients having CPB who exhibit perioperative bleeding with thrombocytopenia and/or with evidence of platelet dysfunction."*.[33] Conversely, Perek et al. showed that routine transfusion of platelets to patients undergoing elective operations for ascending aortic aneurysm decreased risk of re-intervention due to bleeding[90].

Patients in our study receiving platelet transfusion had significantly longer duration of surgery and time on CPB. Despite larger intraoperative bleeding, platelet count did not differ significantly between platelet transfused and non-transfused patients nor did the postoperative blood loss. The latter finding strongly indicates that platelet transfusion improved platelet function as exemplified with significantly increased TRAPTEM results.

Patients will most likely benefit from any measures taken to reduce platelet activation and hemodilution. Such measures could include minimizing prime volume in the CPB circuit by reducing lengths of the tubings and optimising devises. This would decrease the hemodiluting effect on platelets, erythrocytes and coagulation factors. Centrifugal pumps are also considered to reduce trauma of blood cells and will also contribute to minimizing priming volume. In addition, Khan et al.[91] found that individualised heparin dosage and protamine titration reduced platelet transfusion rate from 50% to 22% of the patients in a retrospective study. Those findings are supported by Shigeta et al. reporting heparin-protamine titration restoring platelet response to thrombin and attenuating platelet granule secretion during reversal of heparin.[92]

5.3 APROTININ IN ISOLATED CORONARY SURGERY PATIENTS ON DAPT

In the retrospective observational Study IV, we presented the administration of aprotinin in patients undergoing isolated first-time coronary surgery in our center between 2006-2014. After 2007, when aprotinin was suspended in Europe, aprotinin's use was limited to patients on DAPT i.e. on clopidogrel and aspirin, and with platelet aggregation <85% on the day of procedure, in whom surgery could not be postponed until aggregation had normalized. A comparison of demographic and procedural variables, and clinical outcome parameters of patients who underwent coronary surgery and administered aprotinin during both time periods indicated that, in spite of a higher operative risk according to EuroSCORE I and a longer CPB

time, patients administered aprotinin between 2008 and 2014 had similar clinical outcomes and transfusion needs as patients given aprotinin during the prior 2 years.

Platelet function testing has been recommended as an alternative to fixed discontinuation times to guide interruption of antiplatelet treatment before coronary surgery.[93] The 2017 EACTS/EACTA guidelines[21] state that "*Platelet function testing may be considered to guide the decision on the timing of cardiac surgery in patients who have recently received P2Y12 inhibitors or who have ongoing DAPT*". In patients administered clopidogrel within 5 days of off-pump CABG surgery, Kwak et al.[24] reported that the platelet inhibitory response to clopidogrel according to thromboelastography platelet mapping predicted augmented bleeding volume and transfusion need independent of the discontinuation time of antiplatelet therapy.[24] In patients on clopidogrel within 7 days before on-pump coronary surgery, Dalén et al. reported that PlateletWorks® ADP-induced platelet aggregation significantly correlated inversely with postoperative bleeding volume and predicted blood loss more consistently than timing of clopidogrel discontinuation.[26] A strategy considering preoperative platelet function (ADP-induced platelet-fibrin clot strength determined by thrombelastography) to define timing of coronary surgery in patients on clopidogrel resulted in shorter waiting times (mean 2.7 days) compared with recommended in 2017 EACTS/EACTA guidelines[21] (≥5 days), without a significant difference in 24-hour postoperative bleeding volume and transfusion needs compared with patients not administered clopidogrel.[25]

In our center, platelet aggregation is tested in all patients with clopidogrel discontinuation ≥5 days before coronary surgery. Patients on clopidogrel with platelet aggregation <85% on day of surgery are regarded as having a high risk for disproportionate perioperative bloods loss and to have indication for aprotinin. Two RCTs have assessed the possible advantages of aprotinin in patients on DAPT before cardiac surgery. Akowuah et al. compared two different strategies in 50 patients in need for urgent coronary surgery: the intervention group remained on aspirin and clopidogrel treatment for 5 days prior to surgery and were given intraoperative aprotinin, while the control group was administered placebo capsules instead of aspirin and clopidogrel for 5 days and placebo infusions during surgery.[59] The number of transfused PRBC units was significantly reduced in the aprotinin group. Additionally, 3 patients in the placebo group versus none in the treatment group had a myocardial infarction. In the other RCT by van der Linden et al., 75 patients with unstable angina administered clopidogrel <5 days before coronary surgery were randomized to either full-dose aprotinin or saline.[60] In patients receiving aprotinin, postoperative bleeding volume, volume of transfused PRBC and platelets, and the rate of patients given blood products were significantly reduced. The possible benefits

of aprotinin still need to be assessed in patients on the newer P2Y12 inhibitors (ticagrelor or prasugrel) who require urgent coronary surgery.

5.4 CLINICAL IMPLICATIONS

Study I, a small pilot RCT, indicated that patients randomized to HeProCalc management obtained a significantly lower protamine dose compared with patients randomized to weight-based dosing. ACT levels after CPB were similar in both groups. HeProCalc management for heparin reversal also suggested less postoperative blood loss, while not differing significantly between the groups. The latter finding needed to be investigated in a larger cohort.

The results of the larger **Study II** indicate that the HeProCalc algorithm does not reduce postoperative blood loss or transfusion rate significantly when compared with heparin and protamine dosed by weight. Further larger multicenter RCTs evaluating primarily postoperative blood loss and transfusion rate are needed to more clearly define the value of individual heparin management. However, **Study II** showed that individual heparin/protamin management with the HeProCalc algorithm is feasible.

Study III suggests that use of ROTEM®*platelet*, in addition to ROTEM, which is widely used to guide transfusions in bleeding patients, may be useful to predict platelet function and if platelet transfusions are needed. Activation with TRAPTEM seems to be sensitive both to decreased and increased platelet function, although this pilot study was based on rather few observations. Larger clinical studies are needed to establish appropriate indications for platelet transfusions in cardiac surgery.

In **Study IV**, the clinical use of aprotinin fell significantly after the closure of the BART RCT in 2007. Nevertheless, highly selected patients taking clopidogrel <5 days before coronary surgery, with platelet inhibition >15 % on day of surgery and in whom surgery was not delayed continued to be administered aprotinin. Notwithstanding a higher operative risk according to EuroSCORE I and time on CPB, patients administered aprotinin between 2008 and 2014 did not have significantly different clinical outcomes and transfusion rates compared with those patients given aprotinin during the prior 2 years. This indicates that aprotinin administration still provides expected control of bleeding and transfusion rate in patient on clopidogrel and aspirin undergoing coronary surgery. Further studies are required to assess the use of aprotinin

in patients administered other platelet inhibitors, e.g. ticagrelor or prasugrel, and in need of urgent coronary surgery.

5.5 LIMITATIONS

In **Study I-IV** blood samples for ACT/HTC measurements were not performed by separate study personnel but were conducted according to standard clinical practice by clinically involved nurses and perfusionists. In the RCT **Study I**, no effort was made to cover group assignment, which potentially may have influenced results. Another limitation of **Study I-II** was that ATIII levels were not included in the study protocol and hence not systematically analyzed before surgery. However, this would have made it more difficult to include patients due to additional costs as it is not part of our clinical routine and sampling had to be undertaken the day before surgery. Additionally, it would have required an algorithm to handle patients with specifically low ATIII levels as well as stratification of patient within the randomization. In the 2011 STS/SCA Blood Conservation Clinical practice Guidelines[70] state that ATIII "*concentrates are indicated to reduce plasma transfusion in patients with AT(III) mediated heparin resistance immediately before cardiopulmonary bypass.*"(Class I, Level A, **Figure 16**). However, heparin resistance was defined as the failure to achieve ACT of at least 400-480 seconds after a heparin dose of 400-1,200 IU/kg and not according to a specific ATIII level.[70] Interestingly, the 2018 published guidelines of STS/SCA/AmSECT for anticoagulation during CPB,[44] do not give any specific recommendation for administration of ATIII, although it states that "*the pharmacodynamics of unfractionated heparin are highly dependent on the level and function of plasma ATIII*". On the contrary, the 2017 EACTS/EACTA guidelines recommend that "*AT supplementation is indicated in patients with AT deficiency to improve heparin sensitivity*", without stating any specific threshold value (Class I, Level B).[21](**Figure 16**)

In **Study I** postoperative blood loss was calculated from end of surgery until the next morning, whereas variable was measured more specifically in **Study II**, by measuring blood loss during the first 12 hours after end of surgery. Additionally, one may argue that the 9 patients in **Study II** who underwent reoperation due to surgical bleeding should not have been excluded. Nevertheless, irrespective of whether these 9 patients were excluded or not, no statistical differences between the 2 groups were found regarding bleeding and transfusion rate.

Study III had several limitations. Besides the observational design of the study and the small sample size, there are some aspects to keep in mind when interpreting the results. A larger sample size might have shown significantly improved results regarding both ARATEM and ADPTEM, thus implying a risk of type II-error.

Another aspect is the fact that conducting platelet function test with a low platelet count may influence the results. Median platelet count in the whole cohort of our study was 101 x 10^9/L after CPB and before platelet transfusion. ARATEM and ADPTEM were below normal range after CPB and before platelet transfusion. A platelet count below normal may influence the results, but only moderately at a platelet count around 100x10^9/L [94, 95]. The median platelet count in our patients who received platelets was 87.5 (77.2/121) x10^9/L after CPB and before platelet transfusion (T1), and after platelet transfusion the platelet count increased to 139 x 10^9/L (124/173, p=0.002). Thus, if the platelet concentration would have had a significant influence on the ROTEM®*platelet* tests after platelet transfusion, one would have expected all three tests to improve significantly. However, as only TRAPTEM AUC improved significantly after platelet transfusion one may argue that the platelet count is unlikely to have had a significant influence.

Study I-IV were performed in a single institution and one group in **Study IV** included a small number of patients (42=6 per year on average) during the 7-year study period during which aprotinin administration was limited to very selected patients. Also, the two groups of **Study IV** differed in demographic and procedural characteristics, including a longer CPB mean time and higher operative risk according to EuroSCORE I in patients undergoing cardiac surgery between 2008 and 2014. Finally, the results of the retrospective **Study IV** should be interpreted with caution due to its observational design.

6 CONCLUSIONS

The specific conclusions were:

- In a randomized controlled pilot study patients, who were administered heparin and protamin according to the HeProCalc algorithm, received a significantly lower dose of protamine compared with a control group, where the protamine dose was dosaged according to body weight.

- The HeProCalc algorithm did not significantly affect postoperative blood loss or transfusion requirement compared with dosage of heparin and protamine based on body weight.

- ROTEM®*platelet* with thrombin activation, TRAPTEM, improved significantly, indicating that platelet transfusion may reverse cardiopulmonary bypass induced platelet dysfunction.

- Despite a significantly higher operative risk and a longer CPB time, patients receiving aprotinin between 2008 and 2014 did not have significantly different clinical outcomes or transfusion rates compared to those receiving aprotinin during the prior two years.

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