COAGULOPATHY IN CARDIAC SURGERY

PHD DISSERTATION

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The PhD thesis is based on work carried out in a six-year period. During this period I have been affiliated at the following institutions:

- Department of Cardiothoracic and Vascular Surgery, Aarhus University Hospital, Skejby, Aarhus
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- The Heart Center, Department of Cardiothoracic Surgery, Rigshospitalet, Copenhagen

To make this work possible I am thankful to numerous people both in my professional sphere as well as in my private life. However, there is only one person to thank for introducing me to the world of coagulation – Benny Sørensen, MD, PhD. He has despite our distance geographically been “by my side” the whole time. The combination of profound knowledge, dedication, enthusiasm, and determination in addition to a great sense of humor as well as good taste in coffee has made him a perfect mentor. Professor emeritus Jørgen Ingerslev contributed with a continuous encouragement in combination with critical questions, thereby enhancing the quality of the research. Professor Vibeke Hjortdal has with her constant support, efficacy, positive attitude, and some degree of impatience been a remarkable help, also in securing research possibilities for me in the department.

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LIST OF PUBLICATIONS

The thesis is based on the following original publications:

**Study I**
Global haemostatic defect peaks six hours after cardiac surgery – a 48 hours observational study
Submitted to British Journal of Anaesthesia, June 2016 - in review

**Study II**
Rational and timely haemostatic interventions following cardiac surgery - coagulation factor concentrates or allogeneic blood products
Submitted to Thrombosis Research, April 2016 - in review

**Study III**
Temporal changes in clot lysis and clot stability following tranexamic acid in cardiac surgery
Accepted for publication in Blood Coagulation and Fibrinolysis, May 2016

ABBREVIATIONS

- **ACT**: activated clotting time
- **CAT**: calibrated automated thrombin generation
- **CT**: clotting time (thromboelastometry)
- **CFC**: coagulation factor concentrates
- **CFT**: clot formation time (thromboelastometry)
- **Cryo**: cryoprecipitate
- **CTI**: corn trypsin inhibitor
- **ECC**: extracorporeal circulation
- **ETP**: endogenous thrombin potential (thrombin generation)
- **FFP**: fresh frozen plasma
- **FIB**: fibrinogen concentrate
- **LMWH**: low molecular weight heparin
- **MaxVel**: maximum velocity for clot formation (calculated from thromboelastometry data)
- **MCF**: maximum clot firmness (thromboelastometry)
- **RoTEM®**: rotational thromboelastometry
- **PCC**: prothrombin complex concentrate
- **PLT**: platelets
- **rVIIa**: recombinant factor VIIa
- **TF**: tissue factor
- **TFPI**: tissue factor pathway inhibitor
- **tPA**: tissue plasminogen activator
- **TXA**: tranexamic acid
- **vWF**: von Willebrand Factor
INTRODUCTION

The coagulation system is subject to considerable challenge during and after cardiac surgery, which increases the risk of postoperative bleeding. Bleeding after cardiac surgery is known to be an individual risk factor for increased morbidity and mortality. Management of postoperative bleeding has traditionally been based on transfusion of allogeneic blood products, which alone is associated with an increased risk of mortality. The increased long term mortality is particularly evident and worrying in low risk patients.

Over the past two decades understanding of the mechanisms in the hemostatic system has evolved. Furthermore, an armamentarium of new laboratory methods such as thrombin generation, thromboelastometry, and platelet function analyses have been developed and evaluated in healthy individuals as well as in a variety of coagulation diseases.

Coagulation factor concentrates were originally developed for use in patients with congenital coagulation factor deficiencies (e.g. recombinant factor VIIa (hemophilia patients with inhibitors), fibrinogen concentrate (congenital a- and dysfibrinogenemia), prothrombin complex concentrate (hemophilia B)). They have been considered a more efficient and targeted hemostatic intervention in patients with perioperative or traumatic coagulopathy than blood products.

However, despite increased knowledge of the hemostatic system, more advanced laboratory diagnostic technologies, and access to coagulation factor concentrates; algorithms for post cardiac bleed management have largely remained stagnant and focused on use of allogeneic blood products. This PhD thesis proposes that improved understanding of the coagulation profile after cardiac surgery may enable development of new algorithms to limit postoperative bleeding and reduce the need for transfusion. Furthermore, potential new treatment options should be investigated in relation to specific changes observed in the coagulation profile.

THE COAGULATION SYSTEM

The coagulation system orchestrates a complex interaction between procoagulants and anticoagulants mediating a balance between bleeding and thrombosis. Key contributors in haemostasis are the endothelial cells of the vessel wall, circulating platelets, coagulation factors, and initiators of coagulation. Together these components interact to maintain the integrity of blood circulation by creating a stable haemostatic plug. To avoid generation of an obstructive thrombosis anti-thrombotic and fibrinolytic factors are activated simultaneously. A haemostatic response to injury is divided into two steps; primary and secondary haemostasis. These two steps should not be thought of as consecutive and individual steps, rather they are highly dependent on each other and should be regarded as concurrent.

**Primary haemostasis**

The steps of primary haemostasis are depicted in figure 1. The reaction starts with an injury to the vessel wall, leading to exposure of collagen to the blood stream followed by unfolding and binding of von Willebrand Factor (vWF). The circulating platelets have a high affinity for vWF. The platelets that bind to vWF get activated and release their granules, containing e.g. ADP and thromboxane A2. Hereby they attract additional platelets to the site of injury. Furthermore, activation also induces a change in platelet shape, whereby they can adhere and aggregate by generating fibrinogen bridges via GIIb/IIIa receptors on other nearby platelets. Vasocostriction and the formation of a primary platelet plug constitute the initial bleed control.

**Secondary haemostasis**

Secondary hemostasis is the formation of insoluble, cross-linked fibrin for stabilization of the primary platelet plug. The process is mediated by coagulation factors, mainly thrombin. The process is illustrated in figure 2. Secondary haemostasis, also referred to as coagulation, is a regulated process involving three overlapping phases for consolidation of a stable clot. TF=tissue factor. Please refer to text, section “Secondary haemostasis” for further details.
sequence of events initiated by the exposure of extravascular tissue factor (TF) to the circulation. TF is released from the injured cell wall and forms a complex with FVII leading to its activation. This TF-FVIIa complex activates both factor FX and FIX (FXa and FIXa). FXa facilitates the conversion of prothrombin to thrombin. This initial and relatively small production of thrombin is called the initiation phase, which in return catalyzes a thrombin mediated burst production by fully activated platelets, as well as cofactors FVa and FVIIa. The autotocytic process is named the amplification phase leading to the last stage, the propagation phase. The accelerated production of thrombin is activated by FVIIa/FIXa (intrinsinc tenase complex) and FXa/FVa (prothrombinase complex). The later is a hundredfold more effective than the initial TF-FVIIa complex. Thrombin converts fibrinogen to fibrin and facilitates the formation of the fibrin strands, thereby inducing the process of clot dissolution and the release of fibrin degradation products, eg. d-dimer.

Antithrombotic activity and fibrinolysis

The regulatory mechanisms aim to localize the haemostatic process to the site of vessel lesion and protect against excessive clot formation. These mechanisms are activated alongside with the secondary haemostasis path. Among many endogenous anticoagulants the principal three will be described here: antithrombin, protein C/protein S and tissue factor pathway inhibitor (TFPI). Firstly, antithrombin, which is produced in the liver and secreted to the circulating blood from where it reaches the surface of endothelial cells. It inhibits thrombin, but also FXa, FXa, FXIa and FVIIa. Antithrombin activity is increased 1000-fold in the presence of heparin or endogenous glycosaminoglycans by enhancing binding to coagulation factors. Secondly, protein C/S contributes to reduction of thrombin production through inactivation of FVa and FVIIIa. Protein C is indirectly activated by thrombin through reaction with thrombomodulin and protein S. Lastly TFPI contributes to reduction of clotting activity by blocking the TF-FVIIa complex and FXa. TFPI is abundant on endothelial surfaces and in platelets, its release is enhanced by thrombin and heparin.

The breakdown of the established clot is referred to as fibrinolysis. The process is depicted in a simplified model below (figure 3). The activation of plasminogen to plasmin is pivotal for fibrinolysis. This process is stimulated by tissue plasminogen activator (tPA), urokinase and kallikreins, released from endothelial cells or circulating in the blood. Noteworthy, plasminogen activation is also facilitated by FXa and FIXa, both part of the coagulation process. Plasmin cleaves the fibrin strands in the clot, thereby inducing the process of clot dissolution and the release of fibrin degradation products, eg. d-dimer.

3.4% of cases and may be as high as 6.8% (Danish Heart Registry). In one third of the cases bleeding is concluded to be due to a coagulopathy. So far allogenic blood products have been the gold standard in treatment of postoperative bleeding in cardiac surgery and are still recommended in guidelines. However, transfusion after cardiac surgery is common with more than 50% of patients being exposed to blood products. However, concerns have been raised with regard to both long-term mortality as well as the hospital morbidity associated with blood product use. For example, the increased risk of infection, transfusion-related lung injury, multiorgan failure in addition to allergic and anaphylactic reactions. Coagulation factor concentrates (CFC) have been suggested as alternative treatment options for postoperative bleeding. At the initiation of the studies in this thesis several case reports on CFCs had been published, however, only a few randomized clinical trials had been performed. One obvious advantage of CFCs is the relatively small reconstitution volume, whereby overhydration (hemodilution) is prevented. Additionally, exact dosing of a known concentration of specific coagulation factors enables goal directed treatment. Concentrated haemostatic intervention further presents an opportunity to exploit the potential pharmacological effect of supra-physiological level. Moreover, the risk of transmission of infectious diseases is reduced due to viral inactivation and heat treatment during production, or use of recombinant technology. On the other hand, coagulation factor concentrates might increase the risk of thromboembolic complications due to excessive or erroneous intervention. Nonetheless, studies comparing the haemostatic effect of CFCs against allogenic blood products are sparse and little data is available. In parallel with the increased availability of new treatment options, diagnostic monitoring has been greatly improved during the last decades with the use of global haemostatic assays, e.g. measuring whole blood thromboelastometry, platelet impedance aggregometry and thrombin generation. These assays are useful in detecting a variety of coagulopathies. In particular they provide dynamic information on clotting profiles. Some of these point-of-care devices are increasingly used in the clinical settings.

![Figure 3: FIBRINOLYSIS](image)

**CARDIAC SURGERY AND BLEEDING DIATHESIS**

Obviously the surgical trauma itself induces a risk of bleeding. However, several other events occurring during surgery may augments the risk. These risk factors include heparinization, hemodilution, fibrinolysis, the use of extracorporeal circulation (ECC), preoperative treatment with platelet inhibitors, consumption of coagulation factors and platelets, as well as acidosis and hypothermia (figure 4). All of these factors alone are known to affect clotting and they may potentiate each other in cardiac surgery. The multifactorial nature of the bleeding diathesis challenges diagnostics as well as treatment. Bleeding after cardiac surgery is common and associated with a negative clinical impact, increased morbidity and mortality. Reexploration due to bleeding occurs in aFter cardiac surgery has a multifactorial background.
Unfortunately many cardiac centres do not have routine access to monitoring by such methods.

Fibrinolysis and alterations in fibrin clot properties are important factors in the development of the coagulopathy. Laboratory assays designed to directly monitor clot lysis are limited and little has been published. Tranexamic acid is widely used in cardiac surgery and trauma and is associated with a reduction in bleeding and transfusion requirements. In the newest guidelines from the Society of Thoracic Surgeons and the Society of Cardiovascular Anesthesiologists the routine use of TXA is now a class I(a) recommendation. During recent years concerns have evolved regarding the fact that TXA may induce postoperative seizures, with evidence of a dose dependent risk. No optimal dosage has been decided so far, and we lack established laboratory methods for evaluating the effect of TXA and measuring fibrinolysis. Furthermore, little is known about the dynamic clot characteristics; especially with regards to the stability of the clot in patients receiving tranexamic acid.

Altogether, cardiac surgery is prone to induce coagulopathy, as demonstrated by many excellent studies, but the underlying causes are not fully understood. Several studies have addressed the haemostatic abnormalities appearing immediately after surgery. There is, however, a general consensus that the development of coagulopathy is multifactorial and not due to an abnormality of a single component of the haemostatic system. Adding to the complexity, the coagulopathy is unlikely to be static, but may display changes over time. The treatment of the post cardiac surgery coagulopathy is therefore neither simple nor monotherapeutic. Information on the most effective treatment options could improve clinical outcome and may reduce transfusion requirements. The routine use of antifibrinolytics should reduce postoperative bleeding. Nevertheless, our knowledge regarding clot stability and facilitated fibrinolysis in these patients is sparse.

To achieve a rational and timely haemostatic intervention, we must expand our understanding of the underlying coagulopathy and these possible time dependent changes.

The Ph.D. project challenges the following hypotheses:

1. Patients undergoing cardiac surgery using ECC, develop a bleeding diathesis defined by abnormal dynamic whole blood thromboelastometry and thrombin generation.

2. Ex vivo spiking with CFCs produce a significantly more potent haemostatic effect compared to allogeneic blood products in blood from patients following cardiac surgery as evaluated by measurement of clot formation and clot stability.

3. Patients undergoing cardiac surgery will develop changes in their clot stability during, and after the operation and these changes are related to the tranexamic acid administration and its elimination.

The aims of the current studies were:

1. Systematically characterize the coagulopathy evolving during cardiac surgery using analysis of dynamic whole blood thromboelastometry, thrombin generation, and clot stability as well as standard coagulation tests and single coagulation factor measurements.

2. Investigate the haemostatic potential following ex vivo spiking of blood with single coagulation factors versus combined haemostatic intervention. Components investigated: fresh frozen plasma, platelets, cryoprecipitate, fibrinogen concentrate, prothrombin complex concentrate and recombinant factor VIIIa.

3. Characterize and evaluate the clot stability and clot lysis, using spectrophotometry, during and after cardiac surgery in patients subjected to prophylactic tranexamic acid.
Blood samples from patients were collected during a 48-hour period, creating a prospective observational study. Blood samples were drawn at seven time points following the flow chart below (figure 4). The blood samples were used in three different sub-studies addressing the three hypotheses listed above. The blood sampling methods as well as laboratory details are listed and described meticulously below.

Patients were recruited at the Department of Cardiothoracic and Vascular Surgery, Aarhus University Hospital, Skejby, Denmark. Only adult (18+ years) patients were enrolled. All those included had a normal preoperative coagulation screen and had discontinued platelet inhibitor and/or anticoagulant therapy five days prior to their elective surgery. Exclusion criteria were: previous heart surgery, hypothermia (<34°C), known congenital bleeding disorders, endocarditis or anemia.

Study I

The study was a prospective observational study addressing the natural patho-physiology of clotting parameters in patients undergoing low risk cardiac surgery. Blood samples were analysed at all sampling time points as depicted in figure 5, facilitating a methodical characterization of the coagulopathy evolution. Moreover, the study enabled a thorough investigation of haemostatic performance at the most common time-points for reexploration. The characterization was based on analysis of dynamic whole blood thromboelastometry, thrombin generation and single coagulation factor measurements. The findings were correlated with clinical bleeding. Additionally, clinical data regarding patient characteristics, reexploration for bleeding, total blood loss, fluid resuscitation and transfusion requirements were collected.

Study II

In this study blood samples from three time points were used - start, 0h and 2h (figure 6). Ex vivo spiking with different haemostatic interventions was performed on blood samples from 0h and 2h mimicking different clinical treatment options.

The following allogeneic blood products and coagulation factor concentrates were investigated: Fresh frozen plasma, platelets, cryoprecipitate, fibrinogen concentrate, prothrombin complex concentrate and recombinant rFVIIa.

The interventions were:

- Monotherapy: FFP, PLT, Cryo, FIB, PCC, or rFVIIa
- Combination therapy: FFP+PLT, FFP+Cryo, PCC+Cryo, PCC+PLT, PCC+FIB, PLT+FIB, PCC+PLT, FIB+PLT, or double concentration FIB+PLT

The haemostatic interventions were designed to reflect daily clinical practice and guideline requirements, both in regard to dosage as well as dilution. Details for dosage are listed in table 2. The haemostatic effect was evaluated by whole blood thromboelastometry (clotting time, maximum velocity of clot propagation and maximum clot firmness) as well as by thrombin generation (lag time, peak thrombin and endogenous thrombin potential).

Study III

Blood samples from the entire 48-hour observation period were investigated in this study (figure 5). Clot formation, clot stability and facilitated clot fibrinolysis were monitored using a customized assay employing simultaneous activation with tissue factor and tissue plasminogen activator. Please confer with section “Clot lysis” below for details. In addition to observing changes in clot lysis activity, this study also evaluated the effect of TXA on clot stability and the duration of resistance to clot lysis.

Laboratory Methods

Blood sampling (all studies)

Baseline blood samples were obtained the day before surgery. Using minimal stasis they were drawn from a peripheral vein, using a 21G butterfly needle, 3.2% citrate tubes (VenoSafe®, 3.4 ml, Terumo, Hatagaya, Japan). Blood samples at all subsequent time points were collected from a central venous line (BD Medical System, Becton Dickinson Critical Care System Ltd., Singapore) using the distal leg (all infusions were stopped and 10 ml of blood was discarded before the sample for analysis was aspirated). The first blood samples after ECC were obtained following heparin neutralisation by protamine when a control aPTT value was comparable to the preoperative level.

Corn trypsin inhibitor (CTI) and contact activation

It is possible to reduce contact activation of the blood by the addition of CTI to the test tubes prior to blood sampling. CTI effectively inhibits FXII thereby subduing the contact pathway of coagulation, as assessed by thrombin generation assays. However, during cardiac surgery contact activation may contribute to the development of a coagulopathy. The use of extracorporeal circulation exposes blood to artificial surfaces, which may induce activation of the coagulation system, independently of the presence of heparin.

Before commencing these studies, we developed a laboratory model of postcardiac coagulopathy and the addition of CTI was shown not to induce any change in clotting parameters. In light of these findings we decided not to adopt CTI in characterisation of the actual haemostatic condition (see supplement for poster).
White blood thromboelastometry (ROTEM®) (study I+II)
The use of white blood thromboelastometry in various clinical settings has increased during the last decades. This is reflected in transfusion guidelines, that currently recommend the use of thromboelastometry in bleeding patients. Thromboelastometry based algorithms have shown to reduce both transfusion requirements and bleeding as well as the use of coagulation factor concentrates.

Thromboelastometry was initially introduced in hepatic surgery in 1970s, and then moved to cardiac surgery in the nineties, however Dr. H. Hartert originally described the method as early as 1948. The strength of thromboelastometry is the continuous measurement in whole blood, thereby giving comprehensive information on clot initiation, clot development, as well as clot firmness and finally the kinetics of clot degradation (fibrinolysis). There are two registered thromboelastometric devices - TEG® (Haemonetics, Braintree, MA, USA) and RoTEM® (TEM International, Munich, Germany). For these studies only RoTEM® was used, therefore only this device will be described in detail. RoTEM has been investigated in both management of perioperative bleeding, trauma as well as explored in patients with congenital bleeding disorders and thrombocytopenia.

Citrated whole blood is added to a prewarmed plastic cup (37° C). Following addition of coagulation reagents (e.g. tissue factor) and calcium chloride for re-calcification, a plastic pin is submerged in the plastic cup with whole blood and reagents. The pin oscillates +/- 4.75°. The coagulation process results in fibrin strands forming between the plastic cup wall and plastic pin and thus changing the oscillation of the pin (figure 7). A digital optimal signal is transmitted and translated to a symmetric thromboelastometric coagulation curve (figure 8). From the coagulation profile several parameters are derived as listed below in table 1.

For study I+II whole blood clot formation and clot stability were evaluated according to a previously established method using citrated whole blood ROTEM® thromboelastometry activated with minute amounts of tissue factor. In brief, citrated whole blood rested 30 minutes prior to analysis. A volume of 300 µl of whole blood was loaded to each cup followed by addition of 20 µl of buffer (20 mM HEPES, 150 mM NaCl, pH 7.4). The coagulation process was activated with tissue factor (Innovin®, Dade Behring, Marburg, Germany - final dilution of 1:17000) followed by recalcification with 200 mM CaCl2. All tests were run in duplicate and the analyses were allowed to run for at least 45 minutes. Raw data were processed using the DyCoDerivAn software (Avordusol, Risskov, Denmark) for determination of dynamic clotting profiles: maximum velocity (MaxVel [mm*100/s]) and time until maximum velocity (t,MaxVel[sec]).

### TABLE 1: THROMBOELASTOMETRIC PARAMETERS DERIVED FROM THE ROTEM

<table>
<thead>
<tr>
<th>Profile</th>
<th>Definition</th>
<th>Influenced by</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clotting time (CT) (sec)</td>
<td>Time from start of reaction until clot firmness of 2 mm is reached. Reflects lag time of clot initiation</td>
<td>Coagulation factors, Anticoagulants</td>
</tr>
<tr>
<td>Clot formation time (CFT) (sec)</td>
<td>Time from 2 mm to 20 mm clot firmness Reflects the clot propagation phase</td>
<td>Coagulation factors, Platelets, Fibrinogen</td>
</tr>
<tr>
<td>Maximum Clot Firmness (mm)</td>
<td>The maximum clot firmness obtained</td>
<td>Platelets, Fibrinogen, FXIII</td>
</tr>
</tbody>
</table>

**FIGURE 7: ROTEM PRINCIPLE**
The pin oscillates while the sample cup is static. The changes in rotation reflect viscoelasticity are monitored using a LED light source and a detector. (Picture from homepage RoTEM (www.rotem.de))

**FIGURE 8: PARAMETERS FROM A ROTEM CURVE**
For these studies the following parameters were used: CT-clotting time (sec), CFT - Clot formation time (sec), MCF Maximum clot firmness (mm). Picture from homepage RoTEM (www.rotem.de)
Calibrated automated thrombin generation (CAT) (study I-II)

The final common pathway of coagulation activation is the conversion of prothrombin to thrombin. Thrombin acts a potent key enzyme with multiple functions: cleavage of fibrinogen to fibrin, activation of platelets, auto-amplification with activation of FV, FVIII, FXI, as well as facilitating clot stabilization via activation of FXIII and thrombin activated fibrinolysis inhibitor (TAFI). Finally, thrombin interacts with thrombomodulin to activate protein C and thereby inhibiting its own production by inactivating FV and FVIII. Measuring the dynamics of thrombin generation thus provide substantial information on the overall plasma coagulability. Calibrated automated thrombin generation assays are so far primarily used for research purposes. The automated method was invented by HC Hemker (Thrombinoscope BV (Maastricht, Netherlands))56 by HC Hemker (Thrombinoscope BV (Maastricht, Netherlands))56.

The general principle is based on platelet poor plasma transferred to a microplate well followed by incubation with a coagulation activator. The activator in the present studies is a mixture of tissue factor and phospholipid. After a short incubation period automated addition of a fluorogenic thrombin substrate takes place. The parameters generally recorded are: lagtime (min), peak thrombin (nM), time to peak thrombin (min), and endogenous thrombin potential (nM) as illustrated in figure 9.

Thrombin generation in study I-II was performed employing standard reagents and software from Thrombinoscope BV (Maastricht, Netherlands) performed on a Fluoroskan device (Thermo Electron Corp., Copenhagen, Denmark).

Blood samples were centrifuged at 2800G for 20 minutes to obtain platelet poor plasma (PPP). Aliquots were kept at -80°C until bulk analyses. Prior to testing all samples were centrifuged for 3 minutes at 12000G. The assay was performed in a 96-well microplate (Immulon 2HB, round bottom, Jencons, VWR International Ltd., Sussex, UK). A total of 80 µl of platelet poor plasma was spiked with 20 µl of PPP reagent 5 pM (lot PPP0121/01). The plate was placed in the fluorometer and prewarmed to 37°C followed by automatic addition of 20 µl thrombin substrate (Fluo-substrate, Fluca-kit, lot FC1004/01). All samples were run as double determinations in parallel with a thrombin calibrator (activity 580nM, lot TC1005/01).

Ex Vivo haemostatic interventions (study II)

To evaluate the haemostatic capacity, aliquots of 2000 µl whole blood were transferred to Eppendorf tubes and subsequently spiked with different haemostatic interventions. Plasma and cryoprecipitate were obtained unfrozen from the local blood bank, reconstituted and stored at -80°C.

The plate was placed in the fluorometer and prewarmed to 37°C followed by automatic addition of 20 µl thrombin substrate (Fluo-substrate, Fluca-kit, lot FC1004/01). All samples were run as double determinations in parallel with a thrombin calibrator (activity 580nM, lot TC1005/01).

FIGURE 9: THROMBIN GENERATION CURVE WITH THE DIFFERENT PARAMETERS MEASURED

Clot formation and degradation in study III was monitored turbidimetrically every minute for 120 minutes at 37°C by absorbance at 405 nm using a multimode microplate reader (FLUOstar Omega® (BMG Labtech, Offenburg, Germany)).

Blood samples were centrifuged at 4000 rpm for 20 minutes to obtain platelet poor plasma (PPP) and aliquots were kept at -80°C until bulk analyses. Prior to testing all samples were centrifuged 3 minutes at 12000G. Clot formation, clot stability and facilitated clot fibrinolysis were recorded using a custom designed assay employing simultaneous activation with tissue factor (TF, final dilution was 1:5000 (Innovin®, Dade Behring, Marburg, Germany, lot 5391311)) and tissue plasminogen activator (tPA, final concentration 3nM and 6nM, Actilyse, Boehringer Ingelheim, UK) diluted in a reaction mixture of 20 mM HEPES, 150 mM NaCl, pH 7.4, phospholipids at 0.5nM (Roxisin®, Mölnland, Sweden (final concentration 4µM)) and 0.1% bovine serum albumin24. Changes in absorbance at 405 nm were recorded every minute for 120 minutes using a multimode microplate reader (FLUOstar Omega® (BMG Labtech, Offenburg, Germany)) calibrated at 37°C. All measurements were run in duplicates. Dilutions of TF and tPA were performed in the reaction mixture described above and recalcification of the citrated blood was done with automated addition of calcium chloride 25 mM.

Dosage was calculated based on clinical recommendations. Example of calculation: FFP would normally be given as 2 units of 300 ml which would dilute the patient (250g/1000ml=0.8, 75 in dilution factor. Interventions were added in 2000 µl whole blood; 2000 µl/8.75=238.8 µl FFP = 230 µl was added as an intervention.

TABLE 2: THE HAEMOSTATIC INTERVENTIONS TESTED

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Dosage in 70 kg person, blood volume 5.25L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh frozen plasma</td>
<td>2 x 300 ml ~ dilution factor 8.75</td>
</tr>
<tr>
<td>Platelets</td>
<td>1 x 300 ml ~ dilution factor 17.5</td>
</tr>
<tr>
<td>Cryoprecipitate</td>
<td>1 x 100 ml ~ dilution factor 52.5</td>
</tr>
<tr>
<td>Fibrinogen Concentrate</td>
<td>2 g ~ 30 mg/kg →2.65 mg/ml ~ 3 mg/ml</td>
</tr>
<tr>
<td>Prothrombin complex concentrate</td>
<td>25-40 U/kg ~ 0.5 U/ml</td>
</tr>
<tr>
<td>Recombinant activated FVII</td>
<td>50-80 µg/kg ~ 0.70 µg/ml</td>
</tr>
</tbody>
</table>

Dosage was calculated based on clinical recommendations. Example of calculation: FFP would normally be given as 2 units of 300 ml which would dilute the patient (250g/1000ml=0.8, 75 in dilution factor. Interventions were added in 2000 µl whole blood; 2000 µl/8.75=238.8 µl FFP = 230 µl was added as an intervention.

Dilutions of TF and tPA were performed in the reaction mixture described above and recalcification of the citrated blood was done with automated addition of calcium chloride 25 mM.

Traditionally, clot lysis has been evaluated by biomarkers of fibrinolysis, such as D-dimer, tissue plasminogen activator, plasminogen activator inhibitor, antiplasmin and plasminogen57,58. However, these biomarkers do not demonstrate the dynamic and functional features of fibrinolysis. In recent years, clot stability and fibrinolysis have been evaluated by means of thromboelastometry and by performing automated plasma clot lysis time using a spectrophotometry microplate reader24,46.
The effect of TXA on tPA facilitated fibrinolysis was evaluated by calculating the percentage of patients showing no sign of fibrinolysis at any time-point (i.e. the number showing total clot preservation). The presence of fibrinolysis was defined as at least a 10% reduction in optical density after reaching the peak level. Changes in overall clot stability were assessed by calculating the area under the curve (AUC) of the turbidity measurement. Finally, clot formation (in the absence of tPA) was assessed by measuring lag time [min] to initiation of clot formation. Examples of clot lysis curves are depicted in figure 10.

Routine coagulation samples (all studies) and single factor measurements (study I)
Preoperatively all patients were screened using a series of routine parameters, such as full blood count, platelet count, PT, APTT, D-dimer, INR, antithrombin and fibrinogen. The first two days after surgery routine blood samples were performed in the morning. Parameters evaluated were among others erythrocyte volume fraction, platelet count, creatinine, urea and fibrinogen concentration.

At the seven different time points during and after surgery the following parameters were also assessed: single coagulation factors, fibrinogen, thrombin time, protein C, antithrombin and anti-Xa. Single factors FII, FVII, FVIII, FIX and FX were measured on a Ceveron® Alpha coagulation analyzer (Technochrom GmbH, Vienna, Austria), by one stage assays using factor deficient plasma employing the following clotting activators: FVIII and FIX: kaolin/sulphate (Dapttin®), FII, FVII and FX: thromboplastin (Technochrom HHS®). A multimode microplate reader (FLUOstar Omega® (BMG Labtech, Offenburg, Germany) measuring change in absorbance at 305 nm was used in measurement of FXIII. Fibrinogen was measured by the Clauss method (Technochrom fibrinogen Reagent®) and fibrin degradation products were evaluated using the Technochrom fibrinogen degradation products assay (Technochrom Fibrinogen Degradation Products®). Protein C was recorded using a one-stage aPTT based clotting assay including a direct protein C activator (Prota®). Thrombin time was performed with a standard thrombin time reagent produced from bovine thrombin (Technochrom Thrombin Reagent®). Anti-Xa and antithrombin were measured by chromogenic assays (Technochrom anti-Xa Reagent®) and Technochrom AT-III Reagent®.

PRE-, INTRA- AND POSTOPERATIVE CHARACTERISTICS
Anesthesia
Patients received premedication with diazepam and acetaminophen. Perioperative monitoring included: ECG, pulse oximetry, capnography, temperature, cardiac index, mixed venous saturation and transesophageal echocardiography. General anaesthesia was induced and maintained using propofol, sufentanil and rocuronium.

Heparin and protamine sulphate
The initial dose of unfractionated heparin (Heparin LEO®, Leo Pharma Nordic, Malmö, Sweden) was 300 IU/kg. Target activated clotting time (ACT) was kept above 400 sec and additional heparin was administered. Reversal of heparin was done with protamine sulphate (Leo Pharma Nordic, Malmö, Sweden) using 1 mg of protamine sulphate per 100 IU of the initial heparin dose.

Tranexamic acid (TXA)
TXA (Cyklokapron®, Meda AS, Allerød, Denmark) is a synthetic derive of the amino acid lysine and inhibits fibrinolysis by competitive inhibition of the conversion of plasminogen to plasmin. Prophylactic use of TXA is recommended in cardiac surgery. In our department we routinely administer a total dosage of 4 grams (2 g at start surgery, 1 g during ECC, and 1 g after reversal of heparin).

Bleeding and transfusion requirements
Data on blood loss, volume resuscitation, reexploration due to bleeding and transfusion requirements were collected. Total blood loss was defined as intraoperative blood loss plus postoperative blood loss. Bleeding was evaluated hourly for minimally initial 12 hours postoperatively (ml/hour). Chest tubes were removed no earlier than 12 hours postoperatively, and only if bleeding was less than 50 ml/hour in two consecutive hours. Time specific data on transfusion of allogeneic blood products and volume therapy was achieved from our institution electronic patient data management system.

Surgery and postoperative treatment
All patients were operated with the use of ECC and cardiac arrest was induced with cold crystalloid cardioplegia. Priming solution was Ringer-Acetate® (Fresenius Kabi AB, Uppsala, Sweden), total priming volume 1600 ml. Perioperative body temperature was not below 34°C and hypothermia was only permitted during ECC. Rewarming to minimum 36°C was done before weaning from ECC. Surgical characteristics are listed in table 3. All patients received antithrombotic treatment with low molecular weight heparin (LMWH) (Fragmin®, Pfizer, Ballerup, Denmark), 5000 IU per day initiated 12-18 hours postoperatively and continued until day five after surgery or until full mobilization. Aspirin was initiated on day one after surgery with no loading dose; clopidogrel was discontinued until day three. Warfarin treatment was given to patients with mechanical heart valves and/or atrial fibrillation, and was initiated in the evening of the first postoperative day.
Study I
Based on previous studies and pilot experiments made in our research unit, we assumed that major cardiac surgery would induce a minimum of 40% change in the whole blood clotting parameters clotting time and/or maximum velocity of clot formation with variability of the results not increasing 25% (CV%). Hence, to test the hypothesis a minimum sample size was expected to be n=30 (assumptions: Delta=40%, B=90%, A=0.05).

Data distribution was assessed by histograms and Q-Q plots. Thromboelastometry and thrombin generation data followed a Gaussian distribution. Data were illustrated as mean and standard deviation (SD). Differences in results over time were analysed by 1-way ANOVA.

Study II
Potential effective haemostatic intervention should induce at least a 20% improvement, thus the minimum sample size should be n=26 (assumptions: delta=20%, B=90%, A=0.05).

Data distribution was assessed by histograms and Q-Q plots. The descriptive data on thromboelastometry and thrombin generation followed a Gaussian distribution. Some of the intervention experiments did not follow a Gaussian distribution. All data are illustrated giving mean and standard deviation (SD). Differences over time and following ex vivo spiking experiments were tested by Student’s paired t-test if data was normally distributed and by Mann-Whitney U test if not. A P-value less than 0.05 was considered statistical significant.

Study III
We did not perform a pre-study sample size assessment for the clot lysis assay since data previously published were sparse. We assumed a sample size necessary would reflect the sample sized needed for study I and II.

Data distribution was assessed by histograms and Q-Q plots. Clot lysis data followed a Gaussian distribution except for the time point 6h and 24h. Results are illustrated as mean and standard deviation. Differences in results over time were analysed by Student’s t-test. Differences in proportions were analysed by Fisher’s exact test. A P-value less than 0.05 was considered statistical significant.

ETHICAL CONSIDERATIONS

The Danish Biomedical Ethics Committee (#M-2009-0078) approved the protocol and all patients provided informed consent. All patients received the standard treatment of the department unaffected by their participation in the project. All haemostatic interventions were done ex vivo and therefore assumed not to cause any risk for the patients. The total blood volume used for sampling was less than 100 ml.
RESULTS

Study subjects
30 patients were initially included in this study. One patient was subsequently excluded due to complicated surgery followed by major bleeding and massive transfusion. The results are listed in sections from each study and figures and tables are positioned at the end of each section.

STUDY I

Thromboelastometry results are illustrated in figure 11 and table 4.

Clot initiation
The mean clotting time (CT) was prolonged by 12% postoperatively, the value more than doubling (192 sec vs. 454 sec (p<0.001)). However, already at the end of surgery the time was prolonged by 20% (232 sec) and at 2h by more than 70% (334 sec). There was a spontaneous normalization 12h, yet it did not reach preoperative levels. The calculated dynamic velocity parameters (MaxVel and tMaxVel) did also progressively deteriorate from time point 0h. MaxVel was halved at time point 6h, nevertheless a partial normalization was evident at 12h. Renewed impairment followed the initiation of LMWH treatment. The time until maximum clot propagation was reached (tMaxVel) demonstrated changes similar to what was observed for the CT, with significant prolongation at 2h and 6h, but in contrast to CT a complete normalization at 12h postoperatively was noted. The initiation of LMWH treatment did affect both CT and MaxVel, but the effect was less remarkable when compared to 2h and 6h. The tMaxVel was significantly prolonged during LMWH treatment.

Clot propagation
Three parameters reflect the clot propagation (CFT, MaxVel and tMaxVel) and all displayed the same tendency. The most explicit change in CFT was seen 6h postoperatively, the value more than doubling (192 sec vs. 454 sec (p<0.001)). However, already at the end of surgery the time was prolonged by 20% (232 sec) and at 2h by more than 70% (334 sec). There was a spontaneous normalization 12h, yet it did not reach preoperative levels. The calculated dynamic velocity parameters (MaxVel and tMaxVel) did also progressively deteriorate from time point 0h. MaxVel was halved at time point 6h, nevertheless a partial normalization was evident at 12h. Renewed impairment followed the initiation of LMWH treatment. The time until maximum clot propagation was reached (tMaxVel) demonstrated changes similar to what was observed for the CT, with significant prolongation at 2h and 6h, but in contrast to CT a complete normalization at 12h postoperatively was noted. The initiation of LMWH treatment did affect both CFT and MaxVel, but the effect was less remarkable when compared to 2h and 6h. The tMaxVel was significantly prolonged during LMWH treatment.

Clot stability
The mean MCF exhibited significant changes immediately postoperatively, dropping from 55 mm to 50 mm (p=0.016). This tendency continued with values being 46 mm at 2h (p<0.001) and 44 mm at 6h (p<0.001). Again, there was a spontaneous change at 12h, with a mean value of 53 mm. The MCF was unaffected by thromboprophylaxis, and a gradual increase was seen, terminating with an MCF of 56 mm at 48h.

Thrombin generation
Thrombin generation parameters are displayed in table 4 and illustrated in figure 12. Changes over time in thrombin generation parameters resembled those seen with whole blood thromboelastometry with a gradual impairment of thrombin generation in the first six hours after surgery. Normalization or near normalization was again present at 12h. The influence of thromboprophylaxis was evident, in particular in parameters peak thrombin and ETP.

Log time
The mean log time increased by 20% after surgery and was further prolonged at 2h. At 6h the logtime had more than doubled compared to baseline. After another six hours (12h), the mean logtime was back to the immediate postoperative level (4.6±0.4). An expected increase in the logtime was seen after initiation of LMWH.

Time to peak
The peak thrombin was unaffected by the end of surgery, remaining at preoperative levels. However, there was a significant increase in tPeak during the following hours, with a 2-fold increase at 2h and a 3-fold increase at 6h, respectively. Notably, there was a reduction again at 12h with values only 10% above baseline values. The tPeak thrombin rose in response to LMWH treatment.

Peak thrombin
There was no impairment of peak thrombin levels right after surgery (234 nM (±16) vs. 212 nM(±16), however impairment was lucid at 2h and 6h. At this time point peak thrombin was significantly reduced to 129 nM (±19) and 50 nM (±15), respectively (both p<0.001). The peak thrombin normalized at 12h with yet another reduction emerging at 24h and 48h due to LMWH.

Endogenous thrombin (ETP)
The total thrombin generation illustrated by ETP was impaired by 10% at the end of surgery and this change continued during the following hours. Despite a 4-fold reduction in ETP at 6h there was almost complete normalization at 12h. LMWH induced a significant reduction in ETP at time points 24 and 48h.

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TABLE 3: DEMOGRAPHIC AND SURGICAL CHARACTERISTICS

<table>
<thead>
<tr>
<th>Types of surgery</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>CABG</em></td>
<td>12 (40)</td>
</tr>
<tr>
<td><em>AVR</em></td>
<td>7 (23)</td>
</tr>
<tr>
<td><em>CABG and AVR</em></td>
<td>6 (20)</td>
</tr>
<tr>
<td><em>Other</em></td>
<td>5 (17)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Operation time, min (mean/median)</th>
<th>177/145</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiopulmonary bypass time, min (mean/median)</td>
<td>92/73</td>
</tr>
<tr>
<td>Cross-clamp time, min (mean/median)</td>
<td>54/49</td>
</tr>
<tr>
<td>Cardioplegia amount ml (mean/median)</td>
<td>1474/1400</td>
</tr>
<tr>
<td>Perioperative crystalloids, incl. priming ml (mean/median)</td>
<td>2622/2650</td>
</tr>
<tr>
<td>Balance postoperative, ml (mean/median)</td>
<td>1103/2650</td>
</tr>
<tr>
<td>pH after reperfusion (mean/median)</td>
<td>7.39/7.39</td>
</tr>
<tr>
<td>Temperature at end of surgery, °C (mean/median)</td>
<td>36.4/36.7</td>
</tr>
</tbody>
</table>

In general, a reduction of coagulation factors to approximately 2/3 of the respective baseline concentration was observed for all coagulation factors by the end of surgery, except for FXIII. FXIII showed a gradual, but insignificant decrease in concentration from initial values postoperatively until 6h. This was followed by an increase and normalization at 24h (figure 13).

Regardless of some relative differences, almost all coagulation factors started a gradual increase from 0h but returned to levels greater than 80% of the start value at time point 48h. Interestingly, FVII did not follow the pattern of continued improvement, showing a new decrease again at 12h and 24h. Despite improvement at 48h it did not reach more than 70% of preoperative value.

Thrombin time did increase initially postoperatively, however not significantly and normalized spontaneously as early as two hours postoperatively (figure 13).

Antithrombin levels were reduced to almost half of concentration after ECC. This was followed by an incline in concentration reaching preoperative levels at 24h. Results not depicted.

Fibrinogen concentration declined significantly immediately after surgery (baseline: 4.0 g/L (±1.8) vs 0h: 3.1 g/L (±2.28g(0.001)), and subsequently demonstrated a gradual improvement. Normalization was however not reached until 6h postoperatively. The incline in concentration continued terminated at levels significantly increased compared to baseline values (48h: 7.2 g/L (±1.3)) (figure 14).

To exclude that the coagulopathy was due to residual heparin, we recorded levels of anti-Xa. At no time points did we find evidence for heparin to be the cause of the coagulopathy. There was, however, an increase in anti-Xa in response to LMWH treatment. Results not shown in tables or figures.

**TABLE 4: CHANGES IN THROMBOELASTOMETRIC AND THROMBIN GENERATION PARAMETERS IN THE 48H OBSERVATION PERIOD**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Start (SD)</th>
<th>End surgery (SD)</th>
<th>2 hours (SD)</th>
<th>6 hours (SD)</th>
<th>12 hours (SD)</th>
<th>24 hours (SD)</th>
<th>48 hours (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT (sec), mean (SD)</td>
<td>495 (197)</td>
<td>555 (±212)</td>
<td>646 (±209)*</td>
<td>789 (±260)*</td>
<td>775 (±1120)</td>
<td>879 (±1675)*</td>
<td>771 (±2165)*</td>
</tr>
<tr>
<td>CFT (sec), mean (SD)</td>
<td>192 (±79)</td>
<td>235 (±132)</td>
<td>648 (±193)</td>
<td>554 (±218)*</td>
<td>228 (±91)</td>
<td>297 (±125)*</td>
<td>136 (±270)*</td>
</tr>
<tr>
<td>MaxVel (mm x 100 sec⁻¹), mean (SD)</td>
<td>11.2 (±3.7)</td>
<td>9.8 (±4.1)</td>
<td>7.4 (±2.3)*</td>
<td>5.6 (±2.3)*</td>
<td>5.6 (±2.5)</td>
<td>8.8 (±2.5)*</td>
<td>9.0 (±3.3)*</td>
</tr>
<tr>
<td>T, MaxVel (sec)</td>
<td>952 (±227)</td>
<td>915 (±295)</td>
<td>1049 (±192)</td>
<td>1225 (±1959)*</td>
<td>906 (±1154)</td>
<td>1086 (±2131)*</td>
<td>1207 (±1496)*</td>
</tr>
<tr>
<td>MCF (mm), mean (SD)</td>
<td>55.4 (±12)</td>
<td>50.1 (±8.3)*</td>
<td>46.4 (±7.0)*</td>
<td>44.4 (±7.2)*</td>
<td>55.2 (±7.7)</td>
<td>51.6 (±8.6)</td>
<td>55.9 (±9.3)</td>
</tr>
<tr>
<td>Lagtime (min), mean (SD)</td>
<td>3.6 (±0.9)</td>
<td>6.3 (±1.3)</td>
<td>6.2 (±1.1)*</td>
<td>9.3 (±0.7)*</td>
<td>9.0 (±2.1)</td>
<td>10.5 (±3.8)*</td>
<td>8.4 (±4.4)*</td>
</tr>
<tr>
<td>Peak thrombin, mean (SD)</td>
<td>234 (±85)</td>
<td>212 (±89)</td>
<td>129 (±99)*</td>
<td>50 (±76)*</td>
<td>50 (±76)</td>
<td>101 (±112)*</td>
<td>112 (±91)*</td>
</tr>
<tr>
<td>Peak thrombin (min), mean (SD)</td>
<td>6.7 (±1.3)</td>
<td>6.8 (±1.8)</td>
<td>12.4 (±1.13)</td>
<td>21.6 (±16.1)*</td>
<td>40 (±4.7)</td>
<td>16.7 (±1.29)*</td>
<td>13.1 (±6.2)*</td>
</tr>
<tr>
<td>ETP (mM), mean (SD)</td>
<td>1383 (±1805)</td>
<td>1180 (±465)</td>
<td>952 (±396)*</td>
<td>641 (±949)*</td>
<td>1289 (±535)</td>
<td>682 (±499)*</td>
<td>839 (±399)*</td>
</tr>
</tbody>
</table>

* Indicates value significant different from value start surgery. CT=clotting time, CFT=clot formation time, MaxVel=maximum velocity of clot propagation, MCF=maximum velocity of clot propagation, Lagtime=time to initiation thrombin generation, ETP=endogenous thrombin potential. Please refer to section “Study I results” for further details.

**Results**

Antithrombin levels were reduced to almost half of concentration after ECC. This was followed by an incline in concentration reaching preoperative levels at 24h. Results not depicted.

Fibrinogen concentration declined significantly immediately after surgery (baseline: 4.0 g/L (±1.8) vs 0h: 3.1 g/L (±2.28g(0.001)), and subsequently demonstrated a gradual improvement. Normalization was however not reached until 6h postoperatively. The incline in concentration continued terminated at levels significantly increased compared to baseline values (48h: 7.2 g/L (±1.3)) (figure 14).

To exclude that the coagulopathy was due to residual heparin, we recorded levels of anti-Xa. At no time points did we find evidence for heparin to be the cause of the coagulopathy. There was, however, an increase in anti-Xa in response to LMWH treatment. Results not shown in tables or figures.

**TABLE 5: TRANSFUSION REQUIREMENTS IN THE SEVEN PATIENTS TRANSFUSED**

<table>
<thead>
<tr>
<th>Patient no</th>
<th>RBC (unit)</th>
<th>FFP (unit)</th>
<th>PLT (unit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 reexploration</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2 reexploration</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>3 reexploration (surgical cause)</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Three amongst patients underwent reexploration for bleeding. RBC=red blood cells, FFP=fresh frozen plasma, PLT=platelets.

Bleeding and transfusion requirements

Transfusion was required in eight patients (27%); three of these underwent reexploration for bleeding (10%). The median time for reexploration after termination of primary surgery was 5 hours, 6 hours and 12 hours, respectively (median 7.7 h). Surgical bleeding was found to be the underlying source in one case. One patient was massively transfused and therefore excluded, as previously mentioned. Transfusion requirements in the remaining seven patients were: See table 5.

There was a tendency towards increased bleeding between four and eight hours postoperatively, followed by cessation at 12 hours. The trend was unchanged when excluding patients undergoing reexploration for bleeding. Eight patients had a blood loss exceeding 1500 mL, however, clotting parameters in this group were indifferent from patients bleeding less.
Cardiac surgery induces a coagulopathy characterized by significant reduction in whole blood clot firmness, prolonged clotting time, compromised clot propagation as well as abnormal thrombin generation.
Cardiac surgery induces a coagulopathy characterized by significant reduction in whole blood clot firmness, prolonged clotting time, compromised clot propagation as well as abnormal thrombin generation.

Of note, the whole blood coagulation abnormalities demonstrated considerable change over time. Thus, the whole blood coagulation profile deteriorated progressively until reaching a maximum six hours after surgery. However, at the time point 6h levels of fibrinogen as well as the measured single coagulation factors were recovering.

Both whole blood coagulation and thrombin generation showed a 12% increase in mean CT at 0 hours and was further prolonged by 40% at 2h. Restoration of the clotting time was accomplished with monotherapy with rFVIIa, PCC, PLT, or FFP. PLT combined with either PCC, FIB or FFP all restored the CT at close to the preoperative levels. The most pronounced effect on CT was seen with rFVIIa+PFB and PCC+FIB (figure 15+16, panel A).

Clot propagation
The velocity of clot propagation reflected by CFT and MaxVel was diminished immediately after surgery. The mean CFT was prolonged by 20% at time 0h after surgery and was further significantly affected at 2h (70% prolongation compared to baseline). At time point 0h all interventions reduced the CFT, however, some significantly below baseline (table 6). Following the more pronounced impairment at 2h, monotherapy was less efficient. All combinations did significantly improve CFT at 2h, the most efficient being any combination with PLT as well as FIB+rFVIIa. Of note, FIB+PLT did reduce CFT significantly below preoperative values. It was possible to correct the significantly impaired MaxVel at 2h with PLT or FIB, while no other monotherapy did improve it significantly. All combination treatments successfully corrected the MaxVel, however, FIB+PLT resulted in values above the preoperative values. The mean MaxVel showed the same tendency with significant prolongation at 2h. Improvement was possible with most interventions. Only monotherapy with FIB and PCC or combination FIB+PCC did not significantly improve mean MaxVel (figure 15+16, panel B).

Clot stability
The clot firmness was significantly affected already at the end of surgery, showing a reduction of 9%. After 2h the effect was even more noticeable being reduced by 16%

STUDY II
Ex vivo haemostatic interventions were carried out at the time points 0h and 2h. However, only interventions from 2h are depicted in figures 15-18. Parameters from both time points with all interventions are displayed in table 5 (interventions time point 0h) and table 6 (interventions time point 2h).

Summary results study i
Cardiac surgery induces a coagulopathy characterized by significant reduction in whole blood clot firmness, prolonged clotting time, compromised clot propagation as well as abnormal thrombin generation.

Thromboclastometry
Clot initiation
We found a 12% increase in mean CT at 0 hours and was further prolonged by 40% at 2h. Restoration of the clotting time was accomplished with monotherapy with rFVIIa, PCC, PLT, or FFP. PLT combined with either PCC, FIB or FFP all restored the CT at close to the preoperative levels. The most pronounced effect on CT was seen with rFVIIa+PFB and PCC+FIB (figure 15+16, panel A).

Clot propagation
The velocity of clot propagation reflected by CFT and MaxVel was diminished immediately after surgery. The mean CFT was prolonged by 20% at time 0h after surgery and was further significantly affected at 2h (70% prolongation compared to baseline). At time point 0h all interventions reduced the CFT, however, some significantly below baseline (table 6). Following the more pronounced impairment at 2h, monotherapy was less efficient. All combinations did significantly improve CFT at 2h, the most efficient being any combination with PLT as well as FIB+rFVIIa. Of note, FIB+PLT did reduce CFT significantly below preoperative values. It was possible to correct the significantly impaired MaxVel at 2h with PLT or FIB, while no other monotherapy did improve it significantly. All combination treatments successfully corrected the MaxVel, however, FIB+PLT resulted in values above the preoperative values. The mean MaxVel showed the same tendency with significant prolongation at 2h. Improvement was possible with most interventions. Only monotherapy with FIB and PCC or combination FIB+PCC did not significantly improve mean MaxVel (figure 15+16, panel B).

Clot stability
The clot firmness was significantly affected already at the end of surgery, showing a reduction of 9%. After 2h the effect was even more noticeable being reduced by 16%.
### Table 5: Changes in Thromboelastographic Parameters and Thrombin Generation End Surgery (Time Point 0h). Effect of Haemostatic Interventions, Both Monotherapy and Combination Therapy at This Time Point

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Start</th>
<th>1h</th>
<th>FFP</th>
<th>PLT</th>
<th>Cryo</th>
<th>FIB</th>
<th>FFP + PLT</th>
<th>PLT + Cryo</th>
<th>FIB + PCC</th>
<th>Cryo + PCC</th>
<th>PCC + FIB</th>
<th>Cryo + PCC + FIB</th>
<th>PLT + Cryo + PCC</th>
<th>FIB + PCC + FIB</th>
<th>FIB + PCC + FIB + rFVIIa</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETP (nM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MaxVol (max ± 30 sec)</td>
<td>35.4</td>
<td>±2.3</td>
<td>30.3</td>
<td>±2.2</td>
<td>34.3</td>
<td>±1.7</td>
<td>32.7</td>
<td>±2.6</td>
<td>31.6</td>
<td>±2.8</td>
<td>27.6</td>
<td>±1.9</td>
<td>26.4</td>
<td>±1.9</td>
<td>29.6</td>
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<tr>
<td>MaxVol (min ± 5 sec)</td>
<td>5.9</td>
<td>±0.3</td>
<td>5.6</td>
<td>±0.2</td>
<td>6.3</td>
<td>±0.1</td>
<td>6.3</td>
<td>±0.1</td>
<td>6.0</td>
<td>±0.1</td>
<td>5.7</td>
<td>±0.2</td>
<td>6.0</td>
<td>±0.2</td>
<td>5.8</td>
</tr>
<tr>
<td>Lag time (min)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MaxVol (peak ± 1 min)</td>
<td>1.9</td>
<td>±0.1</td>
<td>2.1</td>
<td>±0.1</td>
<td>1.4</td>
<td>±0.1</td>
<td>1.5</td>
<td>±0.1</td>
<td>1.4</td>
<td>±0.1</td>
<td>1.3</td>
<td>±0.1</td>
<td>1.6</td>
<td>±0.1</td>
<td>1.5</td>
</tr>
<tr>
<td>ETP (min)</td>
<td>1.85</td>
<td>±0.1</td>
<td>1.85</td>
<td>±0.1</td>
<td>1.85</td>
<td>±0.1</td>
<td>1.85</td>
<td>±0.1</td>
<td>1.85</td>
<td>±0.1</td>
<td>1.85</td>
<td>±0.1</td>
<td>1.85</td>
<td>±0.1</td>
<td>1.85</td>
</tr>
</tbody>
</table>

**Note:** Significant difference from value end surgery (0h)

### Results

| Parameter                | Start | 1h | FFP | PLT | Cryo | FIB | FFP + PLT | PLT + Cryo | FIB + PCC | Cryo + PCC | PCC + FIB | Cryo + PCC + FIB | PLT + Cryo + PCC | FIB + PCC + FIB + rFVIIa |
|--------------------------|-------|----|-----|-----|------|-----|---------|-----------|----------|-----------|----------|------------------|-------------------|-----------------|----------------------|
| MaxVol (max ± 30 sec)    | 1.4   | ±0.1 | 1.3 | ±0.1 | 1.4 | ±0.1 | 1.3 | ±0.1 | 1.5 | ±0.1 | 2.0 | ±0.2 | 2.0 | ±0.2 | 2.0 | ±0.2 |
| MaxVol (min ± 5 sec)     | 3.4   | ±0.2 | 3.1 | ±0.2 | 3.1 | ±0.2 | 3.1 | ±0.2 | 3.1 | ±0.2 | 3.1 | ±0.2 | 3.1 | ±0.2 | 3.1 | ±0.2 |
| Lag time (min)           |       |    |     |     |      |     |         |           |          |           |          |                  |                   |                 |                      |
| MaxVol (peak ± 1 min)    | 1.8   | ±0.1 | 1.9 | ±0.1 | 1.8 | ±0.1 | 1.9 | ±0.1 | 1.8 | ±0.1 | 1.8 | ±0.1 | 1.9 | ±0.1 | 1.8 | ±0.1 |

**Note:** Significant difference from value end surgery (0h)
FIGURE 15: THROMBOELASTOMETRIC CHANGES AFTER CARDIAC SURGERY AND THE EFFECT OF MONOTHERAPY WITH ALLOGENIC BLOOD PRODUCTS OR COAGULATION FACTOR CONCENTRATES

Panel A – Clotting time (CT)
Panel B – Maximum Velocity (MaxVel)
Panel C – Maximum clot firmness (MCF)

Parameters were evaluated prior to surgery (start), at the end of surgery (0h) and two hours postoperatively (2h). Interventions were performed at time point 2h. Haemostatic interventions were: FFP – Fresh frozen plasma, PLT – Platelets, Cryo – Cryoprecipitate, Fib – Fibrinogen concentrate, PCC – Prothrombin complex concentrate, rFVIIa – recombinant activated factor VII.

FIGURE 16: THROMBOELASTOMETRIC CHANGES AFTER CARDIAC SURGERY AND THE EFFECT OF COMBINATION THERAPY WITH ALLOGENIC BLOOD PRODUCTS AND COAGULATION FACTOR CONCENTRATES

Panel A – Clotting time (CT)
Panel B – Maximum Velocity (MaxVel)
Panel C – Maximum clot firmness (MCF)

Parameters were evaluated prior to surgery (start), at the end of surgery (0h) and two hours postoperatively (2h). Interventions were performed at time point 2h. Haemostatic interventions were: FFP – Fresh frozen plasma, PLT – Platelets, Cryo – Cryoprecipitate, Fib – Fibrinogen concentrate, PCC – Prothrombin complex concentrate, rFVIIa – recombinant activated factor VII.

FIGURE 17: CHANGES AFTER CARDIAC SURGERY IN THROMBIN GENERATION AND THE EFFECT OF MONOTHERAPY WITH ALLOGENIC BLOOD PRODUCTS OR COAGULATION FACTOR CONCENTRATES

Panel A – Lagtime thrombin
Panel B – Peak thrombin
Panel C – Endogenous thrombin potential (ETP)

Parameters were evaluated prior to surgery (start), at the end of surgery (0h) and two hours postoperatively (2h). Interventions were performed at time point 2h. Haemostatic interventions were: FFP – Fresh frozen plasma, PLT – Platelets, Cryo – Cryoprecipitate, Fib – Fibrinogen concentrate, PCC – Prothrombin complex concentrate, rFVIIa – recombinant activated factor VII.

FIGURE 18: CHANGES AFTER CARDIAC SURGERY IN THROMBIN GENERATION AND THE EFFECT OF COMBINATION THERAPY WITH ALLOGENIC BLOOD PRODUCTS AND COAGULATION FACTOR CONCENTRATES

Panel A – Lagtime thrombin
Panel B – Peak thrombin
Panel C – Endogenous thrombin potential (ETP)

Parameters were evaluated prior to surgery (start), at the end of surgery (0h) and two hours postoperatively (2h). Interventions were performed at time point 2h. Haemostatic interventions were: FFP – Fresh frozen plasma, PLT – Platelets, Cryo – Cryoprecipitate, Fib – Fibrinogen concentrate, PCC – Prothrombin complex concentrate, rFVIIa – recombinant activated factor VII.
Summary results study II
Ex vivo haemostatic interventions at both 0h and 2h showed a more potent effect of coagulation factor concentrates compared to allogeneic blood products. At the end of surgery the coagulopathy was primarily characterized by reduced whole blood clot firmness. This could be corrected by monotherapy with FIB or PLT, as well as combination therapies FIB+PLT, FIB+rVIIa and FIB+PCC. Two hours postoperatively monotherapy was ineffective, due to the fact that the coagulopathy now was more complex. At this point in time the clotting time and lag time due to the fact that the coagulopathy was more complex.

Two hours postoperatively monotherapy was ineffective, due to the fact that the coagulopathy now was more complex. At this point in time the clotting time and lag time due to the fact that the coagulopathy was more complex.

STUDY III

Study subjects
30 patients were initially included in this study. One patient was excluded due to a shift in treatment from TXA to aprotinin and one due to massive transfusion and coagulation factor treatment, leaving 28 patients for further analysis. Amongst these, 23 patients received TXA prior to baseline blood sample while five patients received the first administration after the initial blood sampling.

TXA effect on fibrinolysis
Fibrinolysis was completely abolished after administration of TXA. The five patients receiving TXA after the first sample showed prompt and complete fibrinolysis prior to administration of TXA. After 12 hours seven patients (25.0%) displayed fibrinolysis and after 24 hours 13 patients (46.4%) had regained fibrinolysis. At 48 hours, 19 patients had regained fibrinolytic activity (67.9%) (figure 19). Interestingly, patients with creatinine levels above 120 µmol/L (n=6) were more resistant to fibrinolysis with only two patients (33%) showing signs of fibrinolysis at 48 hours (p=0.13). Seven patients bled more than 1500 ml in total. Six of these patients (85.7%), a higher percentage, displayed fibrinolysis at 48h compared to those with less bleeding. Patients bleeding more than 1000 ml did not show any correlation with clot lysis parameters.

TXA effect on clot stability
Patients receiving TXA displayed a clot stability that was 13-fold higher than that of patients who had not received TXA at baseline sampling (AUC No-TXA 30±8 vs. TXA 409±40, p=0.0002). Despite this effect the TXA group had a four-fold reduction in mean AUC initially in the postoperative period (start: 409±40 vs. 0h: 111±14 p=0.0001). The following six hours clot stability remained unchanged, but at 12 hours there was a restoration to preoperative levels. Thromboprophylaxis initiated the day after surgery decreased clot stability again (figure 20).

Clot kinetics
Administration of TXA is not expected to influence the clotting time, which displayed a progressive prolongation during the initial postoperative hours, being most affected 6 hours postoperatively (p<0.01). There was a partial restoration at 12 hours postoperatively. The initiation of thromboprophylaxis did increase lag time again (figure 21).

Summary results study III
TXA increased clot stability and protected against facilitated fibrinolysis. All patients showed complete resistance to tPA induced fibrinolysis during the first six hours after last administration of TXA. There was a tendency towards a prolonged effect of TXA in patients with reduced kidney function. A higher percentage of patients bleeding >1500 ml showed recurrence of fibrinolysitic capacity at 48h time point compared to patients bleeding less than 1500 ml. Regardless of the reduced fibrinolysis, the overall clot stability (area under the curve) and clot kinetics (clotting time) were severely affected immediately postoperatively and during the following six hours. At the 12h time point recovery toward normal was observed. The initiation of thromboprophylaxis affected all three parameters.
ACCESSION OF THE HYPOTHESES

The three hypotheses raised in this PhD thesis were pursued in the three studies described above. The descriptive study of the coagulopathy (study I) confirmed that cardiac surgery patients develop a coagulopathy as defined by a i) prolonged clot initiation, ii) compromised clot propagation, and iii) reduced maximum clot firmness as well as iv) reduced thrombin generation. The coagulopathy demonstrates temporal changes and the most profound defect of coagulation was approximately six hours post and thus corresponding with the most common timing of reexplanation due to bleeding.

The in vitro effects of coagulation factor concentrates compared to allogeneic blood products were tested in study II. This study concluded a more potent effect of mono- or combination therapy of coagulation factor concentrates compared to fresh frozen plasma, cryoprecipitate, and/or platelets in blood from patients following cardiac surgery. Furthermore, this study suggested that use of global haemostatic assays such as thromboelastometry and/or thrombin generation may provide a more rational and targeted use of haemostatic interventions.

Study III confirmed that patients undergoing cardiac surgery develop substantial loss of clot stability, however excessive fibrinolysis can be successfully prevented by tranexamic acid. Furthermore, it was shown that automated clot lysis assays may be useful in monitoring tranexamic acid and that renal function impact the anti-fibrinolytic effect of tranexamic acid.

INTERPRETATION OF RESULTS

Study I

This study is the first to provide detailed information on post cardiac surgery coagulopathy; this is by virtue of the many time points analysed, the supplementary laboratory elements as well as the duration of observation period.

The single coagulation factor measurements confirm earlier published studies45, showing an initial reduction in concentration due to consumption and dilution followed by a gradual recovery. Of note, the present study reported reduction in the concentration of FVII in the period from 2 to 24 hours postoperatively. Interestingly, the changes in coagulation factor concentrates did not reflect the changes evaluated by thrombin generation and whole blood thromboelastometry. The whole blood thromboelastometry parameters progressively deteriorated with the most pronounced defect recorded at the 6th time point postoperatively. The initial reduction in MCF was in parallel to a drop in the concentration of fibrinogen. Nonetheless, at 2h the fibrinogen concentration was improving while the MCF was iv) declining. This trend was even more pronounced at the 6th time point postoperatively. Fibrinogen is the main contributor to clot firmness. Thus, the reduced MCF may be explained by three alternative mechanisms.

Firstly, factor XIII concentration continued to decline and did not improve until 12 hours postoperatively (start $98 \pm 35$% vs. 6 h $87 \pm 21$% p=0.08). FXIII is important in cross-linking fibrin, thereby stabilizing the clot. Activation of FXIII is caused by thrombin. Therefore the reduced MCF may be due to low thrombin activity and reduced FXIII concentration and -activation. Secondly, hemodilution is known to induce abnormal fibrin polymerization as well as clot stability46. Clinical studies support this theory since fibrinogen concentration has been shown to reduce postoperative bleeding after surgery in hemodiluted patients47-49. Conversely, a recently published randomized, placebo controlled study revealed no effect of fibrinogen concentrate (2 g) on postoperative bleeding and transfusion requirements in coronary surgery47. Thirdly, a reduction in platelets, either in numbers or function could also contribute to a decrease in MCF. Platelet counts were significantly reduced; however, mean platelet count was $173 \times 10^9$/L (range 96-340). A function platelet test might strengthen these results. On the contrary, recent studies have shown that platelet MCF appears not to be reduced after cardiac surgery50. Furthermore platelet function has been shown to return to normal three hours postoperatively despite initial reduction after ECP51. Since the most pronounced effect on MCF was seen six hours postoperatively, platelets do not seem to be the primary reason.

Thrombin generation also exhibited a significant reduction in the period from two to six hours postoperatively. Studies in trauma patients have shown reduced thrombin generation due to an increase in systemic anticoagulants. The increment of protein C and thrombomodulin is thought to be a response to hyperfusion52. Hyperfusion might be present during ECC, however arterial gasses did not support this suspicion and protein C levels were actually declining.

A simple explanation for the reduced thrombin generation could be residual heparin or a heparin rebound-effect53,54. The time period of heparin rebound effect has previously been described as four to eight hours postoperatively. It is explained by heparin binding to proteins as well as prolonged half time of heparin compared to protamine sulphate. Measurements of anti-Xa were performed and did not indicate any signs of residual heparin. Anti-Xa levels were augmented in response to thromboprophylaxis.

One may speculate whether or not increased levels of tissue factor pathway inhibitor (TFPI) contribute to the coagulation abnormalities seen after cardiac surgery. Previous studies have demonstrated an increase in TFPI after heparin administration, however with a near normalization after protamine reversal55,56. The distribution between free and bound TFPI did, on the other hand, shift towards a higher percentage of free TFPI, which is known to have more anticoagulant effect. Percy et al also demonstrated increased levels of TFPI after ECC, and furthermore they showed an increase in thrombin generation in response to ex vivo inhibition of TFPI57.

Interestingly, surgical reexploration due to bleeding is very often performed in the time period six hours after initial surgery58. At this point in time the haemostatic capacity is severely impaired. Taking a patient back for surgery may increase risk of pronounced bleeding and lead to augmented transfusion requirements.

Study II

The systematic evaluation of treatment with FCP and allogeneic blood products in treatment of postoperative coagulopathy demonstrated a more potent effect of FCP. The coagulopathy was, as previously described dynamic over time, most prominent at two hours postoperatively. Consequently, demands for optimal treatment also changed over time. Initially postoperative monotherapy efficiently corrected some of the abnormalities; however at two hours postoperatively combination therapy was necessary to fully correct the coagulopathy.

Clinical practice and guidelines advocate balanced transfusion with package treatment containing red blood cells, plasma and platelets in ratios 1:1:1, 3:3:1 or 4:4:159-67 during massive bleeding. Our study, however, indicated a less potent haemostatic effect of allogeneic blood products. In particular plasma displayed very little hemostatic potential. Previously, concern has been raised regarding the unpredictability of plasma in the clinical settings59,60. Platelets were more effective in correcting some parts of the coagulopathy. Studies have shown that the effect of platelets is related to storage time61. In the present study platelets were collected freshly every day. One may speculate whether this fact improved efficacy compared to platelets often used in clinical settings. The CFCs were superior in correction of the coagulopathy. However, in some cases the coagulation parameters were overshooting the preoperative levels. A potential state of hypercoagulability is unwanted after cardiac surgery due to the risk of thromboembolic complications, including graft occlusion. This over-correction was primarily seen in the correction of MCF with fibrinogen concentrate, either as monotherapy or in combination therapy. Study I presented in this thesis as well as other clinical studies have demonstrated a spontaneous increment in fibrinogen concentration after surgery, even to levels beyond the preoperative concentration62,63. Despite an increasing use of fibrinogen in many ongoing clinical trials, safety outcomes have been excellent with only few reports on possible adverse events64,65.

FCC was highly efficient in correcting thrombin generation. Only in combination with cryo did peak thrombin and ETP reach levels significantly greater than preoperative levels. Recently, the safety and efficacy of FCC was compared to FFP in randomized trial66, which demonstrated an increased risk of renal injury and postoperative dialysis in the FCP group. There were no thromboembolic events and it was argued, that the renal affection could be due to less fluid infusion in the non-plasma group giving rise to hypotension and more frequent use of vasocostrictors. Nevertheless, thromboembolic complications are a major concern when treating postoperative bleeding. The only randomized trial with rFVIIa was terminated preterm due to safety issues regarding thromboembolic complications67. However, bleeding and transfusion requirements were lower in the patients receiving rFVIIa.

It is important to notice that most of the patients in the present study did not bleed and correction of the coagulopathy would only have been required in a few of those cases. The results of this study may be used as guideline for possible hemostatic interventions in case of bleeding after cardiac surgery. Bleeding initially after surgery should focus on correcting abnormal fibrin poly-
merization and hemodilution. If bleeding commences two hours after surgery the coagulopathy would possibly still need correction of fibrin abnormalities and additionally restoration of thrombin generation.

Study III
Tranexamic acid demonstrated a significant effect on tPA facilitated clot lysis. Despite this effect, the overall clot stability was still reduced during the postoperative period and not fully restored before 12 hours postoperatively. A small reduction in clot stability was noted in response to introduction of thromboprophylaxis. There was a tendency towards prolonged resistance to fibrinolysis in patients with reduced renal function (creatinine >120µmol/L). Since TXA is primarily metabolized through the kidneys the prolonged effect may simply be due to slower metabolism.

Performing the clot assay in plasma prevented us from evaluating the effects of platelets. Whole blood clot lysis was not available at the time this study was conducted, however other studies have shown that platelets affect fibrinolysis in many ways. One of these is through the release of fibrinolytic factors from alpha granules, another by potentiating thrombin generation thus increasing the conversion of plasminogen to plasmin. Evaluating fibrinolysis with thromboelastometry is currently possible, however signs of fibrinolysis are often not accessible until 45 minutes after initiation of the analysis. Interestingly, five patients did by coincidence not receive TXA prior to the baseline blood sample. All five revealed fast and complete clot lysis, which support our theory, that patients prior to TXA administration have complete clot lysis as evaluated by this assay. We assume that clot lysis activity reflects administration and elimination of TXA; nevertheless exact concentrations of TXA would have supported our assumption and results. Previous studies have demonstrated a rather large variation in plasma concentration of TXA although dosage was weight adjusted. In summary, whilst TXA reduces fibrinolytic activity, it does not improve the overall haemostatic capacity. Most patients display effects of TXA for more than 12 hours. Hence, additional TXA is rarely the treatment for postoperative bleeding initially after cardiac surgery; some patients may benefit from it, yet the risk of over-dosing should be taken into consideration.

EVALUATION OF METHODOLOGIES
Thromboelastometry
During the last two decades thromboelastometry has undergone incessant validation both in clinical settings and as a research tool. It provides unique information due to the continuous observation of the clot formation in whole blood. The advantage of whole blood in comparison to plasma is the contribution of platelets, which we know play a pivotal role in coagulation. Thromboelastometry has a high negative predictive value, however, it has not proved to enable bleeding prediction. Transfusion and hemostatic therapy guided by thromboelastometry has demonstrated to reduce transfusion rates and improve cost-effectiveness. Thromboelastometry provides stable and reproducible results, also when omitting 30 minutes resting prior to analysis. For the studies in this thesis 30 min rest was allowed before testing. Thromboelastometry can, however, not detect all coagulation abnormalities and sensitivity to platelet dysfunction and defects of von Willebrand factor is reduced. Furthermore, tests are performed in prewarmed cups (37°C), which may mask coagulopathy due to hypothermia. Despite the fact that the analyses is made in whole blood it does still not account for the endothelial contribution to coagulation.

We adopted the low-tissue factor assay in our studies. This method has been verified and used in several studies and with different coagulopathies. Additionally, a reference interval is available. The advantage of using a low concentration of tissue factor is an improved sensitivity, making it possible to evaluate small changes in the dynamics of clot formation. On the contrary, the risk of precipitation does increase, therefore all test were performed as double determinations.

Thrombin generation
Calibrated automated thrombin generation (CAT) has been validated in plasma, both platelet-rich and platelet-poor. Thrombin generation contribute with one of the most detailed views of haemostatic capacity in plasma. Thrombin generation continues beyond the point of when clotting is initiated. However, there is still some lack of consensus around variations in the assays used. Additionally no reference interval has been defined for different haemostatic deficiencies. Diagnostics in plasma requires some preparation time; therefore CAT is less suitable as POC device. Development and use of thrombin generation in whole blood will probably still need an ethical dilemma to design a study with a control group. There are some additional limitations to the study. A control group not receiving TXA would have strengthened our study. However, since administration of TXA is a class I (a) recommendation in guidelines, it would have been an ethical dilemma to design a study with a control group. Interestingly, five patients did by coincidence not receive TXA prior to the baseline blood sample. All five revealed fast and complete clot lysis, which support our theory, that patients prior to TXA administration have complete clot lysis as evaluated by this assay. We assume that clot lysis activity reflects administration and elimination of TXA; nevertheless exact concentrations of TXA would have supported our assumption and results. Previous studies have demonstrated a rather large variation in plasma concentration of TXA although dosage was weight adjusted. In summary, whilst TXA reduces fibrinolytic activity, it does not improve the overall haemostatic capacity. Most patients display effects of TXA for more than 12 hours. Hence, additional TXA is rarely the treatment for postoperative bleeding initially after cardiac surgery; some patients may benefit from it, yet the risk of over-dosing should be taken into consideration.

Single factor measurements
The majority of single factor measurements were performed using commercial reagents on a fully automated coagulation analyzer for clotting, chromogenic and turbidimetric assays (Ceveron® Alpha (Technoclone GmbH, Vienna, Austria)). Factor XIII was measured by change in absorbance using a FLUOstar Omega® (BMG Labtech, Offenburg, Germany). Calibration curves and test runs were performed prior to running the study samples. The results provide information on concentrations of coagulation factors and fibrinogen, but do not reflect overall coagulation activity and capacity. The information gained should be regarded as support to the dynamic clot evaluations.

STRENGTH AND LIMITATIONS
Strengths and limitations of the three studies will be addressed collectively, since some of these will be overlapping. Individual limitations for each study have been addressed in the Discussion section for each study separately. There are several strengths of the studies. Firstly, the three studies use various different assays for the evaluation of coagulation after cardiac surgery. No single parameter provides the “perfect” information on clot formation and dissolution. Therefore the quality is raised by the multimodal evaluation. Secondly, the prospective design secures a limited selection and information bias. Thirdly, the homogenous and multiple serial observations makes the study more robust and has provided new knowledge compared to previous studies focusing on the changes of interest.
CONCLUSION

Cardiac surgery induces a multifactorial coagulopathy, which evolves and changes over time. Impairment of clotting is most pronounced six hours postoperatively, characterized by reduced clot initiation, clot propagation and clot firmness in addition to impaired thrombin generation. Interestingly, this time point reflects the most common time point for surgical reexploration.

Coagulation factor concentrates were superior to allogeneic blood products as haemostatic intervention in post cardiac coagulopathy. The treatment of bleeding after surgery should take the time course of bleeding into consideration. Treatment of early bleeding should focus on the correction of abnormal fibrin polymerisation while later bleeding needs additional restoration of thrombin generation. Tranexamic acid therapy, whilst inducing resistance to fibrinolysis, nevertheless fails to improve overall clot stability. The time until regained fibrinolytic activity was prolonged in patients with affected renal function.

PERSPECTIVES

The study has provided new understanding of the optimal management of bleeding in patients following cardiac surgery and may provide a basis for new therapeutic strategies. This is further strengthened by the critical evaluation of the haemostatic potential of CFC as compared to transfusion of allogeneic blood products. The results may be used to design future clinical randomised studies aiming to improve bleeding management in patients undergoing cardiac surgery.

The standard use of tranexamic acid provides resistance to fibrinolysis. Nevertheless, individualised dosage regimes may provide a better treatment profile with less risk of adverse effects such as seizures.

Finally, the knowledge gained may also contribute in utilisation of bedside laboratory equipment such as thromboelastometry and thrombin generation for rational guidance in selecting the most efficient haemostatic intervention in bleeding patients. The future may be a personal therapeutic approach for patients undergoing cardiac surgery.
Following cardiac surgery, bleeding occurs frequently, which may lead to increased mortality and morbidity. The surgical procedure in itself may induce bleeding, however in 1/3 of patients undergoing reexploration due to bleeding, a coagulopathy is detected and thought to be the cause of bleeding. The coagulopathy appears to be multifactorial. Transfusion with allogeneic blood products has been the treatment of choice in case of post cardiac bleeding. During the last decades treatment with coagulation factor concentrates has increased, supported by the use of point-of-care assays, such as thromboelastometry. To reduce bleeding and transfusion requirements, antiﬁbrinolytic treatment with tranexamic acid has been introduced as a routine treatment.

The present thesis is based on three laboratory studies.

The main hypotheses were:

i) Patients undergoing cardiac surgery will develop a complex bleeding diathesis

ii) Coagulation factor concentrates are superior to allogeneic blood products in correction of the coagulopathy

iii) Administration of tranexamic acid will modify clot lysis tendency during and after cardiac surgery.

The objectives of these studies were:

i) to describe the postoperative coagulopathy evolving over time in detail by the use of thromboelastometry and thrombin generation together with selected single coagulation factor measurements

ii) to evaluate the haemostatic potential of coagulation factor concentrate substitution as compared to allogeneic blood products

iii) to evaluate the effect on clot lysis activity in patients undergoing treatment with tranexamic acid using spectrophotometry.

All laboratory work was performed ex vivo on blood samples from patients undergoing cardiac surgery under extracorporeal circulation.

Study I characterized the development of a coagulopathy characterized by delayed and reduced clot initiation, clot propagation and clot ﬁrmness as together with impaired thrombin generation. The coagulopathy was detected immediately after the end of surgery, although, these changes were only moderate. During the following hours there was a continuously ongoing deterioration, reaching being most prominent at 6 hours postoperatively. At 12 hours postoperatively, however there is a spontaneous and almost complete return to preoperative levels.

Study II revealed that the initial impairment of clot stability could be corrected with addition of ﬁbrinogen or platelets. However, at two hours postoperatively correction of clot initiation, clot propagation, and thrombin generation to restore clot stability. Treatment with coagulation factor concentrates was superior to allogeneic blood products in improving the coagulopathy ex vivo. Combinations of ﬁbrinogen with either prothrombin complex concentrate or recombinant factor VIIa were found to be the most effective.

Study III demonstrated a signiﬁcant effect of tranexamic acid on enhanced clot lysis. Despite this, a signiﬁcant reduction in the overall clot stability was still seen.

In conclusion, cardiac surgery engenders a multifactorial coagulopathy, which changes over time, and is partly resistant to the use of tranexamic acid. The most pronounced impairment was seen six hours postoperatively. Our data demonstrated that timely and rational treatment with coagulation factor concentrates may be superior to the use of allogeneic blood products.

DANSK RESUME

Blødning efter hjertekirurgi forekommer relativt hyppigt og medfører øget risiko for komplikationer og død. Hjertekirurgi alene medfører en blødningsrisiko, men i mere end 1/3 af tilfældene skyldes blødningen en forstyrrelse i blodets stærkningsevne (koagulopati). Denne forstyrrelse er multifaktorielt og behandlingen af tilstanden har tidligere været blodprodukter, såsom plasma og trombocyter. Transfusion af blodprodukter medfører i sig selv en øget mortalitet, især er det bekymringsværdigt at denne effekt består i adskillige år efter eksponeringen. I tillegg hertil er risikoen særligt tydelig hos yngre patienter uden kormorbidity. I de senere år har der været tiltægende fokus på anvendelse af koagulationsfaktor koncentrater til undervurdering af harmonien, ikke mindst givet af nye monitoringsmuligheder, som fx. thromboelastometri. I et forsøg på at reducere blødnings og transfusionsbehov anvender man i dag rutinærmest en fibrinolysehæmmer (tranexamsyre) i forbindelse med hjertekirurgi.

Afhandlingen er baseret på tre laboratoriestudier, alle med anvendelse af blodprøver stammende fra hjertekirurgiske patienter under og efter operation.

Hypoteserne for studierne var:

i) Hjertekirurgi medfører udvikling af en kompleks koagulopati

ii) Koagulationsfaktor koncentrater kan korrigere koagulopatiene bedre end blodprodukter

iii) Tranexamsyre øger blodets resistens mod fibrinolyse

Formålet med de tre studier var:

i) i detaljer at karakterisere den postoperative koagulopati, der gradvist udvikler sig. Hertil anvendtes thromboelastometri, thrombin generation og måling af enkelt koagulationsfaktorer

ii) at vurdere koagulations faktor koncentraters kemiske potentiale sammenligning med blodprodukter

iii) at evaluere effekten af tranexamsyre på fibrinolyse ved hjælp af spektrofotometri

Studie I beskriver udviklingen af en koagulopati, der både er kendtegnet ved reduceret clot initiation og clot udvikling i tillegg til nedsat thrombin dannelse og påvirket stabilitet af koaglet. Åndringerne begynder umiddelbart postoperativt, i begyndelsen kun med moderat påvirkninger, der dog gradvist tiltager. Den mest udtalte koagulopati er til stede seks timer efter operationen. Efter 12 timer ses en spontan normalisering nærmende sig det preoperative niveau.


Studie III demonstrerer en eksklatant effekt af tranexamsyre, som tydeligt stabiliserede koaglet. På trods heraf, fandt vi fortsat en nedsat total clot stabilitet.

REFERENCES


Global haemostatic defect peaks six hours after cardiac surgery – a six hours observational study

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Introduction: Bleeding complications after cardiac surgery are associated with increased morbidity and mortality. Re-explo- ration often occurs 6-8 hours post surgery. This study characterizes the coagulopathy at seven time points in the first 48 hours after cardiac surgery.

Methods: 30 consecutive patients undergoing cardiac surgery on cardiopulmonary bypass were included in this prospective case follow-up study. Whole blood thromboelastometry (ROTEM®), calibrated automated thrombin generation (CAT), and single coagulation factors were assessed pre-operatively; immediately post-operative (t=0h); and 2, 6, 12, 24, and 48 hours after end of surgery. In addition, postoperative bleeding, transfu- sions requirements, and need for re-exploration were registered.

Results: A progressive weakening of both ROTEM and CAT peaked at 6 hours postoperatively followed by partial recovery at 12h. Most of the single coagulation factors were reduced to 2/3 of the start value at 0 hours, but then gradually improved during 2-48 hours. They were thus discordant with the defect demonstrated in the global haemostasis assay. Initiation of thrombo- polymyxins triggered prolongation of clotting times and reduced CAT, while whole blood clot firmness continued to increase. Three patients underwent reexploration due to bleeding (10%); seven patients were transfused (27%).

Conclusion: The global haemostatic defect peaks around 6 hours after surgery, correlating with the common median timing of re-exploration. Standard coagulation assays or single coagulation factor levels did not detect the defect. Global assays may facilitate rational and timely haemostatic interventions post cardiac surgery.

Keywords: Blood coagulation disorders; cardiac surgery; hemorrhage; postoperative

Ethics Committee reference number H-48-2009-0078

Introduction

Bleeding complications following major cardiac surgery are common and surgical re-exploration required in 3-4% of all cases.1,2 A coagulopathy is concluded in up to 35% of cases,3 and associated with increased morbidity and mortality.4,5 The most common timing of surgical re-exploration is 6 hours after the initial termina- tion of surgery.6,7

Global haemostatic defects such as dynamic whole blood thromboelastometry (ROTEM®) and calibrated automated thrombin generation (CAT) appear to illu- "trate the overall haemostatic performance in a variety of coagulopathies and used to evaluate the efficacy of different haemostatic interventions.8,9 Coagulation studies have focused on changes occurring during or immediately after cardiac surgery. However, the haemostatic performance may be changing more exten- sively and dynamically in postoperative cause.

Clinical trials have indicated that enhancing thrombin generation 10 or improving fibrin polymerisation 11 within the first hours postoperatively may reduce bleeding and the frequency of re-exploration; however understanding of rational timing of such inter- ventions is missing. We pursued the hypotheses that time dependent abnormalities by ROTEM® and CAT develop after cardiac surgery and can be explained by changes in fibrinogen and single pro- and anticoagu- lant factor levels. This study aimed to map character- istics and mechanisms of the coagulopathy evolv- ing during and after cardiac surgery using analysis of ROTEM®, CAT as well as measurement of mul- tiple coagulation factors and natural anticoagulants. Changes observed were correlated with objective mea- sures of blood loss.

Materials and Methods

Study design

The study was approved by the appropriate Ethics au- thority; The Danish Biomedical Ethics Committee (re- ference number #M-2009-0078). Written informed con- sent was obtained from all subjects. Blood samples from patients undergoing cardiac surgery were collected at seven consecutive time points during a 48-hour period, representing a prospective case follow up design. The time points were: start surgery (start anaesthesia), im- mediately post-operatively (t=0h); and 2, 6, 12, 24, and 48 hours after end of surgery.

Post cardiac coagulopathy is considered associated with platelet inhibitor treatment, heparinization, the surgical trauma, haemodilution, cardiopulmonary by- pass (CPB), consumption of coagulation factors and platelets, and increased fibrinolysis. Development of acidosis and hypothermia may further deteriorate the coagulation system 11.

Global haemostatic assays such as dynamic whole blood thromboelastometry (ROTEM®) and calibrated automated thrombin generation (CAT) appear to illu-
Cardiopulmonary bypass system

The heart and lung machine (HLM) used was Mar-quet HLM® (Marquet, Hirrlingen, Germany) while the tubes and oxygenator were either Quadrox® (Marquet) or Primoxx® (Sorin, Mirandola, Italy). The HLM consisted of tubes, an arterial filter, a hollow fiber membrane oxygenator and a venous and cardi- omyocyte reservoir. The arterial filter and reservoir were heparin coated.

Anticoagulants and antifibrinolytics

Unfractionated heparin (Heparin LK0®, Leo Pharma Nordic, Malmö, Sweden) was given at an initial bolus of 300 IU kg⁻¹ with additional administration to maintain an activated clotting time greater than 400 sec. Heparin was reversed by protamine sulphate (Leo Pharma Nordic, Malmö, Sweden) using 1 mg of prota-mine sulphate per 100 IU of initial heparin dose. The mean dose of heparin was 35300 IU (range 20000-58000) whereas the mean protamine dose was 288 mg (range 200-400). Preoperatively, patients had a mean APTT of 34.3 sec (range 21-43). Postoperatively, fol-low ing heparin reversal, the mean APTT was 33.4 sec (range 28-38).

All patients received antifibrinolytic treatment with transexamic acid, total dose 4 g (Cyclopakaprin®, Meda AS, Allered, Denmark), except for one patient who received aprotinin (Trasylol®, Bayer AG, Leverkusen, Germany).

Postoperative treatment, blood loss and re-exploration

Postoperative bleeding, total blood loss, transfusion re-quirements and fluid administration data are displayed in table 2. Total blood loss was defined as bleeding in-traoperatively plus postoperatively. Bleeding was evalu-ated hourly for the first 12 hours postoperatively (ml hour⁻¹). Chest tubes were removed no earlier than 12 hours postoperatively and only when production was less than 50 ml hour⁻¹ in minimum two successive hours. Information on reexploration for bleeding as well as data on transfusion and coagulation factor treat-ment was collected.

Antithrombotic treatment was initiated 12-18 hrs postoperatively using low molecular weight heparin (LMWH) (Fraxiparin®, Pfizer, Billyard, Denmark) 5000 IU per day for 5 consecutive days or until full mobilization. In addition, aspirin 75 mg was started on day 1 after surgery. Warfarin treatment was reinitiated 24-36 hrs postoperatively in patients (n=7) with mechanical heart valves and/or atrial fi-brillation.

Coagulation analyses

Continuous whole blood clot formation profiles were recorded in parallel using ROTEM® (TEM Interna-tional, Munich, Germany) activated with a low tissue factor assay as previously described. ROTEM® plastic cups were prewarmed to 37° degrees centigrade. Whole blood rested 30 minutes prior to analysis. A volume of 300 µl whole blood was loaded to each cup followed by addition of 20 µl of buffer (20 mM HEPES, 150 mM NaCl, pH 7.4). The coagulation process was activated with tissue factor (Innovin®, Dade Behring, Marburg, Germany - final dilution 1:17000) and started by recalci-fication with 200 µM CaCl₂. Tests were processed for at least 45 minutes in duplicates. The following param-eters were evaluated: clotting time (CT [sec]), propagating phase: clot formation time (CFT [sec]), maximum velocity (MaxVel [mm*100 sec⁻¹]), and time to maxi-mum velocity (tMaxVel [sec]), and clot firmness: maxi-mum clot firmness (MCF [mm]).

Thrombin generation in platelet poor plasma CAT was performed as described by Hemker em-ploying reagents and software provided by Thrombo-nicscope BV® (Maastricht, Netherlands) on a Fluoro-skank® device (Thermo Electron Corp., Helsinki, Finland). Blood samples were centrifuged at 4000 rpm for 20 minutes to obtain platelet poor plasma (PPP). Aliquots were kept at -80°C until bulk analyses. Prior to testing all samples were centrifuged three minutes at 12000 G. The assay was performed in a prewarmed (37°C) 96-well microplate (Immunolon 2HB, round bottom, Jencons, YVR International Ltd., Sussex, UK). A total of 80 µl of PPP was spiked with 20 µl of PPP tissue factor reagent 5 µM (lot PPP12/1/01). In the fluorometer 20 µl of thrombin substrate (Fluo- substrate, Fluka-kit, lot FC1004/01) was automatically added. All samples were run as double determinations with a parallel thrombin calibrator (activity 580mN, lot TC1005/01). CAT parameters included: i) lagtime [min], ii) endogenous thrombin potential (ETP [nM]), iii) peak thrombin level [nM] and iv) time to peak thrombin level (tPeak [min]).

Single coagulation factor measurements

Single coagulation factors: FII, FVII, FVIII, FIX and FX were measured on a Coarol® Alpha coagula-tion analyzer (Technocnome GmbH, Vienna, Austria), by one stage assays using factor deficient plasma em-ploying the following clotting activators: FXII and FIX: kaolin/sulphate (Daptitin® and FII, FVII and FX: thromboplastin (Thrombostat HIS®). FXIII was measured by a multimode microplate reader (FLU- Ostar Omega (BMG Labtech, Offenburg, Germany) measuring changes in absorbance at 305 nm. Fibrino- gen was measured by the Claus method. Protein C was recorded using a one-stage aPTT based clotting assay including a direct protein C activator (Protac®). Anti-Xa and antithrombin were measured by chromo-genic assays.

Pre- and postoperative coagulation screen

Preoperative parameters were aPTT, INR, antithrombin, haemoglobin, platelet count. D-dimer and fibrino-gen whereas postoperative parameters were haemoglobin, erythrocyte volume fraction, platelet count, and fibrinogen concentration. Our accredited university laboratory conducted all assays. Standard coagulation assays using a STA®® Evolution instrument (Diag-nostica Stago S.A.S, Asniéres Sur Seine, France) while hemoglobin, haematocrit and platelet counts were per-formed with Sysmex XE-2100® (Sysmex Corporation, Kobe, Japan). Arterial blood gases were analyzed on the Radiometer ABL 827 Flex® instrument (Radiom-eter Medical, Brønshøj, Denmark).

Data analyses and statistical considerations

Data distribution was assessed by histograms and Q-Q plots. ROTEM® and CAT data followed a Gaussian distribution. Data are illustrated as mean and standard deviation. Difference in results over time were analyzed by 1-way ANOVA. A p-value less than 0.05 was consi-dered statistical significant.

Based on previous studies and pilot experiments made in our research unit, we assumed that major cardiac sur-gery would induce a minimum of 40% change in the whole blood clotting parameters of clotting time and/or or maximum velocity of clot formation with variability of the results less than 25% (CV%). Hence, to test hy-pothesis a minimum sample size will be n=30 (assump-tions: Delta=40%, B=90%, A=0.05).

Results

ROTEM and CAT results are listed in table 2. The concentration of all coagulation factors (FI, FVII, FVIII, FIX, FX and protein C) except for FVIII decreased significantly by approximately 35% at the end of surgery (figure 3). The following hours FI, FIX, and FX slowly returned towards preoperative levels. FVIII increased more rapidly and was significantly higher than preoperative level at 48h. FXII concentra-tion exhibited significant reduction in concentration at two time points, 0h and 24 h (p=0.001) regaining near normal concentration in between at 6h. FIIIX was only slightly reduced postoperatively, however, continued to decrease until 6h (start 98% (±30) vs. 6 h 87% (±21) p=0.08). Later, a gradual improvement was observed.
The reduced MCF observed in the present study is expected to reflect the degree of dilutional coagulopathy seen during CPB. Extensive hemodilution during cardiac surgery, anesthe-
sta and CPB leads to dilutional coagulopathy re-
sulting in abnormal fibrin polymerisation and loss of fibrinogen [19, 20]. Interventional studies employing fi-
brinogen concentrate have shown a correction of the abnormal fibrin polymerisation together with reduced bleeding [19, 20]. An additional explanation could be loss of platelets, however, on day 1 the platelet count was on average 173x10^9/μL (±264) in our study. Furthermore, recent studies have demonstrated limited reduction in platelet MCF or impairment of platelet function after CPB, this is only present immediately after surgery [21, 22]. Hence, platelet dysfunction or loss does not seem to explain the reduced MCF.

Our observation of a profound reduction in thrombin generation from 2h to 6h is speculated to arise from an imbalance amongst pro- and anticoagulant factors. A comparable imbalance has been described in trauma patients due to activated protein C in response to hyperperfusion [23]. Following CPB with non-pulsatile flow as well as a lower mean blood pressure; one could imag-
ine some degree of hyperperfusion with the potential to reduce thrombin generation. However, lactate and pH values were normal post CPB and protein C levels were not excessively altered. Another plausible explanation for the reduced thrombin generation could be a heparin rebound effect, which has been described to occur up to six hours postoperatively [24, 25]. However, results of anti-Xa did not point to residual heparin effects.

The discordance between recovery of single coagulation factor levels and continued deterioration of ROTEM® and CAT suggest that other coagulation inhibiting mechanisms may be involved such as e.g. tissue factor pathway inhibitor (TFPI). In the absence of measurable heparin rebound, it may be hypothesized that endothe-
list stress result in delayed and temporary release of TFPI mostly pronounced around 6 hours postopera-
tively. Previous studies have demonstrated that TFPI increase during CPB, but then normalizes at the end of surgery [26]. Recently, Percy and colleagues showed in-
creased levels of TFPI after CPB, and improvement of thrombin generation after ex vivo inhibition of TFPI [27, 28].

Interestingly, the most common time point for surgic-
al reexploration is around 6 hours postoperatively [29, 30], which coincides with the time point at which pati-
ents display the most pronounced degree of derangement in overall coagulation. It may have important clinical consequences to return patients to surgery while their coagulation system is severely depressed and there-
by challenge the risk of additional blood loss and transfusion. The optimal treatment of post cardiac coagulopathy could be a thrombostatic approach with rational haemostatic interventions guided by point of care testing such as ROTEM® since the aetiology is multifactorial and therefore the treatment should also be so.

The prospective design strengthened the study and lim-
ited selection and information bias. The study group was homogenous and multiple serial observations dur-
ing the first postoperatively 48 hours were applied in order to demonstrate time dependent and dynamic changes over time. The coagulopathy was evaluated systematically using two complementary global assays – ROTEM® and CAT. In addition, serial measurements of single coagulation factors were carried out to elu-
cidate the relationship between the time dependent changes seen in the global assays.

External validity may be reduced as only elective car-
diac patients with little risk of bleeding were enrolled and additional studies could focus on excessively bleed-
ing patients. Our assays using CAT and ROTEM® were performed without the use of corn trypsin in-
hibitor (CTI), whereby contact activation may have affected our results. Importantly, before commencing this study, a laboratory model of post cardiac coagu-
lopasy was developed and the addition of CTI in as-
says was shown not to induce any change in clotting parameters [31]. In light of these findings we decided not to adopt CTI in characterisation of the actual haemo-
static condition.

The platelet count was measured in this study, but CPB may have impaired the function of platelets as previously described [32], but a large variability in platelet function tests after CPB has also been demonstrated [33]. However, the inclusion of platelet function tests would have been advantageous. The study did not include measurements of TFPI, nor levels of histones or polynucleotides that both recently have been described as a key player in regulation and pathologic activation of coagulation, respectively [34, 35].

### Discussion

This prospective observational study describes the “na-
tural history” of time dependent changes in global haemostasis assays during the first 48 hours following major cardiac surgery on CPB and highlights the inter-
val between 2-12 hours as the period with the highest impact on overall haemostatic performance. Our results show that immediately after surgery and within the first 2 hours, the coagulopathy is predominantly character-
ised by deterioration of the MCF. From 2 to 6 hours a profound drop in peak thrombin with a parallelled prolonged initiation and reduced propagation phase of whole blood clot formation. From 6 to 12 hours, a partial recovery from the coagulopathy was observed. Initiation of thromboprophylaxis with LMWH affected both CAT and ROTEM®, although the MCF was unaf-
fected and actually increased postoperatively.

The changes in the coagulation factor levels align with other reports [16, 17]. During the first couple of hours, the alterations in ROTEM® and CAT parameters were parallelled by reductions in single factor levels, fibrino-
gen, and prolonged thrombin time. However, at 6 hours postoperatively the coagulation factors, fibrinogen, and thrombin time had improved while ROTEM® and CAT parameters continued to deteriorate displaying the most pronounced impairment in the entire study period.
In conclusion, the study thoroughly demonstrates that the coagulopathy following major cardiac surgery is dynamically changing during the postoperative course. Maximal impairment is seen six hours after surgery corresponding to the most common time for surgical re-exploration. Additionally, this study elucidates the potential clinical importance of timely and rational haemostatic intervention in patients suffering from post-cardiac surgery bleeding.

## Results

### Study subjects

30 patients were initially included in this study. One patient was subsequently excluded due to complicated surgery followed by major bleeding and massive transfusion, data published as case report. Patient characteristics and surgical details are displayed in table 3. The results are listed in sections from each study and figures and tables are positioned at the end of each section.

| Female n(%) | 8 (27) |
| Age (mean/median) | 63.9 / 69 |
| Preoperative treatment aspirin n(%) | 19 (63) |
| Preoperative treatment clopidogrel n(%) | 2 (7) |
| Preoperative treatment warfarin n(%) | 5(17) |
| Euroscore (mean/median) | 4.05/2.87 |
| Ventricular function, ejection fraction >50% n(%) | 26 (87%) |
| Renal function; creatinine level > 130 mol/L n(%) | 0 (0%) |
| Chronic obstructive pulmonary disease n(%) | 3 (10%) |
| Atrial flutter/fibrillation n(%) | 6 (20%) |
| Hypercholesterolaemia n(%) | 21 (70%) |

### Types of surgery n(%):

- **CABG**: 12 (40)
- **AVR**: 7 (23)
- **CABG and AVR**: 6 (20)
- **Other**: 5 (17)

| Operation time, min (mean/median) | 177/145 |
| Cardiopulmonary bypass time, min (mean/median) | 92/73 |
| Cross-clamp time, min (mean/median) | 54/49 |
| Cardioplegia amount ml (mean/median) | 1474/1400 |
| Perioperative crystalloids, incl. priming ml (mean/median) | 2622/2650 |
| Balance postoperative, ml (mean/median) | 1103/2650 |
| pH after reperfusion (mean/median) | 7.39/7.39 |
| Temperature at end of surgery, °C (mean/median) | 36.4/36.7 |

### Bleeding perioperative, ml (median and range)

- 400 (100-1500)

### Bleeding postoperative, ml (median and range)

- 425 (125-1850)

### Bleeding in total, ml (mean and range)

- 843 (205-2050)

### Colloids 24 h after surgery, ml (median and range)

- 1000 (0-1500)

### All transfusion number of patients (n(%) )

- 7 (24%)

- **Patient 1 (reexploration): 2 RBC**
- **Patient 2 (reexploration): 2 RBC, 1 FFP, 1 PLT**
- **Patient 3 (reexploration, surgical cause): 2 RBC, 3 FFP, 2 PLT**
- **Patient 4: 1 PLT**
- **Patient 5: 1 PLT**
- **Patient 6: 1 RBC**
- **Patient 7: 2 RBC, 1 PLT**
FIGURE 1: THROMBOELASTOMETRIC PARAMETERS

- Changes from start of surgery until 48 hours postoperatively. Initiation of thromboprophylaxis is also indicated. Panel A: Clotting time (CT). Panel B: Maximum velocity (MaxVel). Panel C: Time to maximum velocity (tMaxVel). Panel D: Maximum Clot firmness (MCF).

FIGURE 2: THROMBIN GENERATION PARAMETERS


FIGURE 3: CHANGES IN ACTIVITY OF COAGULATION FACTORS AND THROMBIN TIME DURING THE 48-HOUR OBSERVATION PERIOD

- Changes in percentage [%] of thrombin time for different factors over time.
Authorship

Mariann Tang: Contribution to design of the study in addition to acquisition, analysis and interpretation of data for the work. Drafting and revising of the manuscript. Accountable for all aspects of the work in ensuring that questions related to accuracy or integrity of any part of the work are appropriately investigated and resolved.

Christian Fenger-Eriksen: Contribution to study design and critical revision of manuscript. Final approval.

Jørgen Ingerslev: Contribution to study design and critical revision of manuscript. Final approval.

Vibeke Hjortdal: Contribution to study design and critical revision of manuscript. Final approval.

Benny Sørensen: Contribution to design of the study and interpretation of data for the work. Drafting and revising of the manuscript. Final approval.

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References


Abstract

Background Cardiac surgery may cause a serious coagulopathy leading to increased risk of bleeding and transfusion demands. Allogeneic blood products are commonly first line haemostatic intervention, but has been associated with hazardous side effect. Coagulation factor concentrates may be a more efficient, predictable, and safe treatment. This study investigated the haemostatic potential of ex vivo supplementation of coagulation factor concentrates versus allogeneic blood products in patients undergoing cardiac surgery.

Methods 30 adults were prospectively enrolled (mean age=63.9, females=27%). Ex vivo haemostatic interventions (monotherapy or combinations) were performed in whole blood taken immediately after surgery and two hours postoperatively. Fresh-frozen plasma, platelets, cryoprecipitate, fibrinogen concentrate, prothrombin complex concentrate (PCC), and recombinant FVIIIa (rFVIIa) were investigated. The haemostatic effect was evaluated using whole blood thromboelastometry parameters, as well as by thrombin generation.

Results Immediately after surgery the compromised maximum clot firmness was corrected by monotherapy with fibrinogen or platelets or combination therapy with fibrinogen. At two hours postoperatively the coagulation profile was further deranged as illustrated by a prolonged clotting time, a reduced maximum velocity and further diminished maximum clot firmness. The thrombin lagtime was progressively prolonged and both peak thrombin and endogenous thrombin potential were compromised. No monotherapy effectively corrected all haemostatic abnormalities. The most effective combinations were: fibrinogen+rFVIIa or fibrinogen+PCC. Allogeneic blood products were unable to fully correct the coagulopathy.

Conclusion Coagulation factor concentrates appear to provide a more optimal haemostasis profile following cardiac surgery compared to allogeneic blood products.

Key words Cardiac surgery, coagulopathy, coagulation factor concentrate, blood transfusion.
Introduction

Bleeding problems occurring during cardiac surgery are associated with increased morbidity and mortality[1,2]. Development of post cardiac coagulopathy is multifactorial, and haemostasis management is challenging. Current guidelines for management of bleeding recommend transfusion with human allogeneic blood products such as red blood cell concentrates, fresh frozen plasma, or platelets[3,4]. Unfortunately, transfusion of allogeneic blood products is associated with serious side effects, such as increased mortality[5,6], nosocomial infections, multi organ failure, transfusion related acute lung injury and allergic or even anaphylactic reactions[7,8].

Cardiac surgery using extra corporeal circulation (ECC) induces a complex coagulopathy affecting several components of the coagulation system. Platelet counts and functions are compromised and coagulation factors and natural anticoagulants are diminished due to haemodilution, loss, and consumption[9-11]. Fibrin polymerization is more impaired than total thrombin generation immediately after ECC[12] but subsequently, thrombin generation deteriorates significantly. Approximately 12 hours post-operatively the recorded abnormalities reverse to baseline[13]. Finally, acidosics, hypothermia and hyperfibrinolysis impair the function of the coagulation proteme and irreversibly reduce levels of fibrinogen and platelets[10,14].

In addition to crude blood bank products, haemostatic remedies are available, including antifibrinolics (tranexamic acid or aprotinin), fibrinogen (FIB), prothrombin complex concentrate (PCC) and recombinant factor VIIa (rFVIIa). A clinical trial of rFVIIa in cardiac surgery demonstrated a reduction in bleeding, transfusion requirement and need for re-exploration, however with an increased risk of thromboembolic complications[15]. So far, the potential efficacy and safety of PCC during cardiac surgery has not been investigated, however an emerging number of case reports and retrospective studies has been published[16-18]. FIB has been shown to correct dilutional coagulopathy[19]. Retroactive studies and proof of principle clinical trials argue that FIB may be efficacious as first line treatment in management of critical bleeding during cardiac surgery[20].

It is a major concern that most allogeneic blood products have not undergone mechanistic or experimental test of their haemostatic capacity and controlled prospective clinical trials are very sparse. It may be argued that both fresh frozen plasma and cryoprecipitate reveal limited and unpredictable haemostatic effect[21-23], and that the effect of platelets is highly dependent upon storage time of the product[24].

This present study investigated the haemostatic capacity of allogeneic blood products as opposed to a panel of coagulation factor concentrates following cardiac surgery through ex vivo spiking experiments followed by global coagulation measurements using whole blood thromboelastometry profiles as well as thrombin generation characteristics. The following haemostatic agents were investigated: Fresh frozen plasma (FFP), platelets (PLT), cryoprecipitate (cryo), FIB, PCC and rFVIIa. We also addressed a rational timing of haemostatic interventions in relationship to the risk of development of post cardiac coagulopathy. We challenged the hypothesis that coagulation factor concentrates provide a significantly more potent haemostatic effect compared to FFP, cryo, or PLT following cardiac surgery.

Materials and methods

Blood samples were collected at three different time points: i) baseline prior to surgery, ii) immediately after reversal of heparin (t=0h) and iii) two hours postoperatively (t=2h). Ex vivo interventions were performed at time points t=0h and t=2h.

Patients

Patients were recruited from the Department of Cardiothoracic and Vascular surgery, Aarhus University Hospital, Skejby, Denmark. All patients were adults (18+ years) and scheduled for elective surgery. Patients who had discontinued platelet inhibitors five days prior to surgery, while any other anticoagulation treatment was withheld two days prior to surgery. Low molecular weight heparin could be continued until the day before surgery. Exclusion criteria were: previous heart surgery, hypothermia (<32°C), known congenital bleeding disorders, endocarditis or anemia (defined as hemoglobin below 7.0 mmol/L). Patients with an abnormal preoperative coagulation screen were not enrolled.

The study was approved by the Danish Biomedical Ethics Committee (#M-2009-0078) and all patients provided informed consent. Patient demographics and perioroperative characteristics are listed in table 2.

Blood sampling

Preoperative blood samples were obtained from an antecubital vein, using a 21G butterfly needle, 2.5% citrate tubes (Venosafe®, 3.4 ml, Terumo, Hatagaya, Japan) using minimal stasis. All other blood specimens were withdrawn from a central venous line (BD Medical System, Becton Dickinson Critical Care System Ltd., Singapore) using the distal leg (all infusions were stopped and 10 ml of blood was discarded before the sample for analysis was taken).

After weaning off the ECC, blood samples were collected five minutes after heparin neutralisation by protamine and at the same time point an APTT measurement was done (Haemochron Junior®, ITC Medical, Edmon, NJ).

Ex Vivo haemostatic interventions

The ex vivo haemostatic interventions were performed at two time points (t=0h and t=2h). The interventions were:

- » Monotherapy: FFP, PLT, cryo, FIB, PCC and rFVIIa
- » Combination therapy: FFP+PLT, FFP+cryo, cryo, PCC+cryo, PCC+FIB, PCC+PLT, FIB+PLT and FIB+ rFVIIa, double concentration PCC+FIB, double concentration FIB+FVIIa

Aliquots of 2000 μl whole blood were transferred to Eppendorf tubes and subsequently spiked with different haemostatic interventions.

Plasma and cryoprecipitate were obtained unfrozen from the local blood bank and stored at -80 degrees and thawed at 37°C for 30 minutes prior to use. We randomly used plasma from two different donors while cryoprecipitate was produced from 2 pools. Freshly collected platelets were provided daily from the blood bank. Coagulation factor concentrates were purchased from respective manufacturers: Fibrinogen concentrate (Haemoconjunct®) and PCC (Beriplex®) from CSL.

Example of calculation: FFP would normally be given 2 bags of 300 ml, which would dilute the patient 5250/600=8.75 in dilution factor. Interventions were added in 2000 μL whole blood; 2000 μL/8.75=228.6 μL was added as intervention.

Table 1. The haemostatic interventions. Dosage calculated after clinical recommendations.

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Dosage in 70 kg person, blood volume 5.25L</th>
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<tbody>
<tr>
<td>Fresh frozen plasma</td>
<td>2 x 300 ml – dilution factor 8.75</td>
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<tr>
<td>Platelets</td>
<td>1 x 300 ml – dilution factor 17.5</td>
</tr>
<tr>
<td>Cryoprecipitate</td>
<td>1 x 100 ml – dilution factor 52.5</td>
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<tr>
<td>Fibrinogen Concentrate</td>
<td>2 g – 30 mg/kg – 2.65 mg/ml – 3 mg/ml</td>
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<tr>
<td>Prothrombin complex concentrate</td>
<td>25-40 U/kg – 0.5 U/ml</td>
</tr>
<tr>
<td>Recombinant activated FVII</td>
<td>50 μg/kg – 0.70 μg/ml</td>
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Behring, Marburg, Germany, and rFVIIa (NovoSeven®) from Novo Nordisk A/S (Bagsværd, Denmark). All were reconstituted as recommended by the manufacturer and stored in small aliquots saved at -80 degrees until use. The dosages were according to package inserts while the dosage regime for allogeneic blood products was calculated according to department guidelines and daily practice reflecting the recommendations by the Society of Thoracic Surgeons and The Society of Cardiovascular Anesthesiologists(3,4). All medications and frozen blood products were thawed 30 minutes prior to use. Details on dosages are summarized in table 1.

Anesthesia

All patients were premedicated with diazepam 5-10 mg and a slow release formulation of aortamethadone 60-90 minutes prior to surgery. Standard monitoring was ECG, arterial line, pulse oximetry, capnography and temperature measurement, cardiac index, mixed venous saturation and transesophageal echocardiography. General anesthesia was induced with propofol, sufentanil and rocuronium; maintained with continuous infusion of propofol 20-80 µg/kg/min as well as bolus administration of sufentanil to a total of 3-5 µg/kg.

Medications

Unfractionated heparin (Heparin LEOW®, Leo Pharma Nordic, Malmö, Sweden) was dosed using an initial bolus of 300 IU/kg followed by additional heparin to keep the target ACT above 400 sec. Heparin was reversed with protamine sulphate (Protamine Sulphate, 1 mg of protamine sulphate per 100 IU of the initial heparin dose. The mean dose of heparin was 35300 IU (range 20000-58000) whereas mean protamine dose was 288 mg (range 200-400). The aPTT at the beginning of surgery had a mean value of 34.3 sec (range 21-43 sec) and at the end of the surgery the mean aPTT was 33.4 sec (range 28-38 sec).

Antifibrinolytics were routinely administered to all patients; tranexamic acid (Cyklokapron®, Meda AS, Allerød, Denmark), 2 g when starting surgery, 1 g before ECC and 1 g after protamine.

After cross clamping of the aorta cardiac arrest was induced with cold crystalloidal St Thomas solution (Kardiolipin®, HS Apotec, Copenhagen, Denmark). The median dose of cardioplegia was 1400 ml.

Bleeding, transfusion and reexploration

Bleeding was evaluated both as postoperative bleeding and total blood loss, which is defined as bleeding intraoperatorically plus postoperatively. Bleeding was evaluated hourly for the first 12 hours postoperatively (ml/hour). Chest tubes were removed no earlier than 12 hours postoperatively and when production was less than 50 ml/hour in minimum two hours. Information on reexploration for bleeding as well as data on transfusion and coagulation factor treatment was collected.

Coagulation analyses

Whole blood thromboelastometry

Whole blood clot formation was recorded applying a previously validated highly sensitive method using RoTEM® thromboelastometry (TEM International, Munich, Germany) following activation with minute amounts of tissue factor as previously described [25]. RoTEM plastic cups were prewarmed at 37°C. Whole blood rested for 30 minutes prior to analysis. A volume of 300 µl whole blood was loaded to each cup followed by addition of 20 µl of buffer (20 mM HEPES, 150 mM NaCl, pH 7.4). The coagulation process was activated with tissue factor (Innovin®, Dade Behring, Marburg, Germany - final dilution of 1:17000) and started by re-calification with 200 mM CaCl2. All tests were run in duplicate and recording proceeded for at least 45 minutes.

The following thromboelastometry parameters were evaluated: initiation phase: clotting time (CT [sec]), propagation phase: clot formation time (CFT [sec]), maximum velocity (MaxVel [mm/100 s]), time until maximum velocity (t,MaxVel[sec]), and maximum clot firmness (MCF [mm]).

Thrombin generation in platelet poor plasma

Thrombin generation was performed as described by Hemker et al [26] employing reagents and software from Thrombinoscope BV (Maastricht, Netherlands) on a Fluroskan device (Thermo Electron Corp., Copenhagen, Denmark). Blood samples were centrifuged twice at 2000G for 20 minutes to obtain platelet poor plasma (PPP). Aliquots were kept at -80°C until bulk analyses. Prior to testing all samples were centrifuged 3 minutes at 12000G. The assay was performed in a 96-well microplate (Immulon 2HR, round bottom, Jencons, VWR International Ltd., Sussex, UK). A total of 80 µl of platelet poor plasma was spiked with 20 µl of PPP reagent 5 µM (lot PFP0812/01). The plate was placed in the fluorometer and prewarmed to 37°C followed by automatic addition of 20 µl thrombin substrate (Fluo-Substrate, Fluka-kit, lot FC1004/01). All samples were run as double determinations with a parallel thrombin calibrator (activity 580mU, lot TC1005/01). Thrombin generation parameters included: i) lagtime (min), ii) endogenous thrombin potential (ETP), iii) peak thrombin level (nM), and iv) time to peak thrombin level (min).

Pre- and postoperative coagulation screen

Preoperative parameters were aPTT, INR, pp-relative, antithrombin, haemoglobin, platelet count, dimer and fibrinogen whereas postoperative parameters were haemoglobin, erythrocyte volume fraction (EVF), platelet count and fibrinogen concentration. Our accredited university haemostasis laboratory conducted all assays.

TABLE 2: DEMOGRAPHICS AND PERIOPERATIVE DATA

<table>
<thead>
<tr>
<th></th>
<th>Female (n%)</th>
<th>Age (mean (SD))</th>
<th>Preoperative treatment aspirin (n%)</th>
<th>Preoperative treatment clopidogrel (n%)</th>
<th>Preoperative treatment warfarin (n%)</th>
<th>Euroscore (mean (SD))</th>
<th>Ventricular function, ejection fraction &gt;50% (n%)</th>
<th>Renal insufficiency creatinine level &gt;130 mmol/L (n%)</th>
<th>Chronic obstructive pulmonary disease (n%)</th>
<th>Abial flutter/ fibrillation (n%)</th>
<th>Hypercholesterolemia (n%)</th>
<th>Surgical procedures:</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>8 (27)</td>
<td>63.8 (±14)</td>
<td>19 (63)</td>
<td>2 (7)</td>
<td>5 (17)</td>
<td>4.1 (±3.8)</td>
<td>26 (87%)</td>
<td>0 (5%)</td>
<td>3 (10%)</td>
<td>6 (20%)</td>
<td>21 (70%)</td>
<td>12 (40)</td>
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|                         |            |                |                                   |                                        |                                     |                       |                                               |                                          |                                   |                                      |                                      | 7 (23)
|                         |            |                |                                   |                                        |                                     |                       |                                               |                                          |                                   |                                      |                                      | 6 (20)
|                         |            |                |                                   |                                        |                                     |                       |                                               |                                          |                                   |                                      |                                      | 5 (17)
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Preoperative parameters were aPTT, INR, pp-relative, antithrombin, haemoglobin, platelet count, dimer and fibrinogen whereas postoperative parameters were haemoglobin, erythrocyte volume fraction (EVF), platelet count and fibrinogen concentration. Our accredited university haemostasis laboratory conducted all assays.
Data analyses and statistical considerations
Data distribution was assessed using histograms and Q-Q plots. The descriptive data on thromboelastometry and thrombin generation followed a Gaussian distribution. Some of the intervention experiments did not follow a Gaussian distribution. All data are illustrated giving mean and standard error. Differences over time and following ex vivo spiking experiments were tested by students paired t test if data was normally distributed and by Mann-Whitney U test if not. A P-value less than 0.05 was considered statistically significant.

Results
A series of ex vivo haemostatic interventions were performed at the end of surgery (0h) and two hours postoperatively (2h). For simplicity, only the interventions done at time point 2h are depicted in the figures. All the results, including the ex vivo interventions done on blood samples taken at the end of surgery (0h) are listed in tables 3 and 4.

Clot initiation
The mean CT increased postoperatively by 12% and was further prolonged by 31% at two hours postoperatively compared to baseline.

Ex vivo monotherapy with PCC or rFVIIa revealed the most pronounced shortening in the CT while FIB caused a prolonging effect on the CT. PLT or FFP addition also significantly shortened the CT, while cryoprecipitate only induced small and insignificant changes (figure 1, panel A).

Combined substitutions using PLT (FFP+PLT, PCC+PLT, FIB+PLT) all significantly reduced the CT to close to preoperative levels. The combination of FIB+rFVIIa demonstrated the most pronounced reduction of the CT, even reaching values below the preoperative levels (figure 2, panel A). Increasing the concentrations of FIB+rFVIIa 2-fold did not further reduce the CT.

TABLE 3: CHANGES IN THROMBOELASTOMETRIC PARAMETERS AND THROMBIN GENERATION END SUGERY (TIME POINT 0H). EFFECT OF HAEMOSTATIC INTERVENTIONS, BOTH MONOTHERAPY AND COMBINATION THERAPY AT THIS TIME POINT

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Significance</th>
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<tbody>
<tr>
<td>CT (s)</td>
<td>192</td>
<td>196</td>
</tr>
<tr>
<td>MCF (mm)</td>
<td>46.4</td>
<td>50.3</td>
</tr>
<tr>
<td>CT with Monotherapy</td>
<td>154</td>
<td>122</td>
</tr>
<tr>
<td>MCF with Monotherapy</td>
<td>45.8</td>
<td>50.3</td>
</tr>
<tr>
<td>CT with Combination Therapy</td>
<td>141</td>
<td>126</td>
</tr>
<tr>
<td>MCF with Combination Therapy</td>
<td>46.4</td>
<td>50.3</td>
</tr>
</tbody>
</table>

Clot propagation
The mean MaxVel, as well as mean tMaxVel was affected initially postoperatively. These changes aggravated further at 2h with increasing tMaxVel and reduced MaxVel. The MaxVel was significantly increased following either monotherapy with either PLT or FIB, however intervention with PCC, cryoprecipitate, cryo or FFP failed to induce significant changes (panel B in figure 1+2). All combination therapies demonstrated an ability to correct the MaxVel to preoperative levels. The combination of FIB+PLT increased the MaxVel significantly above preoperative levels.

Likewise the CFT was prolonged by 20% after surgery and being significantly affected at 2h (70% prolonged compared to baseline). At time point 0h all interventions shortened the CFT, in some cases reaching levels significantly below baseline (table 3). Following the more pronounced impairment at 2h monotherapy was less efficient. All combinations did significantly improve the CFT at 2h, the most efficient being any combination with PLT as well as FIB+rFVIIa. Of note, FIB+PLT did reduce the CFT significantly to levels below preoperative values.

Clot firmness
Immediately after ECC, a significant drop in whole blood MCF was observed (figure 1, panel C). The mean preoperative MCF value was 55.4 mm (±7.2), which was reduced to 50.3 mm (±8.3) at 0h (p<0.016). Further deterioration was seen at 2 hours postoperative where the mean MCF was 46.4 mm (±7.6) (p<0.001).

Monotherapy with FIB at 0 h did significantly increase the MCF, in fact above preoperative level (65.8 mm (±5.2)). Intervention with PLT normalised the MCF (54.8 mm (±36.5)), while PCC increased the MCF to 51.5 mm (±7.4). Neither rFVIIa, FFP, nor cryoprecipitate caused any significant improvement in single component interventions.
Combined interventions with PLT (FFP+PLT, PCC+PLT) or FIB (FIB+fRVIIa, PCC+FIB) restored MCF to preoperative levels or greater; the most potent intervention being the combination of FIB+PLT. Likewise, the combination of PCC+cryo improved the paired clot firmness. Only the combination of FFP+cryo did not increase the MCF significantly at 8h postoperatively.

At 2hrs, fibrinogen concentrate was the only monotherapy amongst interventions tested that normalized the MCF while PLT significantly improved the MCF but failed to raise the MCF to the preoperative level (figure 1, panel C).

The combinations with FIB (FIB+PLT, FIB+fRVIIa, FIB+PCC) still showed the potential of restoring MCF, in any case to levels above the preoperative value. The combinations containing PLT (PLT+PCC, PLT+FFP) did not normalize MCF, although they significantly improved the MCF (figure 2, panel C). At both time points double concentration of FIB+PCC (99.1 mm (±55.9)) or double concentration of FIB+fRVIIa (72.7 mm (±4.7)) did increase MCF significantly to levels greater than the preoperative levels.

Lagtime thrombin
The lagtime of thrombin generation displayed minor, but significant, changes immediately post-operatively. The mean preoperative lagtime was 3.76 min (±0.92), which prolonged to 4.31 min (±1.27) (p=0.045) at 0 hrs and further increased to 6.24 min (±4.07) at 2 hrs postoperatively (figure 2, Panel A). Monotherapy with fRVIIa successfully restored the lagtime to preoperative levels both recorded at 0 hrs and at 2 hrs. Both FFP, PLT and PCC reduced the lagtime significantly at 2 hrs, although values failed to reach the corresponding preoperative values (figure 4, panel A). Combinations of FIB+fRVIIa, FFP+PLT, and FFP+cryo significantly decreased the lagtime at 8h and at two hours the same combinations restored the lagtime to preoperative levels (panel A in figure 3 and figure 4).

Peak thrombin
Peak thrombin demonstrated a minor decrease initially after ECC from preoperative mean 233 nM (±85) to 212 nM (±58). At 2 hrs, the peak thrombin was reduced to 127 nM (±97) (p=0.001). PCC was the only successful monotherapy enabling restoration of peak thrombin at this timepoint. fRVIIa caused significant improvements in peak thrombin, but failed to normalize it (figure 3, panel B). Combinations containing PCC (PCC+Cryo, PCC+PLT, PCC+FIB) significantly increased peak thrombin to preoperative levels, and PCC+cryo beyond the preoperative level. All other combination therapies only induced minor increments in peak thrombin (figure 4, panel B).

Endogenous thrombin potential
The reduced ETP at the end of surgery (1180 nM (±465)) was restored to the initial value (1383 nM (±385)) or greater with either PLT or PCC. The combination of FIB+fRVIIa normalized the ETP, while combinations including PCC restored ETP at or above preoperative level.

Two hours postoperatively the ETP was even more deranged (944 nM (±589)) and PCC was still the only monotherapy that normalized the ETP (figure 3, panel C). Combinations containing PCC (PCC+PLT, PCC+Cryo, PCC+PLT, PCC+FIB) were superior to the allogeneic combinations (FFP+PLT, FFP+Cryo), while the combination of FIB+fRVIIa also restored ETP (figure 4, panel C).

Postoperative bleeding
The total blood loss registered until 18 hours after the end of operation was mean 991 ml (range 250-2050 ml) and the postoperative blood loss was 648 ml (range 125-1850). One patient received transfusions and coagulation factor concentrates prior to surgery and was therefore excluded. In total, eight patients (27%) received transfusion during the first 48 hours. Three patients (10%) had re-exploration for bleeding and surgical bleeding was found to be the cause in one of these patients.

Discussion
This is the first study to systematically investigate the haemostatic effect of CFC and allogeneic blood products following cardiac surgery. Data show that CFC or combinations thereof are equally to, or more potent than allogeneic blood products in correcting post cardiac surgery coagulopathy. Immediately after ECC the abnormal MCF could be corrected by monotherapy intervention with i) FIB or ii) PLT. Combinations of i) FIB+PLT, ii) FIB+fRVIIa, or iii) FIB+PCC also

**FIGURE 1:** THROMBOELASTOMETRIC CHANGES AFTER CARDIAC SURGERY AND THE EFFECT OF MONOTHERAPY WITH ALLOGENEIC BLOOD PRODUCTS OR COAGULATION FACTOR CONCENTRATES

**FIGURE 2:** THROMBOELASTOMETRIC CHANGES AFTER CARDIAC SURGERY AND THE EFFECT OF COMBINATION THERAPY WITH ALLOGENEIC BLOOD PRODUCTS AND COAGULATION FACTOR CONCENTRATES
Haemostatic interventions were: FFP-Fresh frozen plasma, postoperative (2h). Interventions were performed at time point prior to surgery (start), at the end of surgery (0h) and two hours after surgery. Panel A: Lagtime thrombin; Panel B: Peak thrombin; Panel C: Endogenous thrombin potential.

FIGURE 3: CHANGES AFTER CARDIAC SURGERY IN THROMBIN GENERATION AND THE EFFECT OF COMBINATION THERAPY WITH ALLOGENIC BLOOD PRODUCTS OR COAGULATION FACTOR CONCENTRATES.

FIGURE 4: CHANGES AFTER CARDIAC SURGERY IN THROMBIN GENERATION AND THE EFFECT OF COMBINATION THERAPY WITH ALLOGENIC BLOOD PRODUCTS AND COAGULATION FACTOR CONCENTRATES.

A common clinical practice is the use of combinations of FFP and PLT. However, in the present study FFP+PLT substitution resulted in a surprisingly modest haemostatic effect. Moreover, cryo or FFP+Cryo displayed limited effect as compared to FIB. A previously published case report confirmed this in the clinical setting[34]. The limited effect may be due to the addition of considerably amounts of diluents as part of the production of blood components. In consequence, the allogeneic blood components are diluted and adding them to blood will cause further dilution[35]. Furthermore, following substitution, the plasma level of coagulation factors in the recipient will only approximate the levels present in FFP and cryo, thus any additional “pharmacological” effect of higher levels of coagulation factors or fibrinogen should not be expected. In contrast, using a concentrated form of lyophilised coagulation factors such as e.g. PCC, FIB or rFVIIa will induce predictable increments in plasma levels of coagulation factors as well as potentially by-passing pharmacological effects. Eventually, the PLT used in the study were freshly collected by the blood bank and had not been stored prior to use. This procedure was done to ensure optimally working platelets since previous studies have indicated considerable time dependent impairment of the function of stored platelets[24].

It should be emphasized that in some cases the haemostatic intervention lead to an over-correction of the coagulopathy as compared to the preoperative parameters. This was particularly noticeable for FIB in correction of MCF, both when used as monotherapy as well as in combination with PCC, rFVIIa and PLT. The potential danger of high levels of fibrinogen and MCF is questionable. Fibrinogen is an acute-phase reactant and post surgery inflammation may raise fibrinogen levels multi fold and even exceed baseline levels[19]. Furthermore, mechanistic studies suggest that high levels of fibrinogen may exert an inhibitory effect on thrombin generation[36]. Finally, although one retrospective study has raised concerns about higher incidences of stroke and acute renal injury in patients treated with fibrinogen[37], while prospective randomized trials aiming to increase MCF to the upper end of the reference range did not show an increased risk of thromboembolism[38,39].

Exaggerated coagulation leading to graft occlusion is a feared complication following cardiac surgery. However, a study investigating pre-operative MCF levels and graft patency did not demonstrate any association between elevated MCF and graft occlusion[40].

In some cases the changes in coagulation profile was more complex and composed of prolonged whole blood clot initiation, reduced clot propagation and further diminishment of clot firmness. In addition, the lagtime of thrombin generation was further prolonged and both peak thrombin and ETP were compromised suggesting that thrombin generation propagation is not compromised at 0h, however certainly at 2h. It may be postulated that the declining haemostatic capacity is due to loss/consumption of procoagulants, heparin rebound effect, dilution and excessive tissue factor pathway inhibitor[27-29]. No monotherapy effectively corrected all haemostatic abnormalities. The most efficacious combinations were: i) FIB+rFVIIa or ii) FIB+PCC. No combination including allogeneic blood products fully corrected the coagulopathy at 2 hrs. Previous studies have shown that loss of fibrin polymerisation and fibrinogen deficiency is the first abnormality developing following cardiac surgery and ECC and is linked with consumption, loss, excessive fibrinolysis and pronounced dilution[9]. The present data suggest that the combined derangement of clot firmness and thrombin generation require multiple interventions targeting both fibrin formation and thrombin generation. Our finding of insufficient thrombin generation is supported by previously published laboratory data[30] and efficacy of rFVIIa in post cardiac coagulopathy[15]. The abnormal MCF is likely a result of dilution, reduced platelet counts, fibrinolysis, or low levels of FXIII. In the present study, we did not investigate the influence of FXIII as a haemostatic agent. A recently published clinical trial did not report any differences in blood loss and transfusion requirements in patients undergoing cardiothoracic surgery randomised to FXIII versus placebo, although FXIII did improve MCF values[31]. The effect of PLT illustrates that substitution could be effective; however the effect was not as noticeable as with fibrinogen. This observation aligns with two randomised placebo controlled clinical trials concluding less bleeding in patients treated with fibrinogen concentrate[32,33]. Fibrinolysis is not expected to be an important factor in this study considering the concomitant antifibrinolytic therapy that all patients received, although it could be speculated that dosing of tranexamic acid might have been inadequate in some cases.

A very common clinical practice is the use of combinations of FFP and PLT. However, in the present study
The strengths of the study include the prospective study design of a homogenous group of patients followed meticulously over time. Comprehensive and systematic laboratory studies of single component as well as combination therapy were performed. The study has some limitations. Firstly, the majorities of patients were non-bleeding and did not require excessive transfusion. “Physiologic” coagulopathy developing in a majority of patients and the ex vivo spiking experiments reflect the capacity of allogeneic blood products and CFC to correct underlying haemostatic abnormalities. However, the lack of a sub-group of excessively bleeding patients makes it more difficult to address the effect of allogeneic versus CFC in such clinical scenarios. It may be argued that if an intervention is not capable of reversing an underlying coagulopathy it appears unlikely to show notable effect in more severe coagulopathic situations. Secondly, previous studies have argued that artificial contact activation may mask the haemostatic effect or fail to disclose the true phenotype of the underlying coagulopathy when using thromboelastometry or thrombin generation assays.[41]. In our study we decided not to adopt inhibition of the contact pathway because we wished to maintain some illustration of the contact activation that is expected to occur from the mechanical stress induced by ECC. Furthermore, experimental model data showed no effect of standard 18.3ug/ml of corn tryptic inhibitor known to inhibit contact activation[42]. Finally, a limitation of our study is the lack of platelet function evaluation, which was not included for logistic reasons. Additionally, laboratory data have a high internal validity, but it can be difficult to extrapolate data to clinical work. In spite of this ex vivo studies represent initial investigations of potential treatment options prior to setting up larger clinical trials.

In conclusion, this study shows that CFC mono- or combination therapy can correct the time dependent, complex and multifactorial coagulopathy following cardiac surgery. The CFCs were more potent than any of the allogeneic blood products. The data support the use of early first line intervention with FIB in case of bleeding immediately after ECC. In case of later occurring bleeding, the results suggest the need for combinations of FIB with PCC or rFVIIa. Based on these ex vivo experiments we envision that haemostatic management of postoperative bleeding can be accomplished with reduced or no use of blood bank products and strongly encourage clinical trials challenging allogeneic blood products with CFCs.

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Reference list


Temporal changes in clot lysis and clot stability following tranexamic acid in cardiac surgery

Running head: Tranexamic acid and clot lysis in cardiac surgery

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Abstract

Introduction

Cardiac surgery induces a multifactorial coagulopathy. Regular use of tranexamic acid (TXA) is becoming standard of care. Clinical challenges include selecting optimal dosing regimen and balancing the benefit versus risk of additional dosing with antifibrinolytics. The objective was to evaluate the effect of TXA by assessing kinetic properties of plasma clot formation, clot stability and clot fibrinolysis.

Methods

Prospective case follow-up of 28 patients undergoing cardiac surgery (mean age 63.9 years, 29% female). Blood samples were analysed at 7 time points during the first 48 hours after surgery. All patients were treated with TXA: 2 g at start surgery, 1 g during extra corporeal circulation, and 1 g after reversal of heparin. An automated clot lysis assay using tissue factor and tissue plasminogen activator (tPA) was performed to evaluate clot formation, stability and fibrinolysis.

Results

TXA protects against facilitated fibrinolysis and inducible plasminogen activator (tPA) was performed to evaluate clot formation, stability and fibrinolysis.

Introduction

Bleeding after cardiac surgery is a frequent phenomenon with need of surgical reexploration in approximately 2.6% of patients. It is enhanced by dysfunction of haemostasis in up to 35% of cases.[1–3]. The presence of bleeding complications and the need for surgical reoperation are associated with increased morbidity and mortality.[4,5] Cardiac surgery and extra corporeal circulation (ECC) induce a multifactorial coagulopathy with distinct laboratory changes during the first 12 hours postoperatively.[6]

The surgical trauma and in particular the use of ECC trigger a series of profibrinolytic processes. Exposure to the artificial surfaces in the ECC circuit leads to the secretion of bradykinin, activation of coagulation factor XII, and increased release of endothelial tissue plasminogen activator (tPA). These events promote the conversion of plasminogen to plasmin and thereby enhancement of fibrinolysis. In consequence, levels of soluble fibrin rise from 1% to 35% during ECC and PAI-1 and D-dimer are elevated.[7,8] Heparinization also facilitates fibrinolysis.[9] In addition, factors taking part in clot stabilization such as levels of FXIII and fibrinogen are compromised beyond haemodilution after ECC.[10]

Clinical trials support systematic use of tranexamic acid (TXA) or aprotinin during cardiac surgery and meta analyses show that antifibrinolytics reduce the need for blood transfusion.[1,12]. However, antifibrinolitics may be associated with serious adverse events such as seizures (TXA).[13] renal failure and concern of possible increased mortality (aprotinin) [14]. An unmet need in use of antifibrinolitics is selecting the optimal dosing regimen and balancing the benefit versus risk of additional dosing with antifibrinolitics in a bleeding patient.[15,16]. At present, TXA can be measured in plasma using advanced mass spectrometry assays and studies have shown that the half-life of TXA is around 2 hours. However, a major difficulty in evaluating pharmacodynamic effects of TXA is the lack of established laboratory methods for measuring fibrinolysis. Biomarkers such as fibrin degradation products[17–19], tissue plasminogen activator, plasminogen activator inhibitor, antiplasmin and plasminogen fail to illustrate the functional and dynamic aspects of fibrinolysis and are not useful for monitoring TXA. The euglobulin lysis time or area clot lysis test are functional tests that measure overall fibrinolysis by mixing citrated platelet-poor plasma with acid or urea in a glass test tube followed by re-cal-cification and recording of clot formation and clot lysis with 10 min interval.[20] These tests may be used to evaluate functions of TXA, but they are cumbersome and rarely done in routine laboratories. During recent years fibrinolysis and clot stability have been evaluated by whole blood thromboelastometry or by performing an automated plasma clot lysis time using a spectro-photometry microplate reader.[21–23].

The present study aimed to investigate the post-operative effect of perioperative use of TXA as quantified by an automated assay suitable for assessing the kinetic of plasma clot formation, clot stability and dynamic evaluation of fibrinolysis. Moreover, since TXA is eliminated via the kidney we explored the effect of renal function upon the pharmacodynamic effects of TXA in cardiac surgery. We hypothesized that i) TXA significantly protects against fibrinolysis and ii) the antifibrinolytic effect of TXA is influenced by kidney function.

Methods

Study design

Blood samples from 28 patients undergoing cardiac surgery and ECC were obtained over a 48-hour period, representing a prospective case follow up design (figure 1). Blood samples were drawn at these time points and labeled: Start after induction of anesthesia, prior to surgery; 0h=5–10 minutes after protamine administration; 2h=2 hours after end of surgery, and 6h, 12h, 24h and 48h being six hours, 12 hours, 24 hours and 48 hours after end surgery, respectively. Patient blood samples were used to evaluate multiple other hypotheses and results including ROTEM and thrombin generation will be published separately.

Patients and ethical considerations

Blood samples from 28 patients undergoing cardiac surgery and ECC were obtained over a 48-hour period, representing a prospective case follow up design (figure 1). Blood samples were drawn at these time points and labeled: Start after induction of anesthesia, prior to surgery; 0h=5–10 minutes after protamine administration; 2h=2 hours after end of surgery, and 6h, 12h, 24h and 48h being six hours, 12 hours, 24 hours and 48 hours after end surgery, respectively. Patient blood samples were used to evaluate multiple other hypotheses and results including ROTEM and thrombin generation will be published separately.

Patients were recruited at the Department of Cardiothoracic and Vascular surgery, Aarhus University Hospital, Skejby, Denmark. Only adults (18+ years) patients with a normal pre-operative coagulation screen who had discontinued platelet inhibitor and/or anti-coagulant therapy five days prior to their elective surgery were enrolled. Exclusion criteria were: previous heart surgery, hypothermia (<34°C), known congenital bleeding disorders, endocarditis or anemia. The Danish Biomedical Ethics Committee (RM-2009-0078) approved the protocol and all patients provided informed consent. Demographic and perioperative characteristics are listed in table 1.
Blood sampling
Baseline blood samples were obtained from a peripheral vein, using a 21G butterfly needle, 3.2% citrate tubes (VenoSafe®, 3.4 ml, Terumo, Hatagaya, Japan) and minimal stasis. All other blood samples were taken from a central venous line (BD Medical System, Becton Dickinson Critical Care System Ltd., Singapore) using the distal leg (all infusions were stopped and 10 mL of blood was discarded before the sample for analysis was aspirated). The first blood samples after ECC were obtained only after heparin neutralisation by protamine when a control aPTT value was comparable to the preoperative level. The blood sample was drawn 5-10 minutes after protamine administration; surgery was not complete at this time point.

Anaesthesia
Patients received diazepam and acetaminophen 60-90 minutes prior to surgery. Perioperative monitoring included: ECG, pulse oximetry, capnography, temperature, cardiac index, mixed venous saturation and transthoracic echocardiography. General anaesthesia was induced using propofol, suxamethonium and rocuronium.

Heparin and protamine
Unfractionated heparin (Leo Pharma Nordic, Malmo, Sweden) was dosed using an initial bolus of 300 IU/kg followed by additional heparin to keep the target ACT above 400 sec. Heparin was reversed with protamine sulphate (Leo Pharma Nordic, Malmo, Sweden) using 1 mg of protamine sulphate per 100 IU of the initial heparin dose. The mean dose of heparin was 35,300 IU (range 20,000-58,000) and the mean protamine dose was 288 mg (range 200-400). The aPTT at the outset of surgery had a mean value of 34.3 sec (range 21-43 sec) and at the end of the surgery the mean aPTT was 33.4 sec (range 28-38 sec).

Tranexamic acid
TXA (Cyklokapron®, Meda AS, Allerød, Denmark) is a synthetic derivate of the amino acid lysine and inhibits fibrinolysis by competitive inhibition of the activation of plasminogen to plasmin. In our department prophylactic TXA is routinely administered using a total dosage of 4 g (2 g at start surgery, 1 g during ECC, and 1 g after reversal of heparin).

In total, 23 patients received the first dose of TXA intravenously prior to blood sampling while remaining five patients had the first administration after the initial blood sampling. One of the patients included was shifted to aprotinin infusion and was therefore excluded from further analysis.

Renal function
Renal affection was defined as an increase of creatinine to above 120 µmol/L. Preoperative levels of crea-
tine was measured as well as preoperative estimated glomerular filtration rate (eGFR). Postoperative daily measurements of creatinine were made and eGFR calculated. Definition of acute kidney injury was a 50% increase in creatinine (RIFFLE).[24]

Surgery and postoperative treatment
Surgical characteristics are listed in table 1. All patients were operated with the use of ECC and cardiac arrest was induced with cold crystalloid cardioplegia (mean 1474 ml (±549)). Priming solution was Ringer-Acetate® (Fresenius Kabi AB, Uppsala, Sweden), total priming volume 1600 ml. Perioperative body temperature was not below 34°C, which was only during ECC. Rewarm volume 1600 ml. Perioperative body temperature was induced with cold crystalloid cardioplegia (mean calculated. Definition of acute kidney injury was a 50% increase in creatinine by 50%, one of these had clot lysis at 24h.

Partial lysis, a more than 10% reduction from peak amplitude. Large area under the curve and no reduction over time. Part C: Complete lysis, showing total clot preservation. The area under the curve (AUC) was assessed using concentrations of tPA at 3nM versus 6nM (data with 6nM not shown).

Other laboratory parameters
Preoperative parameters were aPTT, INR, PT (prothrombin time), antithrombin, haemoglobin, platelet count, d-imerin and fibrinogen whereas postoperative parameters were haemoglobin, EFE, platelet count and fibrinogen concentration. All assays were conducted by our accredited university laboratory. All patients pre-operatively had a creatinine level below 120 µmol/L. Anti-Xa levels were measured using a chromogenic assay to control to assess residual concentration and activity of heparin.

Data analyses and statistical considerations
Data distribution was assessed by histograms and Q-Q plots. Clot lysis data followed a Gaussian distribution except for the time point 6h and 24h. Data are illustrated as mean and deviation. Difference in results over time were analysed by student t-test. Differences in proportions were analysed by Fisher's exact test. A P-value less than 0.05 was considered statistical significant.

Results
Effect of TXA on facilitated fibrinolysis
Preoperative administration of TXA completely abolished fibrinolysis. Thus, 100% of patients receiving TXA 2 g showed no sign of fibrinolysis, whereas the 5 patients who had not received TXA before blood sampling showed prompt and complete fibrinolysis. Fibrinolysis was measurable again in 7 of the 28 patients after 12 hours. In total 13 of the 28 patients demonstrated clot lysis after 24 hours. At 48 hours 9 patients still showed clot lysis resistance (figure 3). Patients with a postoperative creatinine level above 120 µmol/L (n=8) showed a trend towards increased resistance to fibrinolysis with none of the patients demonstrating tPA induced fibrinolysis at 12 hours and only one patient showing fibrinolysis at 24 hours. At 48 hours 5 of the 8 patients were still resistant to fibrinolysis (data not significant, Fisher exact test, p=0.13) (figure 3). Mean eGFR at baseline was 68.4 (range 38-90), eight patients had an eGFR below 60 preoperatively. Half of these patients did not regain fibrinolytic activity during the observation period (Fisher 0.08). Four patients had acute renal injury with an increase in creatinine by 50%, one of these had clot lysis at 24h. The remaining three did not recover clot lysis during the observation period (Fisher 0.08).

Changes in clot stability following use of TXA
Compared to individuals not receiving TXA prior to blood sampling, those who did receive TXA had a mean AUC that was 13-fold higher, reflecting better clot stability (AUC No TXA 30±8 vs TXA 409±40, p=0.0002). During the surgical procedures and ECC there was an almost four fold decrease in clot stability (AUC) when looking at the patients who had received TXA at baseline (start: 409±40 vs 8h:111±14 p=0.0001). Clot stability remains at a plateau level during the first 6 hours postoperatively and then increase after another six hours beyond the preoperative level. Thromboprophylaxis with LMWH triggered decreased clot stability (figure 4).
The lag times of clot formation progressively prolong from baseline up to 6 hours postoperatively (p < 0.01). At 12 hours the lag time is partially corrected and following start of thromboprophylaxis the lag time increases again (figure 5). Anti-Xa levels were unchanged during the first 12 hours postoperative and prolonged at 24h and 48h in response to the initiation of thromboprophylaxis.

**Bleeding, transfusion and reexploration**

The mean total blood loss was 991 ml (range 250-2050) and the postoperative blood loss 648 ml (range 125-1850). One of the patients received transfusions and coagulation factor concentrates prior to the blood sampling two hours postoperative and was therefore excluded. In total, eight patients (27%) received transfusion during the first 48 hours, three patients (10%) had reexploration for bleeding and surgical bleeding was found to be the cause in one of these patients.

A reduced resistance to clot lysis might reflect bleeding patients and of note is that in patients bleeding more than 1500 ml (n=7) only one patient did not demonstrate clot lysis at any time. 11 patients had more than 1000 ml of total bleeding, however there was no correlation with clot lysis parameters.

**Other laboratory parameters**

APTT was measured at the induction of anaesthesia, prior to surgery and again after reversal of heparin with protamine sulphate. Mean APTT values prior to surgery was 34.3 sec (range 21-43 sec) and postoperatively 33.4 sec (range 28-38 sec).

Mean fibrinogen concentration was 10.9 ± 2.8. The first day after surgery fibrinogen was unchanged 10.0 ± 2.2) followed by a significant increase to 15.8 ± 3.7 the second day after surgery (p < 0.001). The mean platelet count decreased significantly from 255 x 109 (t08) preoperatively to 173 x 109 (t08) the day after surgery (p < 0.001) and then stabilized (171 x 109 (t62) day 2). EVF declined from 0.418 (±0.03) to 0.316 (±0.04), improving somewhat before discharge (0.328 (±0.04).

**Whole blood thromboelastometry** using tissue factor as activator displayed no sign of fibrinolysis in any of the postoperative measurements.

**Discussion**

Antifibrinolytic treatment with TXA protects against facilitated fibrinolysis and induces a multifold increase in clot stability. All patients show complete fibrinolytic resistance during the first 6 hours after cardiac surgery. This then declines to 33% at 48 hours. Reduced kidney function is associated with prolonged resistance to Ila facilitated fibrinolysis.

Despite inhibition of fibrinolysis with TXA, the overall clot stability as measured by AUC declines and the kinetics of clot formation worsens during cardiac surgery and is restored 12 hours after surgery. Our measurement of clot stability, using AUC, has been selected to reflect the combined effect of alterations in clot formation and resistance to lysis. Decreased clot stability despite use of TXA may be due to loss of fibrinogen and dysfunctional fibrin polymerisation affecting the quality of the clot formed[27,28]. In a recent laboratory study clot stability was shown to be linked to the concentration of fibrinogen within the model system, increasing in a dose dependent manner as fibrinogen concentration increased[29]. Furthermore, both the structural quality and resistance to lysis may be affected by changes in FXIII or TAFI concentrations peripherally[10]. TXA will not affect fibrinogen, TAFI or FXII activation explaining the observation that clot lysis can be effectively inhibited, yet overall clot stability remains impaired intraoperatively in the group of patients studied.

The lowest effective concentration of TXA is not entirely clear; thus pharmacokinetic data may not be sufficient to estimate potential benefit of additional dosing. Moreover, inter individual differences in renal clearance of TXA may interfere with the pharmacokinetic profile[30]. Hence, a relatively rapid and more functional assessment of resistance against facilitated fibrinolysis may be a more attractive laboratory measure to guide dosing and thereby avoid serious side effects. In view of the fact that the risk of seizures after cardiac surgery is related to TXA administration in a dose-dependent fashion the need for individualized treatment and dosing regimens is of clinical value especially since seizures after cardiac surgery is independently associated with several adverse events[31].
A major strength of the present study is the multiple observations in common cardiac surgery cases within the first 48 hours postoperatively. Furthermore, the study adopts a custom designed functional assay to illustrate the pharmacodynamic effect of TXA rather than pharmacokinetic properties as well as changes in clot stability over time following cardiac surgery and ECC. However, there are also several limitations. The use of plasma rather than whole blood in evaluating resistance to fibrinolysis over looks contribution from platelets [21]. A new assay has been developed for evaluating resistance to fibrinolysis and clot stability in whole blood[32], however this could not be adopted in the present study for logistic reasons.

Evaluation of hyperfibrinolysis by viscoelastic tests is increasingly common in evaluating patients undergoing liver transplantation and trauma patients [33]. However, standard TEG/RoTEM assays are not sensitive to low grade fibrinolysis[34]. Additionally fibrinolysis parameters are not available until approximately 45 min after starting the analysis. Pomerening et al have suggested to use velocity curves for fibrinolysis evaluation [35], which may improve usability of standard TEG/RoTEM assays in predicting excessive fibrinolysis, transfusion and mortality. It also has been indicated that low early values of maximum clot firmness were associated with increased fibrinolysis[36], but others argue that standard viscoelastic assays lack sensitivity to demonstrate fibrinolysis [37].

Currently, there is a lack of international consensus on laboratory assessment of clot and lysis and clot stability. Natafka et al defined a 50% reduction in clot turbidity as marker of susceptibility to fibrinolysis[38] whereas Jankun et al used area under the curve to evaluate the process of clot lysis[39]. In our study we adopted multiple assessments. AUC was selected to reflect the mutual contributions on clot stability from both clot formation and resistance to lysis. This was assessed in parallel with individual parameters of clot formation and clot lysis.

The study has several limitations. First, a control group not receiving TXA would have strengthened the study. However, administration of TXA in cardiac surgery is a class I(a) recommendation and thus it would have been unethical to have a control group [40]. Secondly, it would have been optimal to know the exact concentrations of TXA in our study and advanced methods for measuring serum concentrations of TXA have been developed. Previous studies have, however, shown a large variation in plasma concentration despite weight adjusted dosage regimes[30]. In the study five patients unintentionally did not receive TXA dosage prior to blood sampling. However, these cases proved useful to confirm that patients prior to surgery display normal clot lysis in the customized assay. In the exploratory subgroup analyses the number of patients is low, hence results should be regarded as hypotheses generating rather than conclusive.

The number of patients experiencing excessive bleeding is rather limited and the types of surgical intervention are considered low risk. Therefore the results cannot be applied directly to high-risk cardiac cases. Importantly, other assays for evaluating thrombin generation and clot lysis are available and may be able to add additional insight into mechanisms and associations [41].

The results of this study indicate that TXA can be used in cardiac surgery to improve resistance to clot lysis. However, it is important to recognize that the use of TXA improves resistance to clot lysis, but does not prevent loss of clot stability and derangements in the kinetics of clot formation. If a structurally normal clot is formed, then TXA will help to prevent lytic activity, but it will not aid haemostasis if other mechanisms of clot formation and clot stability, such as FXIII/TAI activation or fibrinogen levels, are deranged. Monitoring the level of resistance to fibrinolysis may reduce risk of overdosing in particular in patients with reduced kidney function. In addition, it should be noted that bleeding patients may be adequately treated with antifibrinolytics, but continue to bleed due to impaired clot formation and impaired overall clot stability.

In conclusion TXA induces a multifold increase in clot resistance to fibrinolysis but does not affect clot formation or clot stability.

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Reference list


Development of a laboratory model of post cardiac coagulopathy

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Introduction
Cardiac surgery is a major challenge to the haemostatic system due to the surgical trauma, heparinisation, haemodilution, the use of cardio-pulmonary bypass (CPB), and consumption of coagulative factors and platelets, as well as polyvalent and hypothermia. Bleeding complications following major cardiac surgery make endstage a frequent and serious clinical problem. Surgical re-exploration due to haemorrhage occur in 3% to 4% and may be as high as 10% when looking at daily practice. In up to 95% of cases, intensive bleeding is caused by a coagulopathy. Haemostatic regimens would be desirable in management of bleeding after cardiac surgery.

Aims
The aims of this study were to:
1. Develop an experimental standardized laboratory model of post cardiac coagulopathy (MPCCS) mimicking the challenges blood and blood coagulation is exposed to during cardio-pulmonary bypass.

Materials and methods
See Figure 1. Blood samples (platelet 3.2%, 2500 µL) from 8 healthy volunteers were spiked with heparin (40 µL, 40 U/mL) and exposed to mechanical stresses by centrifugation (3000 g, 15 minutes, fixed acceleration, 50% deceleration). In total, 200 U/L of the supernatant containing platelets and plasma was removed. 100 U/L of the pelleted red cells was aspirated and haemolysed with Versene treatment, and 100 µL of thrombin were measured. Mimicking the haemorheology and loss of the red blood cells that take place during CPB, the blood was used with 2 ml of Heparin’s lactate, with the platelet in haemostatised due to priming volume in the CPB machines and the cardioplegia. Heparin induced intracellular release of TFP was achieved by adding a final concentration 100 µM. Finally, haemolysed was neutralised by protamine sulphate (1 mg or 100 U of heparin, 200-1000 U). Whole blood (WB) thrombocytopenia and platelet aggregation was recorded using aggregation by whole blood. Description of methodology and methods using von-Willebrand-Antigen test.

Results
Whole blood coagulation (Tables 1 and Figure 2, Panels A and C). Changes in whole blood clot formation were primarily characterised by reduced maximum clot firmness. Activating the haemostasis of the red cells did not influence WB clot formation.

Bone cell counts (Table 2): All blood cells decreased.

Causes improve additive (Figure 2): Correlation window (CTI) to obtain contact activation had no impact on the clotting profiles in MPCCS.

Conclusion
Compared to studies in patients after CPB, the MPCCS seems to lead the closest to demonstrate similar patterns in whole blood clot formation. Future studies aim at direct comparison with patient blood samples and in vivo investigation of repetitive performances of haemostasis intervention. The established model can be used to evaluate novel haemostatic interventions.

Table 1
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