The use of fibrinogen
in cardiac surgery patients

Clinical and experimental studies

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Cover illustration: "Brunt mönster med trianglar" by Andreas Waldén

"Skäms inte för att du är människa, var stolt!

Inne i dig öppnar sig valv efter valv oändligt.

Du blir aldrig färdig, och det är som det skall."

Tomas Tranströmer

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ABSTRACT

BACKGROUND Cardiac surgery with cardiopulmonary bypass impairs hemostasis due to hemodilution and consumption of platelets and coagulation factors, such as fibrinogen. The aim of this thesis was to study the role of fibrinogen in bleeding complications in cardiac surgery patients.

METHODOLOGY Patients who underwent cardiac surgery at Sahlgrenska University Hospital from 2009 to 2017 were included in four studies.. Study I assessed the importance of preoperative plasma fibrinogen concentration for excessive bleeding and the need for red blood cell transfusion in an observational study in 1954 patients. Study II was a double-blinded placebo-controlled study where 48 patients were randomized to prophylactic administration of fibrinogen concentrate or placebo. Primary endpoint was postoperative bleeding volume. Study III was an observational study in 5408 patients that assessed if patients who had received perioperative fibrinogen concentrate due to bleeding had a higher risk of thromboembolic complications or death. Study IV was an in vitro study where fibrinogen concentrate was added to blood samples from 15 patients to investigate if concomitant tranexamic acid and fibrinogen administration has additional effects on clot formation compared to fibrinogen alone.

RESULTS The preoperative plasma fibrinogen concentration correlated inversely to increased postoperative bleeding but not to RBC transfusion. Prophylactic infusion of 2 g fibrinogen concentrate did not reduce postoperative bleeding volume. Patients who received fibrinogen concentrate due to perioperative bleeding did not have a higher risk of thromboembolic complications or death during the first year after surgery. The combination of tranexamic acid and fibrinogen did not have additional effects on platelet-independent clotting time or clot firmness than fibrinogen alone.

CONCLUSION Preoperative plasma fibrinogen concentration is associated with excessive bleeding after cardiac surgery. Preoperative supplementation with fibrinogen concentrate did not significantly influence postoperative bleeding in low risk patients undergoing coronary artery bypass grafting. Perioperative administration of fibrinogen concentrate in case of bleeding appears safe. The enhancing effects of fibrinogen concentrate on clot firmness in blood samples from cardiac surgery patients was not further increased in the presence of tranexamic acid.

KEYWORDS fibrinogen, cardiac surgery, bleeding, transfusion, thromboembolism, tranexamic acid

POPULÄRVETENSKAPLIG SAMMANFATTNING

Hjärtats och lungornas funktioner kan, vid behov, till exempel under hjärtoperationer, tas över av en så kallad hjärt-lungmaskin. I hjärt-lungmaskinen pumpas blodet förbi hjärtat samtidigt som det syresätts. Hjärtat kan då tillfälligt stoppas utan att patientens liv hotas. En nackdel vid användandet av hjärt-lungmaskin är att blodets förmåga att stoppa blödning och samtidigt motverka blodproppar påverkas negativt.

Blodets förmåga att balansera blödning och proppbildning kallas för hemostas. Hemostasen under och efter hjärtkirurgi påverkas av flera faktorer relaterade till både patienten, som till exempel ålder och övrig sjuklighet, samt till vilken typ av kirurgisk metod som används och hur lång tid operationen tar. När man använder en hjärt-lungmaskin måste patientens blod spädas ut och blodet utsätts också för kroppsfrämmande ytor, bland annat plastslangar. Detta drar igång en aktivering och förbrukning av koagulationsfaktorer samt en aktivering av inflammatoriska reaktioner. Denna påverkan på koagulationssystemet kan orsaka allvarlig blödning både under och efter kirurgi och en del patienter kan behöva opereras på nytt på grund av dessa blödningar.

Ungefär hälften av de hjärtkirurgiska patienterna behöver behandlas med blodtransfusion. En akut operation på grund av blödning innebär en ökad risk för sjuklighet och död efter hjärtkirurgi. Det är därför viktigt att kunna förutse och minska dessa blödningskomplikationer. Fibrinogen är den koagulationsfaktor som finns i störst mängd i blodet. Fibrinogen är ett protein som vid aktivering bildar ett nät där blodplättar och röda blodkroppar fastnar och därmed bildas ett koagel på platsen för skadan. Vid större blödning förbrukas tillgängligt fibrinogen och därför har fibrinogenkoncentrat under de senare åren använts allt oftare för att förbättra koagulationen under och efter operationen. Fibrinogenkoncentrat tillverkas av plasma från blodgivare.

Syftet med avhandlingsarbetet är att belysa fibrinogenets roll när det gäller att förutse blödning, om profylaktisk behandling med fibrinogenkoncentrat före operationen minskar blödning, om behandling med fibrinogen till blödande patienter är säker och om fibrinogen tillsammans med standardbehandling med läkemedel som hämmar blodkoaglets nedbrytning, så kallad fibrinolyshämmare, ger förbättrad koagulation.

I delarbete I undersöktes om det fanns ett samband mellan patienternas fibrinogenkoncentration i blodet före operationen och mängden blödning efter operationen samt behovet av blodtransfusion. Vi fann att patienter med låg fibrinogenkoncentration hade en signifikant ökad risk för allvarlig blödning. Däremot sågs inget samband mellan fibrinogenkoncentration och behovet av blodtransfusioner.

Delarbete II syftade till att undersöka om förebyggande behandling med fibrinogenkoncentrat innan kirurgi kan bidra till att minska blödningsmängden efter operationen. Patienterna delades in i två grupper där den ena gruppen behandlades med fibrinogenkoncentrat och den andra med placebo. Studien kunde inte visa att förebyggande infusion av fibrinogenkoncentrat minskar blödningsmängden efter kranskärlsoperationer.

I delarbete III studerades 5408 patienter som hjärtopererats. Vi jämförde de patienter som erhållit fibrinogenkoncentrat på grund av blödning under eller efter hjärtoperationen med de patienter som inte erhållit detta. Jämförelsen gjordes med avseende på risk att drabbas av blodproppar och död. Patienter som erhållit fibrinogenkoncentrat hade inte en högre risk för blodproppar och död upp till ett år efter kirurgi.

Delarbete IV var en experimentell studie som beskriver effekten av fibrinogentillsats till blodprover som tagits från hjärtkirurgiska patienter före och efter infusion av tranexamsyra. Tranexamsyra är en fibrinolyshämmare, det vill säga ett läkemedel som förhindrar nedbrytning av blodkoagel och därmed minskar blödningar. Tranexamsyra ges rutinmässigt till patienter som hjärtopereras. I studien förbättrade fibrinogentillsats koagelbildning och koagelstyrka i blodproverna, men effekten skiljde sig inte i blodprover som samlats före eller efter att tranexamsyra givits.

Sammanfattningsvis har patienter med låg koncentration av fibrinogen i blodet en ökad risk för allvarlig blödning efter hjärtkirurgi. Att förebyggande ge fibrinogenkoncentrat till patienter med normala fibrinogennivåer och låg förväntad risk för blödning minskade ej signifikant blödningsmängden när de genomgick kranskärlskirurgi. Användandet av fibrinogenkoncentrat till patienter med pågående blödning i avsikt att förbättra koagulationen förefaller sig säkert och ökar inte risken för blodproppar. Den förstärkta koagelstyrkan som fibrinogenkoncentrat bidrar till i blodprover från hjärtkirurgiska patienter blir inte ytterligare förbättrad i närvaro av fibrinolyshämmare.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I.   Waldén K, Jeppsson A, Nasic S, Backlund E, Karlsson M.

     Low preoperative fibrinogen plasma concentration is associated with excessive bleeding after cardiac surgery.

     Ann Thorac Surg 2014 Apr;97(4):1199-206.

II. Jeppsson A, Waldén K, Roman-Emanuel C, Thimour-Bergström L, Karlsson M.

     Preoperative supplementation with fibrinogen concentrate in cardiac surgery: a randomized controlled study.

     Br J Anaesth 2016 Feb;116(2):208-14.

III. Waldén K, Jeppsson A, Nasic S, Karlsson M.

     Fibrinogen concentrate administration to cardiac surgery patients with ongoing bleeding does not increase the risk of thromboembolic complications or death.

     (Submitted manuscript)

IV. Waldén K, Jeppsson A, Shams-Hakimi C, Karlsson M.

     Effects of fibrinogen supplementation on clot formation in blood samples from cardiac surgery patients before and after tranexamic acid administration.

     Transfus Med 2019 May 22 10.1111/tme .12604
     (Epub ahead of print).

CONTENT

ABBREVIATIONS

ACT	Activated clotting time
ANOVA	Analysis of variance
APTT	Activated partial thromboplastin time
ASA	Acetyl salicylic acid (aspirin)
CABG	Coronary artery bypass grafting
CI	Confidence interval
CPB	Cardiopulmonary bypass
CT	Clotting time
DAPT	Double anti platelet therapy
ECC	Extra corporeal circulation
GUCH	Grown up congenital heart
HCT	Hematocrit
HB	Hemoglobin
HR	Hazard ratio
LMWH	Low molecular weight heparin
MCF	Maximal clot firmness
ML	Maximal lysis
OR	Odds ratio
PCI	Percutaneous coronary intervention
PLT	Platelets

PS	Propensity score
PT	Prothrombin time
RBC	Red blood cell
SD	Standard deviation
TXA	Tranexamic acid
vWF	Von Willebrand factor

1 INTRODUCTION

Excessive bleeding is common during and after cardiac surgery. It can be caused both by the surgical trauma and by an impaired hemostasis. It is of great importance to address the causes to reduce complications related to bleeding. The coagulation factor fibrinogen is the substrate in the coagulation system with the highest plasma concentration and essential in blood clot formation. However, there is limited knowledge about the role of fibrinogen to predict and prevent bleeding in cardiac surgery.

1.1 OVERVIEW OF THE HEMOSTASIS AND THE COAGULATION SYSTEM

In the presence of a vascular injury, there is an immediate response by smooth muscle cells in the vessel wall, initiated by sympathetic receptors. This causes a vasoconstriction at the site of injury, reducing the blood flow through the injured area. In the damaged vessel, subendothelial cells and collagen are exposed to plasma. The von Willebrand factor (vWF), a protein circulating in plasma, binds to receptors on the exposed collagen. Further on, vWF attracts platelets to the site, which connects to vWF through receptors (Ib) on their surface. This activates the platelets, making them change shape and stimulates to degranulation. The release of adenosine diphosphate (ADP), calcium (Ca) thromboxane A2 (TXA2) and thrombin promotes attraction, activation and adhesion of more platelets. Additional vWF, fibrinogen and coagulation factor V and XIII are also released. Fibrinogen, readily circulating in plasma and released from platelets, binds to activated glycoprotein receptor IIb/IIIa (GPIIb/IIIa) on the platelet surface , forming a rather weak plug (1-3).

At the site of injury tissue factor (TF), a glycoprotein, expressed by subendothelial and perivascular cells is exposed. TF and its interaction with circulating coagulating factor VII in plasma, initiate a chain reaction that activates several other coagulation factors. These coagulation factors interact with activated platelets and forms a complex both attached to the vessel wall and directly to the platelets. The product of this cascade is an extensive conversion of prothrombin to thrombin. Thrombin cleaves fibrinogen to fibrin, as well as activates factor XIII. Together, fibrin and factor XIII, form a stable network and a mature blood clot is achieved.

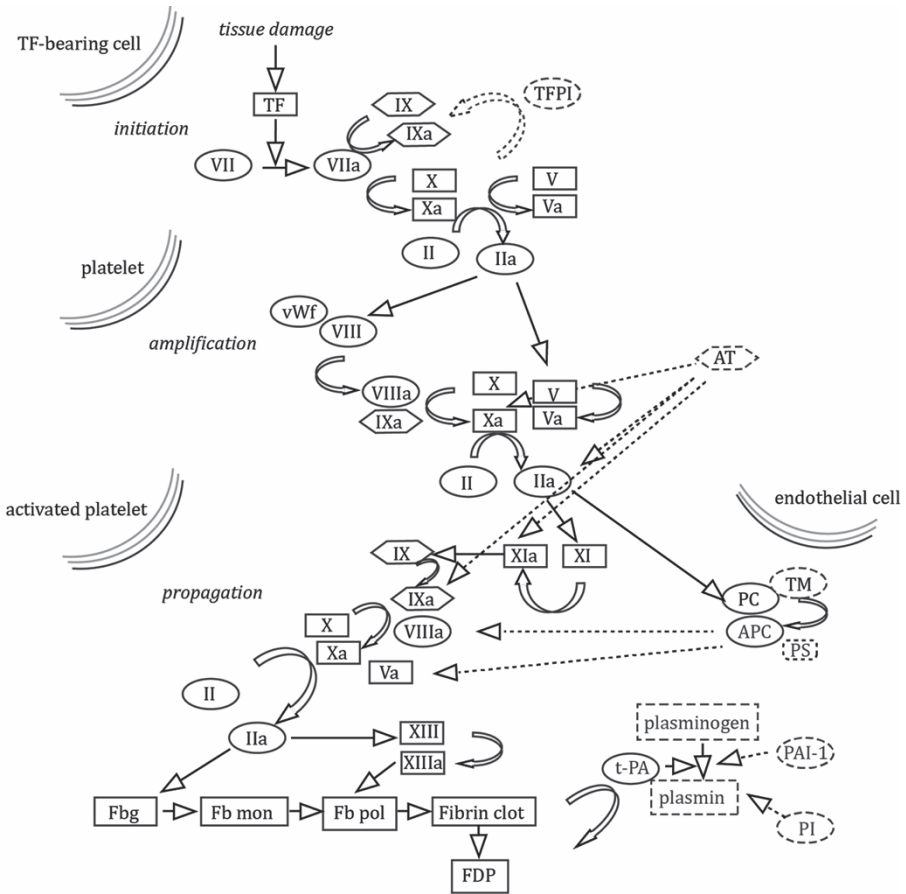During this process, circulating red blood cells (RBCs) are trapped in the clot (1, 4, 5).

Figure 1. Illustration of the coagulation cascade where the end product is an insoluble fibrin clot. Image with the courtesy of Aleksandra Antovic, Dept. of Medicine, Karolinska Institute.
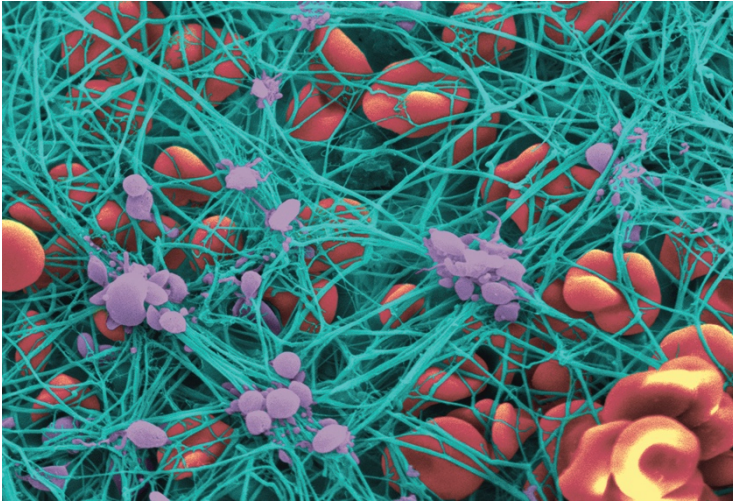
Figure 2. Colorized scanning electron micrograph of a whole blood clot. Fibrin strands in green, platelets in purple and red blood cells (RBCs) in red. Image with the courtesy of Yuri Veklich and John W Weisel, Dept. of Cell and Developmental Biology, Perelman School of Medicine, University of Pennsylvania.

1.2 FIBRINOGEN

The glycoprotein fibrinogen is also known as coagulation factor I. It was discovered over 350 years ago when Malphigi in 1666 describes the fibrin strands in a microscope. During the 19th century, Virchow named the protein fibrinogen and later in the 19th century, Hammarsten was able to purify it (6).

Fibrinogen has a molecular weight of 340 kilodalton (kDa) and is a key factor in the human coagulation system (7). Approximately 2-5 g of fibrinogen is synthesized daily the by the hepatic cells in the liver. The same quantity is catabolized through normal protein degradation, the coagulation process and other unidentified pathways (8). Fibrinogen has an estimated half time of three to four days (9). The plasma concentration in healthy humans is approximately 1.8-4.5 g/L depending on measurement device and reagent (8, 10). The concentration is usually determined by the Clauss method which measures coagulation time in diluted plasma in the presence of excess thrombin, an environment where

the amount of fibrinogen is the limiting factor and coagulation time is inversely proportional to fibrinogen concentration (11, 12).

Fibrinogen is a physiological substrate for thrombin, factor XIII and plasmin. A fibrinogen molecule is a dimer containing pairs of chains linked together with disulfide bridges. These chains are cleaved by thrombin, thus releasing fibrinopeptides from the terminals, forming fibrin. The fibrin molecules begin to assemble to form fibrin polymers. These fibrin polymers are further crosslinked and stabilized by activated factor XIII, forming branched fibrin fibers (13, 14). The fibrin clot is degraded by plasmin. As mentioned above, fibrinogen also supports platelet aggregation in primary hemostasis by acting as a ligand to GPIIb/IIIa, which is expressed on the surface of activated platelets (14).
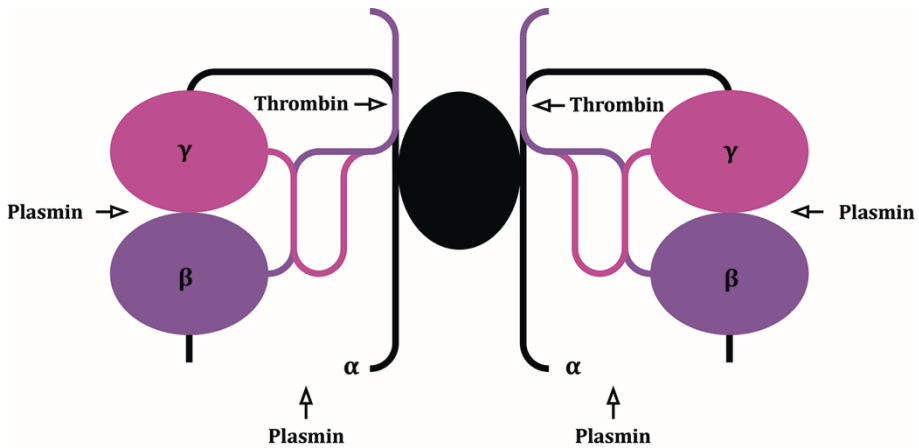

Figure 3. Schematic illustration of a fibrinogen molecule.
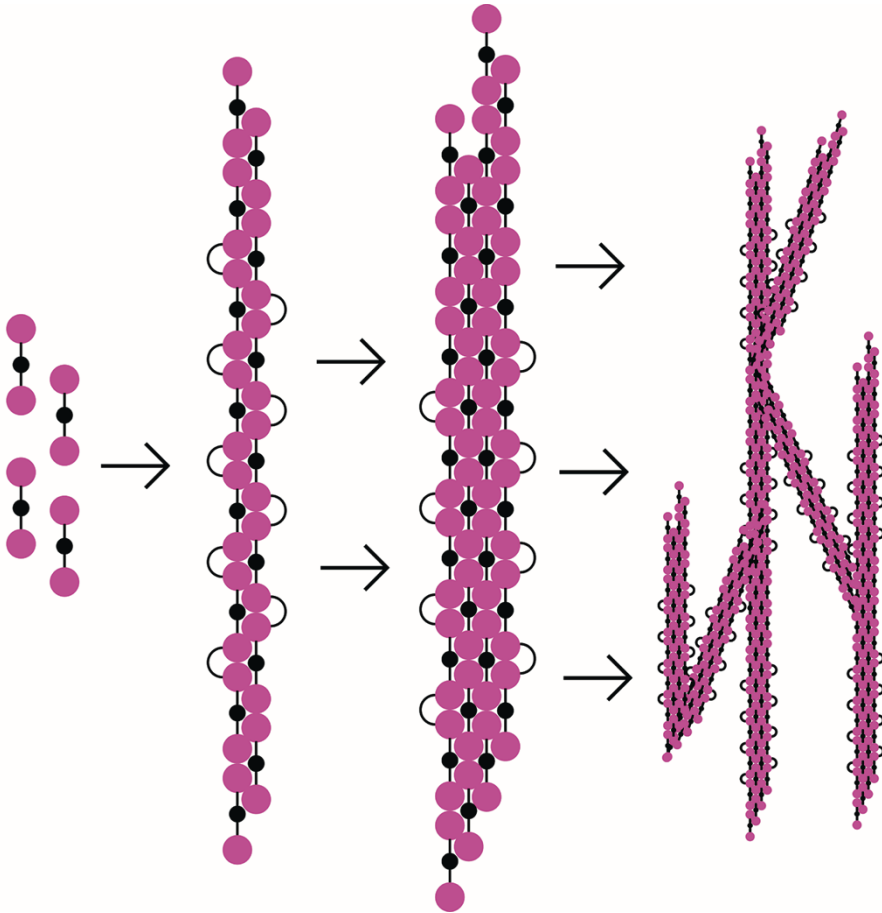
Figure 4. Schematic illustration of fibrin assembling. Upon fibrinogen peptide cleavage by thrombin, fibrin monomers assemble to form fibrin polymers. During this process, activated FXIII stabilizes peptide bond formation between the fibrin polymers, forming branched, crosslinked, stable fibrin fibres.

Besides its hemostatic effect, fibrinogen is an acute phase reactant and regulates inflammatory responses (15). Fibrinogen and its degradation products have multiple receptors which interact with and activate a wide range of immune cells, both in the blood stream and in the perivascular space (15). Fibrinogen plasma levels are normally elevated with age, in females and during pregnancy and are also associated with lifestyle factors such as tobacco- and alcohol use (16). Epidemiological studies have shown a strong association between elevated fibrinogen

concentration and the risk of major cardiovascular disease and mortality, but a causal relationship has not been established (16, 17).

Hypofibrinogenemia

The presence of low levels of circulating fibrinogen, hypofibrinogenemia, may have different causes. Acquired hypofibrinogenemia is most frequently caused by consumption of clotting factors due to bleeding and in cases of hemodilution. It may also be present in patients with reduced synthesis due to hepatic failure as well as in acute leukemia (18).

In major hemorrhage, fibrinogen is one of the coagulation factors that first fall to critical levels, since the increase in synthesis rate is most often insufficient to compensate the use and breakdown of the coagulation factor (19-21). Further on, acidosis and hypothermia that accompany massive bleeding, induce coagulopathy and seem to aggravate an increased breakdown and decreased synthesis of the coagulation factor (22). Hemodilution due to volume resuscitation further reduces available fibrinogen in plasma, causing reduced clot stability (23, 24). Bleeding and hypofibrinogenemia seem to be related to both increased morbidity and mortality in both trauma and in the postoperative setting (25-27).

Congenital disorders of fibrinogen are rare. It can present as hypofibrinogenemia, dysfibrinogenemia and afibrinogenemia and the severity of symptoms is dependent upon the level of dysfunction or deficiency of fibrinogen. It can be as little as no symptoms at all to fatal hemorrhages (28). Major and spontaneous bleeding is generally reported in patients with hypofibrinogenemia and a plasma level below 0.5 g/L and patients with a level over 1 g/L do not generally experience unprovoked bleeds (29, 30). Contractively, the incidence of both venous and arterial thrombosis among these patients is elevated. The reason is not completely understood, but seems to be a cause of excess thrombin generated in absence of fibrinogen. The thrombin is instead available for platelet activation and aggregation, leading to a large and loosely packed platelet thrombus (31-33).

Treatment of hypofibrinogenemia

There are three different ways to administer fibrinogen: plasma, cryoprecipitate and fibrinogen concentrate. Plasma for transfusions contains about 1-3 g fibrinogen per litre. Large doses are therefore

required to restore a fibrinogen deficit and supplementation with plasma is often accompanied by a risk of hypervolemia (34). If the target level of fibrinogen concentration is higher than that of the administered plasma, it cannot be reached (35, 36). Cryoprecipitate is produced from plasma by precipitating the coagulation factors FVIII, vWF and fibrinogen by their weight. The concentration of fibrinogen in cryoprecipitate is about 16 g/L (27). Since the product is constituted by plasma from multiple donors and does not undergo any antiviral processing, it has been withdrawn for safety concerns in several European countries, but is still widely used in the United States and in the United Kingdom (37). Pasteurized fibrinogen concentrate is derived from human plasma and is delivered as a freeze-dried powder and dissolved to a concentration of about 20 g/L. Fibrinogen is processed through viral inactivation processes, which also remove antigens and antibodies that reduce the risk of immunological and allergic reactions (38). Fibrinogen concentrate has been commercially available since the 1960's for prophylaxis and treatment of bleeding episodes in patients with congenital hypo- or afibrinogenemia. In these patients, supplementation is usually made below 1 g/L, since this level seems to be sufficient to keep hemostasis when no other coagulation deficiency is present (31).

In recent years, infusion of fibrinogen concentrate has become an established method to treat patients with ongoing bleeding after surgery and trauma (39, 40). For decades, a plasma concentration of above 1g/L was considered to be enough for effective clot formation and maintaining hemostasis even in patients with acquired hypofibrinogenemia due to bleeding(19, 41). However, in situations of major bleeding, more recent data suggests that a higher plasma concentration of fibrinogen and a more liberal use of fibrinogen concentrate might be desirable to restore or improve hemostasis (35, 42, 43).

1.3 CARDIAC SURGERY

In the late 19th century, the first reported successful suture on a human heart was reported (44). The gained knowledge of the importance of sterile technique and development of anesthesia helped to make the procedure successful (44). In the 1950's, the development of the cardiopulmonary bypass (CPB), i.e. heart-lung machine, together with the start of commercial manufacture of the anticoagulant heparin paved the way for open heart surgery. CPB and anticoagulation with heparin

facilitated extracorporeal circuits without deleterious clot formation and thereby the "modern" cardiac surgery was born (45). Since then, the development of techniques has made it possible to perform surgery on e.g. valves, coronary arteries and complex congenital heart defects.

The use of CPB provides a bloodless field during cardiac surgery. The CPB circuit consists of pumps, tubing, cannulas, reservoir, oxygenator, heat exchanger and an arterial line filter, described in Figure 5.
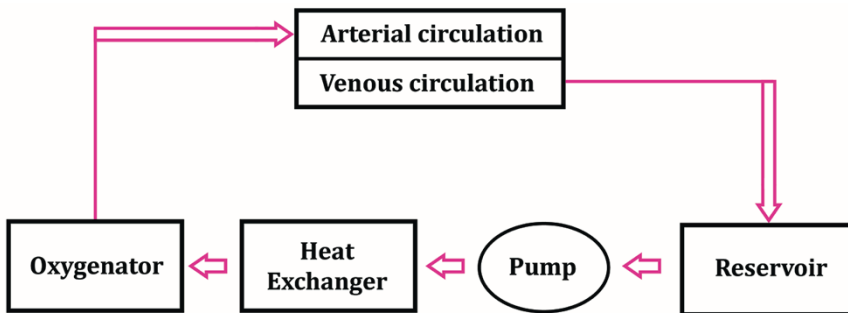

Figure 5. The CPB circuit. Venous blood is drained either from the right atrium or from v. cava superior and inferior. Through gravity, the venous blood drains into a reservoir. The roller pump moves blood from the reservoir, through a heat exchanger to an oxygenator. After oxygenation, the blood returns to the arterial circulation through a cannula in the aorta.

In 2017, 5800 open cardiac surgery procedures were performed in Sweden, compared to nearly 9400 in the early 1990's (46). The decline in the number of procedures is largely due to the progress of endovascular treatment by percutaneous coronary interventions (PCI) in patients with coronary artery disease. Despite this, cardiac surgery as a treatment of coronary artery disease and valvular disease is still a needed intervention in selected patients with more complicated disease. The number of open heart surgery has been fairly constant since 2013. Coronary artery bypass grafting (CABG) accounts for 45%, isolated valve surgery for 25% and a combined CABG and valve surgery for 9% of all cardiac procedures in Sweden (46).

The 30-day mortality is about 2.5% after cardiac surgery (46). There are still substantial risks of complications after cardiac surgery such as bleeding, stroke, renal and heart failure as well as pulmonary dysfunction (47-54).

Cardiac surgery and bleeding

Postoperatively, the bleeding volume is measured using chest tubes, inserted in the pleura and pericardium at the end of surgery. Postoperatively, a cardiac surgery patient may be expected to bleed about 500-1000 ml. The drainage is usually removed the day after surgery. Bleeding can be caused by the surgical trauma, impaired hemostasis or both. Hemostasis during and after surgery is influenced by several factors, some of which are patient related, while others are related to the surgical procedure. The use of CPB induces activation of the hemostatic system, mostly because of the contact between the patient's blood and the artificial surfaces of the circuit. This leads to enhanced platelet activation, increased fibrinolysis and substantial inflammatory response interacting with the coagulation system (55, 56). The required hemodilution further lowers the concentration of coagulation factors (57, 58). Preoperative medication with anticoagulants and antiplatelet therapy may also contribute to the compromised hemostasis (59).
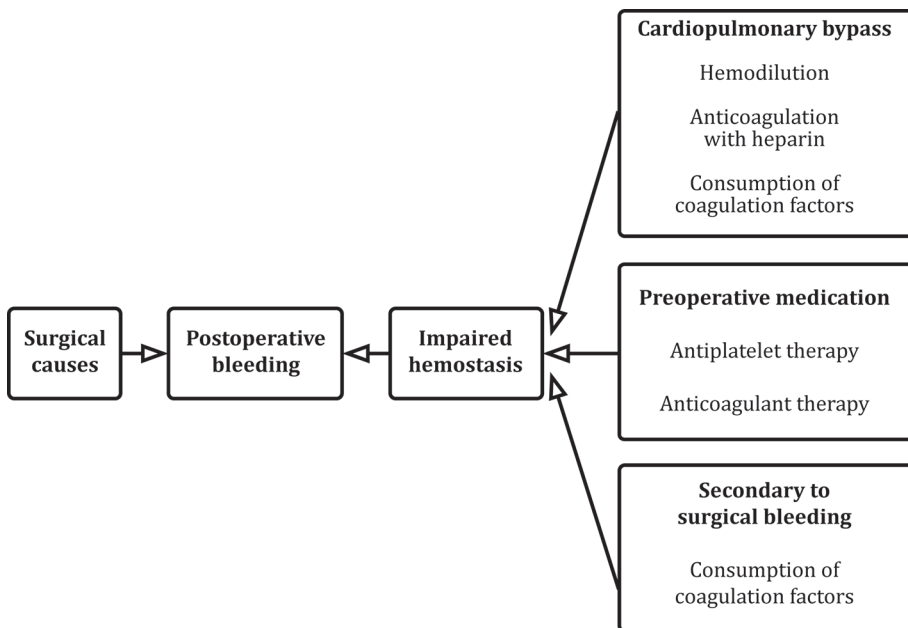

Figure 6. Overview of factors causing postoperative bleeding in cardiac surgery. Factors involve the surgical trauma, impaired hemostasis or both.

Despite progress in surgical techniques and the gained understanding of factors affecting perioperative hemostasis, approximately one in twenty patients is still in need of re-exploration due to bleeding (46, 60), where a surgical cause of bleeding is found in 60-80% of the cases(60, 61). This leaves a substantial fraction of patients with bleeding due to coagulopathy, where surgical intervention may be avoided if effective countermeasures are initiated. Re-exploration for bleeding is an independent risk factor for increased morbidity and mortality after cardiac surgery and data suggest a two to threefold increase in the risk of death after re-exploration (62, 63). Major bleeding in itself and the re-operation due to bleeding increase the risks of renal failure and arrythmias, as well as the risk of prolonged ventilatory support and prolonged stay at the intensive care unit (64-66).

Blood transfusions in cardiac surgery

Transfusion of blood products is common in cardiac surgery where the use in case of severe bleeding is lifesaving. However, blood transfusions may involve undesirable adverse effects. Today, the risk of transmission of infectious agents is low, but cannot be completely ignored. Immunologic reactions of varying degree, such as hemolysis and anaphylaxis as well as transfusion related lung injury (TRALI) and transfusion associated circulatory overload (TACO) are detrimental side effects. Fatal reactions are rare, occurring in about one in 200 000-400 000 transfused units (67). When stored in a blood bank, the cells do change properties. After a few days, red blood cells gradually lose the ability to bind, transport and deliver oxygen to the tissues, mostly due to a reduction in 2,3-diphosphoglycerate (2,3-DPG) and altered deformability (68). The clinical implications of these so called "storage lesions" are debated, where older RBC's have been suggested to cause more harm than fresh ones (69). However, recent studies have not been able to find associations between length of storage of the RBCs and mortality, nor any increased risk of adverse events such as renal failure, infections, stroke, embolisms, thrombosis or respiratory failure as previously described (70, 71).

 The prevalence of transfusions differs extensively between institutions and reported transfusion rates do vary between 10-85% (72, 73) but are usually around 50-60%. Most of the transfused blood products are allocated to about one fifth of the patients, receiving massive transfusions

(74). Recognized risk factors for the need of allogenic blood transfusion in cardiac surgery are preoperative anemia, the need for re-sternotomy or acute surgery, low body mass index, higher age and preoperative use of drugs affecting hemostasis (74). Since avoidance of unnecessary transfusions is of high priority, not only due to undesireble side effects, but also has health economic benefits, determining safe thresholds for transfusion is crucial. In observational retrospective studies, transfusion of blood products have been associated with poor outcome and reductions of avoidable transfusions during and after cardiac surgery have been suggested to reduce both morbidity and mortality (75-77). On the contrary, recent data from two large randomized studies of restrictive versus liberal transfusion strategies after cardiac surgery did not establish any difference in their primary outcome regardless of transfusion approach (78, 79), although older patients (>75 years) seem to benefit from a more restrictive strategy (78).

Treatment with antifibrinolytics in cardiac surgery

The use of anti-fibrinolytic drugs is widespread in cardiac surgery, since they have been shown to significantly reduce blood loss and allogenic blood transfusion (80). The use also seems to lower the rate of re-operations due to bleeding (81). A causal relationship between the use of antifibrinolytics and mortality has not been established. At our institution, tranexamic acid (TXA) is used as a routine pre- and post-CPB. TXA is usually well tolerated, and has a low incidence of side effects, including thromboembolic complications (80, 81).

TXA prevents the activation of plasminogen to plasmin (82). Consequently, the degradation of fibrin clots is inhibited, Figure 7. The action is mainly competitive, but can be non-competitive with high doses. The inhibition of plasmin generation and activation also seem to reduce plasmin induced platelet activation during CPB (83, 84). Patients receiving TXA perioperatively appear to have a lower grade of inflammatory response postoperatively, which may be a result from reduced fibrinolysis. During CPB, a reduced fibrinolysis leads to a lower plasmin activity along with attenuated plasmin activated pro inflammatory cytokines and complement proteins (85-87). Furthermore, TXA may partly improve platelet aggregation in patients treated with acetyl salicylic acid and clopidogrel due to a mechanism not yet established (88).
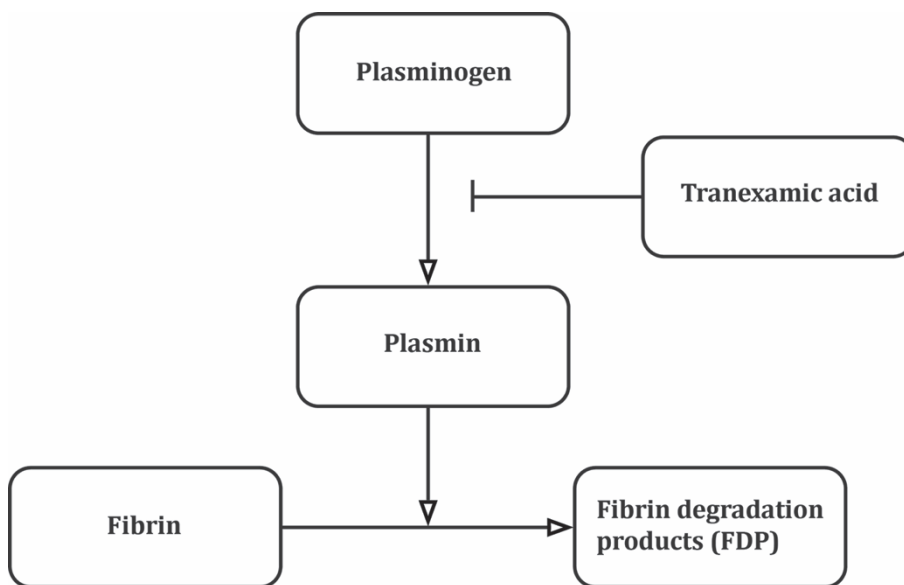
Figure 7. In the fibrinolytic process, fibrin is degraded by plasmin. TXA inhibits the activation of plasminogen to plasmin, thus inhibiting fibrinolysis.

Thromboelastometry in cardiac surgery

In the case of substantial perioperative bleeding, it is important to differentiate between a surgical and a coagulopathic cause. The development of bedside methods to assess coagulation has increased in interest during the last decade since it allows evaluation of the coagulation and can address type of coagulopathy within minutes. Thromboelastometry (ROTEM®) is a viscoelastic analysis method to assess blood coagulation in real time, a technique first described in 1948 (89). A small blood sample is analyzed regarding clot formation, strength and lysis by a rotating sensor in the sample tube. As the viscoelastic strength of the blood sample increases, the sensor is exposed to an increasingly larger force. The result is presented as a graphical reaction curve showed in Figure 8 (90). A clot formation dysfunction leading to coagulopathy can be evaluated after 10-15 minutes in comparison to standard coagulation tests in which the response time can be approximately 60 minutes (91). To evaluate the contribution of fibrinogen to the clot, the analysis ROTEM® FIBTEM is used, where platelets are inactivated by cytochalasin D. In cardiac surgery, thromboelastometry guided transfusion protocols are associated with reduced blood loss and

the need for transfusion of blood products in bleeding patients (92, 93), but there is yet to establish whether ROTEM®-guided transfusion protocols result in reduction in morbidity or mortality.
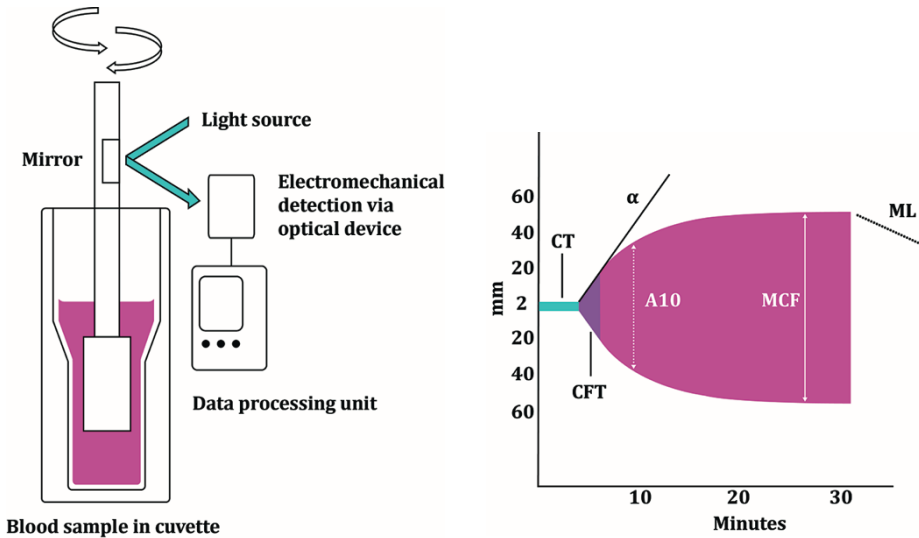

Figure 8. Illustration of the thromboelastometry instrument and an example of a graph. CT (clotting time)= the time it takes for the initial trace of coagulation to reach 2 mm, CFT (clot formation time)= the time it takes for the clot amplitude to increase from 2 to 20 mm, MCF (maximum clot lysis)= the peak amplitude of the clot, ML (maximum lysis)= percent reduction in MCF.

1.4 STUDY OBJECTIVES

Excessive bleeding and re-operation due to bleeding after cardiac surgery remain a risk factor for increased morbidity and mortality (62-64, 94). Being able to identify patients with increased risk of bleeding complications is appealing as it may reveal possibilities to undertake preventative actions. There has been an increasing interest in the relationship between bleeding and plasma concentrations of individual coagulation factors. Several small studies found a significant correlation between preoperative plasma fibrinogen concentration and bleeding volume (95-101), whereas others did not (102-104).

In a previous study from our group, preoperative plasma level of fibrinogen was shown to be an independent risk factor, not only for postoperative bleeding but also for blood transfusion in patients

undergoing elective CABG (100). The study had obvious limitations due to the small-sample size and was conducted on an isolated cohort of elective CABG patients with a low risk of peri- and postoperative bleeding. In the light of the above, our objective in **study I** was to investigate the potential correlation between preoperative plasma fibrinogen levels and postoperative bleeding complications and transfusions in a larger study population consisting of various types of cardiac surgery procedures. A prospective observational study was designed where plasma fibrinogen concentration was measured the day before surgery and evaluated the correlation between fibrinogen concentration and bleeding volume and transfusion of RBC's. In addition, we sought to determine other risk factors for excessive bleeding and transfusions.

To further investigate the role of fibrinogen in postoperative bleeding, a small pilot study (n=20), to test the tolerability and feasibility of prophylactic administration of fibrinogen concentrate to CABG patients with low-normal preoperative plasma fibrinogen concentration, has been performed at our centre. In that study, there were no adverse events related to fibrinogen supplementation and the intervention seemed to reduce the postoperative bleeding volume (105). The study was conducted on a limited number of patients without placebo control, since infusion of fibrinogen to patients without ongoing bleeding had not been studied before. In **study II**, our objective was therefore to confirm the safety and efficacy of prophylactic fibrinogen infusion in patients with fibrinogen levels in the lower normal range undergoing CABG. For this purpose, a randomized placebo-controlled double-blinded single centre study was designed, where the primary endpoint was postoperative bleeding volume.

The use of fibrinogen concentrate to reduce bleeding in patients undergoing cardiac surgery has increased rapidly but little is known about potential side effects and the possible risk of thromboembolic events among patients with acquired hypofibrinogenemia. Data based on spontaneous reports of side effects when fibrinogen concentrate is used, mainly in patients with hereditary hypofibrinogenemia, indicates a beneficial safety profile, with a low incidence of adverse drug reactions such as thromboembolic events (106). More recent reports in acquired hypofibrinogenemia cardiovascular surgery patients have shown diverging results. Two studies confirm the beneficial results(107, 108), but one study reports an association between fibrinogen concentrate administration and ischemic stroke (109). Hence, the study objective in **study III**, was to retrospectively explore the safety of administrating

fibrinogen concentrate to cardiac surgery patients in a large study population.

The use of antifibrinolytics, such as TXA, reduces both peri- and postoperative blood loss and the need for transfusion of blood products in cardiac surgery (80, 81). Consequently, both fibrinogen and TXA improve hemostasis but it is not known whether these procoagulants have additional effects on hemostasis when administered concomitantly, compared to what can be achieved individually. In **study IV**, an in vitro study was designed, hypothesising that the effect of fibrinogen combined with TXA to blood samples from cardiac surgery patients may have an additive pro-coagulant effect compared to fibrinogen alone.

2 STUDY AIMS

I. To investigate the relationship between the preoperative plasma concentration of fibrinogen and the postoperative bleeding volume and transfusions after cardiac surgery (**study I**).

II. To investigate if preoperative administration of fibrinogen concentrate to patients with low normal fibrinogen plasma concentration reduces bleeding and transfusion requirements after elective CABG (**study II**).

III. To investigate if administration of fibrinogen concentrate increases the risk of thromboembolic complications and death after cardiac surgery (**study III**).

IV. To investigate if in vitro fibrinogen supplementation combined with tranexamic acid has an additive pro coagulant effect compared to fibrinogen alone in blood samples from cardiac surgery patients (**study IV**).

3 PATIENTS AND METHODS

3.1 PATIENTS

The four studies were approved by the Regional Ethics Committee and were conducted in accordance with the declaration of Helsinki. In study I and III, the committee waived patient consent due to the observational nature of the studies. In studies II and IV, patients were included after oral and written consent. Study II was approved by the Swedish Medical Products Agency and registered at clinicaltrials.gov (NCT00968045) prior to enrollment. The studies were all performed at the Department of Cardiothoracic Surgery at Sahlgrenska University Hospital, Gothenburg, Sweden. Patient characteristics in the four studies are displayed in Table 1.

	Study I	Study II	Study III	Study IV
n	1954	48	5408	15
Age	66±12	64±8	67±11	65±4
Female gender	489 (25%)	1 (2%)	178 (25%)	1 (7%)
BMI, kg/m2	27±4	27±3	27±4	28±4
Smoking	228 (12%)	20 (4%)	-	3 (20%)
Diabetes	402 (21%)	7 (15%)	1074 (20%)	3 (20%)
EuroSCORE I	5.1±3.0	1.9±1.8	5.3±3.1	-
Platelet inhibition				
with aspirin	1362 (70%)	46 (96%)	3907 (75%)	15 (100%)
DAPT	636 (33%)	12 (25%)	2127 (39%)	-
Fibrinogen g/L	3.8±1.0	3.0±0.4	3.9±1.7	3.3±05
Platelet count x10^9	260±78	231±62	251±77	236±115
Creatinine umol/L	89±55	81±12	91±67	93±4
Hemoglobin g/L	137±15	145±9	137±15	148±11
CPB time, min	95±42	68±19	90±38	73±24
Acute surgery	88 (5%)	-	366 (7%)	-
Operative procedure				
CABG	1075 (55%)	48 (100%)	3069 (57%)	15 (100%)
Valve	541 (28%)	-	1212 (22%)	-
CABG+Valve	205 (10%)	-	651 (12%)	-
Other	133 (7%)	-	476 (9%)	-

Table 1. Patient characteristics in the four studies. Continuous data are shown as mean±SD, categorical data as number (%).

3.2 METHODS

Clinical management

CPB was performed in all patients. Anesthesia was induced with fentanyl, propolipid and rocuronium and maintained with sevoflurane in most cases. During CPB, anesthesia was maintained with propolipid. Before cannulation, heparin was given to keep activated clotting time (ACT) above 480 s. After CPB, protamine was administered to reverse the heparin effect reducing ACT to less than 130 s. All patients received 2 g of tranexamic acid after induction of anesthesia and at the end of surgery.

The CPB circuit consisted of a hollow fibre membrane oxygenator and roller pumps with a standard non-pulsatile technique and hemodilution. The operations were performed in normothermia or mild hypothermia (bladder temperature 35-36°C). Cardio protection was achieved with cold blood cardioplegia. Weaning of CPB was performed after re-warming to a bladder temperature of 36°C. Preoperative aspirin (ASA) was not discontinued before surgery. Ticagrelor and clopidogrel were discontinued three and five days before surgery, respectively, in non-emergent cases.

The local transfusion protocol specified that RBCs should be transfused when hemoglobin (Hb) was below 60-70 g/L or when a clinically significant anemia occurs. Plasma should be transfused in case of significant bleeding and a prolonged clotting time (CT) on thromboelastometry. Platelets should be transfused in case of significant bleeding and a known or suspected platelet dysfunction. Sustained effect of heparin was tested with ROTEM®-HEPTEM or ACT and reversed with protamine. Fibrinogen was administered to patients with ongoing bleeding and signs of impaired fibrinogen function on ROTEM®-FIBTEM analysis. Overall, the final decision regarding transfusions and fibrinogen administration was left to the anestethist in charge.

Postoperative bleeding was defined as the total amount of chest tube drainage during the first 12 postoperative hours after closure of the sternum, or until re-exploration. A bleeding volume >1000 ml in 12 hours was defined as excessive bleeding.

Methods Study I

At total of 2208 adult cardiac operations were performed at Sahlgrenska University Hospital from February 2009 to January 2011. After excluding 170 patients (6.1%) due to missing data, a total of 1954 patients were included in the study. All elective and acute procedures were included: CABG, valve surgery, combined CABG and valve surgery, GUCH surgery, and arrhythmia operations, with an exception of surgical procedures on the ascending aorta. If a patient underwent more than one operation during one hospital admission only the first operation was included in the analysis. We sought to investigate the correlation between preoperative plasma fibrinogen concentration and bleeding and transfusion of RBC's respectively. The included patients were also arbitrarily divided into five groups according to their preoperative fibrinogen concentration ($\leq$2.5, 2.6-3.0, 3.1-3.8, 3.9-4.5 and $\geq$4.6 g/L).

Blood samples were collected the day before surgery in elective cases or immediately before surgery in acute cases. Plasma fibrinogen concentration was determined according to the method by Clauss (5) with a reference value at the time of 2.0-4.5g/L. Activated thromboplastin time (APTT), serum creatinine, Hb, platelet count (PLT) and prothrombin time (PT) were measured preoperatively and analyzed with standard clinical methods. The total amount of RBC's, plasma and platelets during the hospital stay was recorded.

Methods study II

From April 2009 to February 2012, 52 patients were included in the study. Patients were eligible if they were scheduled for first time elective CABG and had a plasma fibrinogen concentration of $\leq$3.8 g/L. Predefined exclusion criteria were known liver- or kidney disease or known bleeding disorder. Low molecular weight heparin (LMWH) was discontinued the day before surgery. Clopidogrel and warfarin were withdrawn at least five days prior to surgery. Acetyl salicylic acid (ASA) was not discontinued.
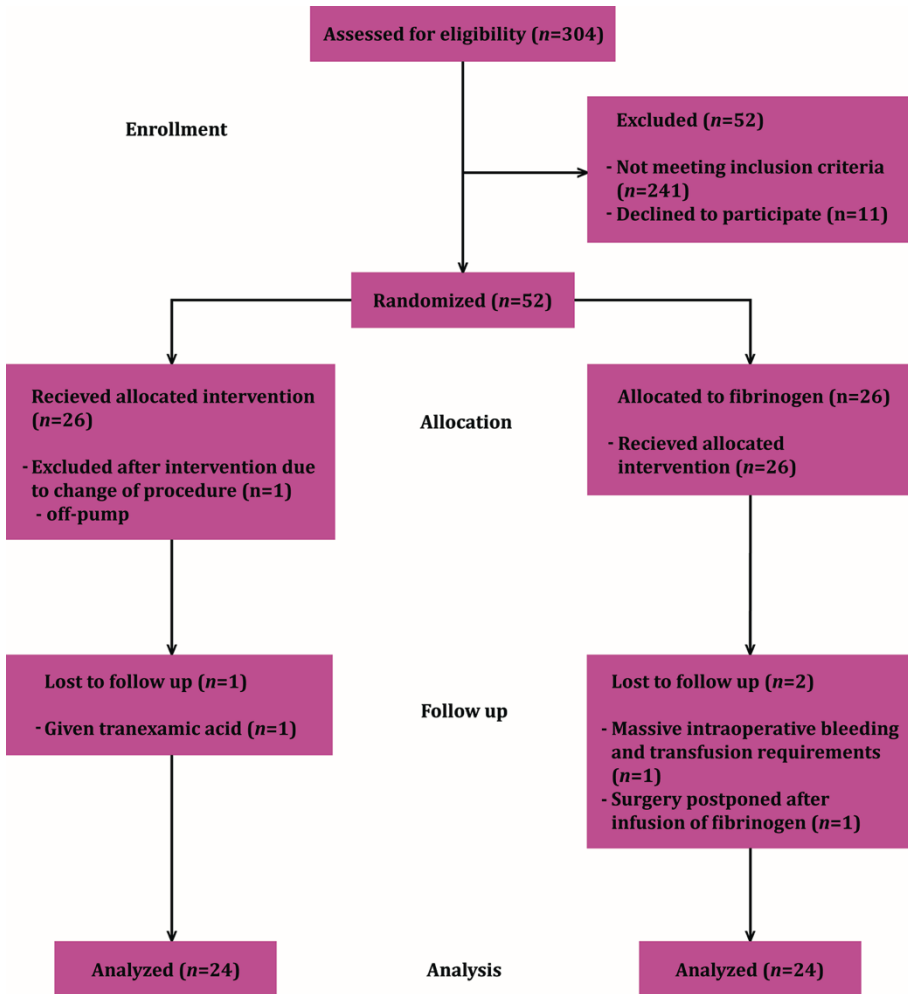
Figure 9. Flow chart for enrollment, allocation, follow up, and analysis.

Patients were randomized to receive either 2 g of fibrinogen concentrate (Haemocomplettan®; CSL Behring, Marburg, Germany) or placebo, Figure 9. Bleeding during the first 12 postoperative hours was compared between the groups. Secondary endpoints were estimated intraoperative bleeding volume, total perioperative bleeding volume, total number of transfused allogenic blood products, proportion of transfused patients, hemoglobin concentrations two and twenty-four hours after CPB, plasma

fibrinogen concentration, PLT, APTT, and PT before, during and after CPB. Preoperative blood samples were collected on the day before surgery.

Subjects were randomized using block randomization with six patients per group where three patients received the study drug and three patients received placebo. A blinded infusion of either the study drug or placebo, prepared by the hospital pharmacy, was administered after induction of anesthesia but before skin incision. Patients and staff were blinded regarding group allocation. The allocation list was not broken until completion of the study and after data analysis.

Plasma fibrinogen concentration was measured according to the method of Clauss with a reference interval of 2.0-4.5 g/L. Hb, PLT ,PT, APTT and serum creatinine level were measured with standard clinical methods. Thromboelastometry was not used routinely, but was utilized in case of bleeding. Fibrinogen was administered outside the study protocol to patients with ongoing bleeding and a ROTEM®-FIBTEM analysis result of less than 10 mm.

Methods Study III

All patients who underwent first-time cardiac surgery at our department between 2009 and 2014 were included in a retrospective observational study. All types of cardiac surgery were included: CABG, valve surgery, combined CABG- and valve surgery, GUCH surgery and arrhythmia operations. Patients who underwent surgery on the ascending aorta, and re-sternotomies were excluded. In total, 6442 patients were assessed for inclusion, and 5408 were included, Figure 10.
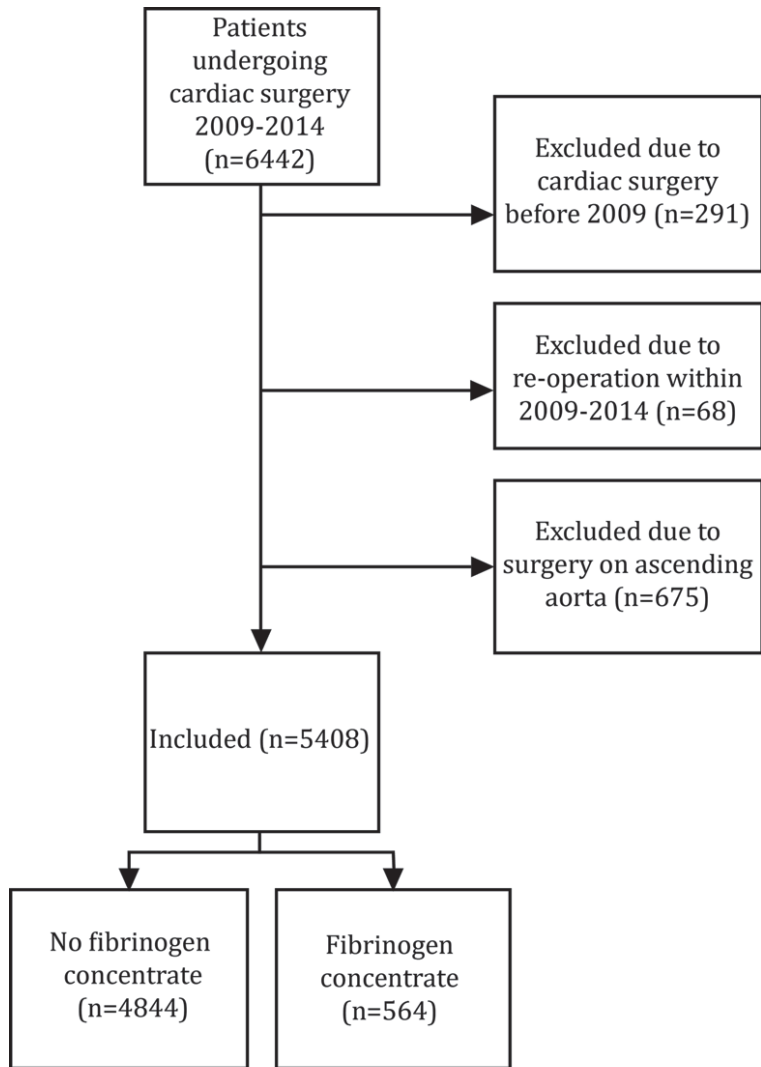
Figure 10. Patients assessed for inclusion in study III.

Patient characteristics, type of surgery, pre- and postoperative laboratory testing, bleeding volume, number of transfusions and fibrinogen administration were retrospectively collected from patient records and the Swedish Heart Surgery Registry (110). The National Patient Registry (NPR) and the Cause of Death Registry supplied data on thromboembolic complications and death (111, 112). The merging of data was possible by using the social security number given to each Swedish citizen.

The incidence of a composite of thromboembolic events and mortality at one year after surgery were compared between patients who received perioperative fibrinogen concentrate and patients who did not. Secondary endpoints were the incidence of the composite endpoint at 30 days and mortality at 30 days and 1 year respectively.

Diagnosis	ICD code
Myocardial infarction	I21
Revascularization	FN
Ischaemic stroke	I63
Mesenterial ischemia	K55
Peripheral arterial embolization	I74
Pulmonary embolization	I26
Deep vein thrombosis	I80
Portal vein thrombosis	I81.9

Table 2. ICD-codes in the composite endpoint.

Methods Study IV

Fifteen patients scheduled for first-time elective CABG between November 2017 to January 2018 and a plasma fibrinogen concentration within the normal range (1.8-3.8 g/L) were included. At the time of this study, the hospital implemented a new reference interval for plasma fibrinogen concentration due to both a new instrument and a new reagent. All included patients were preoperatively treated with ASA which was not discontinued before surgery. Exclusion criteria were known bleeding disorder, renal disease, and on-going treatment with platelet inhibiting agents and anti-coagulant agents that was not discontinued according to institutional practice.

In accordance with our institutional protocol, two grams of TXA was administered after induction of anesthesia, but before surgical incision, and at the end of surgery. Blood samples for clot formation analyses were

collected before and five minutes after both TXA doses. Samples for platelet count, hemoglobin and plasma fibrinogen concentration analyses were collected the day before surgery and immediately prior to the second dose of TXA.

For each subject, two portions at each sample occasion were prepared for ROTEM®-FIBTEM analysis, giving a total of eight samples, presented in Figure 11 and 12. All samples collected before surgery were diluted with Ringer's Acetate to achieve a hemodilution of 21% in order to mimic hemodilution during CPB. After dilution, fibrinogen concentrate was added in a dose corresponding to 3 g fibrinogen given to a 70 kg patient.
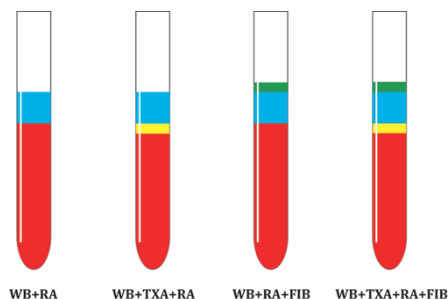
Figure 11. Preoperative samples prepared for thromboelastometry. Baseline sample contains blood diluted with Ringer's Acetate. WB=whole blood(red); RA=Ringers Acetate (blue); TXA=tranexamic acid (yellow); FIB=fibrinogen (green)

Figure 12. Postoperative samples prepared for thromboelastometry. WB=whole blood(red); TXA=tranexamic acid(yellow); FIB=fibrinogen(green)

The clot formation process was assessed with thromboelastometry (ROTEM®). ROTEM®-FIBTEM in which clot formation is activated by tissue factor was used. Changes in clotting time CT), maximum clot firmness (MCF) and clot lysis (ML) were compared between the samples.

3.3 STATISTICAL ANALYSES

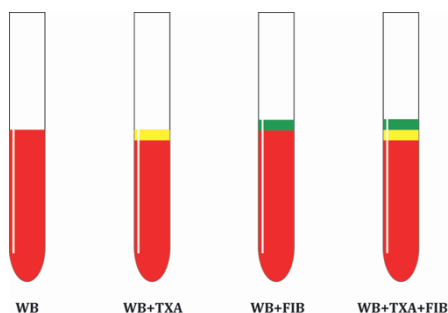For all studies, data are presented as mean with standard deviation (SD), median with interquartile range (IQR) or proportions with percentage (%) as described. Any p-value <0.05 was considered statistically significant.

Study I

Categorical variables were compared between the groups with χ2-test and continuous variables with one way analysis of variance (ANOVA). The importance of fibrinogen concentration for postoperative bleeding and transfusion were analyzed by handling the explanatory and the outcome variable as continuous variables by univariate testing and multiple models with fibrinogen as a continuous variable and bleeding as a dichotomous variable (<1000 ml/12 h and >1000 ml/12 h). Bleeding and transfusions of RBCs were also compared between the subgroups according to preoperative plasma concentration of fibrinogen.

To explore risk factors for bleeding >1000 ml in 12 hours and transfusions, univariate and multivariable logistic regression was performed and presented as odds ratios (OR) with 95% confidence intervals (CI). All factors univariately associated with bleeding or transfusion were used in the multivariable models.

Study II

Normality of distribution was tested with Shapiro-Wilk test. Group comparisons in normally distributed continuous data were made with the Student's t-test and in non-normally distributed data with the Mann-Whitney U-test. The χ2-test was used to compare categorical data. For group comparisons of variables analyzed at more than one occasion, ANOVA for repeated measurements was used, followed by the Students t-test if ANOVA indicated a difference. Changes from baseline within a group were analyzed with a paired t-test.

A power calculation, based on the previously described pilot study was made (105). To show a 30% reduction in bleeding volume in the treatment group, 25 patients were needed in each group with 80% power and a significance level of 0.05.

Study III

Categorical variables were compared between the groups with the χ^2-test or Fisher's test if small numbers. Continuous variables were compared between the groups with the t-test or the Mann-Whitney test depending on variable distribution. To assess the importance of fibrinogen concentrate administration for the composite event and mortality at 30 days postoperatively, univariate and multivariable logistic regression analyses was used. To assess the importance of fibrinogen concentrate administration for survival time and time to the composite event up to one year, univariate and multivariable Cox regression analyses were used. The variables that were statistically significant in the univariate model were included in the multivariable logistic regression model and in the Cox regression model. Two propensity score-based sensitivity analyses were performed, based on the probability of administration of fibrinogen concentrate.

Study IV

To be able to detect a 20% (2 mm) change in FIBTEM-MCF with a standard deviation of 2.5 mm, 80% power and a significance level of 0.05, 12 patients were needed. To achieve marginal, 15 patients were included. The non-parametric Wilcoxon signed paired test was used to compare results from baseline and after addition of TXA, fibrinogen and TXA plus fibrinogen, respectively.

4 RESULTS

4.1 PREOPERATIVE PLASMA CONCENTRATION OF FIBRINOGEN, BLEEDING AND TRANSFUSIONS (I)

The mean plasma fibrinogen concentration in the cohort was 3.8±1.0 g/L. Eight patients (0.41%) had a fibrinogen concentration below the normal reference limit (2.0 g/L) and 370 patients (17%) had a concentration above the normal reference limit (4.5 g/L). Table 3 illustrates patient characteristics in relation to preoperative fibrinogen concentration.

Variable	Fibrinogen Concentration, g/L				
	≤2.5 (n = 105)	2.6-3.0 (n = 340)	3.1-3.8 (n=729)	3.9-4.5 (n = 410)	≥4.6 (n = 370)
Age, y	57±18	63±13	67±11	68±10	67±11
Female gender	16(15)	69 (20)	169 (23)	130 (32)	106 (29)
BMI, kg/m^2	25±4	23±3	27±4	28±4	28±5
Weight, kg	76±13	79±13	82±14	82±14	83±17
CABG	29 (28)	158 (46)	418 (57)	238 (58)	232 (63)
Diabetes	9 (8.7)	46 (14)	149 (20)	101 (25)	97 (27)
CPB time, min	101±48	95±45	94±40	96±43	95±40
Smoking	4 (4)	26 (8)	81 (11)	60 (15)	61 (17)
Acute operation	5 (5)	9 (3)	27 (4)	15 (4)	32 (9)
Aspirin	43 (41)	211 (62)	529 (73)	300 (73)	279 (75)
Clopidogrel (<5 days pre-op)	5 (5)	24 (7)	58 (8)	38 (9)	58 (16)
APTT, s	38±19	36±10	36±6	36±5	37±11
PT (INR)	1.12±0.20	1.09±0.19	1.11±0.28	1.12±0.30	1.11±0.32
Platelet count, ×10⁹/L	235±74	236±63	253±65	270±78	295±99
Serum creatinine, μmol/L	83±20	83±20	87±34	91±55	99±72
eGFR, mL/min	95±33	91±30	89±31	85±31	84±33
Hemoglobin, g/L	143±12	141±13	138±14	137±14	129±16

Table 3. Patient characteristics in relation to preoperative fibrinogen concentration. Continuous data are shown as mean±SD, categorical data as number (%).

Bleeding

The mean bleeding volume was 563±374 ml/12 hours and 96 patients (4.9%) underwent re-exploration due to excessive bleeding or tamponade. Ten percent of the patients had a postoperative bleeding volume of more than 1000 ml/12 hours. There was a weak inverse correlation between preoperative fibrinogen concentration and postoperative bleeding volume (r=-0.17, p<0.001). Patient characteristics and univariate risk factors for bleeding >1000 ml/12 hours are presented

in Table 4. Independent risk factors for excessive bleeding were low fibrinogen concentration, low BMI, longer CPB time, clopidogrel intake <5 days before surgery, low preoperative platelet count and acute operation.

Variable	Bleeding, mL/12 Hours		OR (95% CI)	p Value
	≤1,000 (n = 1,749)	>1,000 (n = 205)		
Age, y	66±12	67±12	1.00 (0.99-10.2)	0.33
Female gender	450 (26)	39 (19)	0.67 (0.47-0.98)	0.037
BMI, kg/m^2	27.1±4.3	25.6±4.0	0.91 (0.88-0.95)	<0.001
Smoking	204 (12)	24 (12)	1.02 (0.65-1.59)	0.95
Diabetes	365 (21)	37 (18)	0.84 (0.58-1.23)	0.38
Pre-op medication				
Aspirin	1,220 (70)	142 (69)	0.97 (0.71-1.33)	0.87
Clopidogrel <5 days pre-op	156 (9)	27 (13)	1.55 (1.001-2.39)	0.0496
Pre-op analyses				
Fibrinogen, g/L	3.8±1.0	3.6±1.0	0.75 (0.64-0.89)	0.001
aPTT, s	36.1±9.1	36.5±5.2	1.00 (0.99-1.02)	0.47
PT (INR)	1.1±0.3	1.1±0.2	1.04 (0.63-1.74)	0.87
Platelet count, ×10^9/L	262±77	245±88	0.997 (0.995-0.999)	0.004
eGFR, mL/min	88±31	84±32	0.996 (0.99-1.001)	0.092
Hemoglobin, g/L	137±15	136±15	0.99 (0.98-1.01)	0.52
CABG	980 (56)	95 (46)	0.68 (0.51-0.91)	0.009
Acute operation	71 (4)	17 (8)	2.14 (1.23-3.70)	0.007
ECC time, min	94±41	110±53	1.01 (1.004-1.010)	<0.001

Table 4. Univariate risk factors for bleeding ≤1000 ml or >1000 ml/Hours (n= 1954). Continuous data are shown as mean±SD, categorical data as number (%).

When dividing patients into subgroups according to their preoperative fibrinogen concentration, the patients with the lower fibrinogen levels had both a higher postoperative mean bleeding volume and a higher percentage of excessive bleeding. Re-operation for bleeding was more common in patients with the highest and the lowest fibrinogen levels, illustrated in Figure 13. In multivariable analysis, patients with the lowest fibrinogen concentration still had a significant increased risk of bleeding >1000 ml/12 hours compared to patients with the highest levels, presented in Table 5.
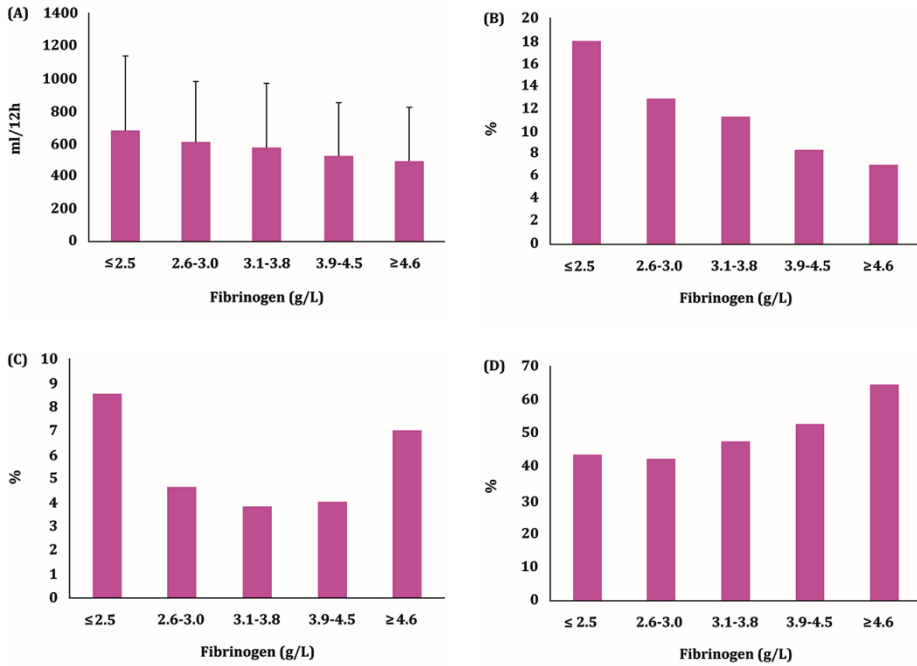
Figure 13. (A)Unadjusted mean postoperative blood loss, (B) blood loss exceeding 1000 ml/12 h (C) re-exploration rate (D) proportions of patients receiving RBC in patients with different fibrinogen concentrations. Error bars indicate SD.

Fibrinogen (g/L)	Univariate OR (95% CI)	p Value	Multiple Model OR (95% CI)	p Value
≤ 2.5	2.92 (1.55-5.53)	0.001	2.31 (1.16-4.59)	0.017
2.6-3.0	1.97 (1.18-3.27)	0.009	1.64 (0.95-2.83)	0.074
3.1-3.8	1.68 (1.06-2.66)	0.028	1.55 (0.96-2.5)	0.075
3.9-4.5	1.20 (0.70-2.03)	0.51	1.17 (0.68-2.03)	0.57
≥4.6	1	-	1	-

CI = confidence interval;   OR = odds ratio

Table 5. Unadjusted and adjusted Odds Ratios (OR) for bleeding over 1000 ml/12 h in subgroups with different fibrinogen concentrations.

Transfusions

There was a weak correlation between preoperative fibrinogen concentration and the number of RBC transfusions (r=0.14, p= <0.001).

Patient characteristics and univariate risk factors for RBC transfusion are presented in Table 6. In the multivariable analysis, low fibrinogen levels were not associated with an increased risk of RBC transfusion (OR 1.10; 95% CI 0.89-1.28 per g/L, p=0.49). Independent factors associated with RBC transfusion were acute operation, impaired renal function, long CPB-time, clopidogrel intake <5 days before surgery and low preoperative hemoglobin concentration. Within the fibrinogen concentration subgroups, there was no significant difference in risk of transfusion of RBC and fibrinogen concentration in the multivariable analysis.

Variable	No Red Cell Transfusion (n = 964)	Red Cell Transfusion (n = 990)	OR (95% CI)	p Value
Age, y	64±12	68±11	1.03 (1.02-1.04)	< 0.001
Female gender	158 (16)	331 (33)	2.57 (2.06-3.18)	< 0.001
BMI, kg/m^2	27.4±4	26.5±4	0.95 (0.93-0.97)	< 0.001
Smoking	106 (11)	124 (13)	1.19 (0.90-1.57)	0.223
Diabetes	183 (19)	219 (22)	1.22 (0.98-1.52)	0.073
Pre-op medication				
Aspirin	682 (71)	680 (69)	0.91 (0.75-1.11)	0.36
Clopidogrel <5 days pre-op	58 (6)	125 (13)	2.26 (1.63-3.12)	< 0.001
Pre-op analyses				
Fibrinogen, g/L	3.7±0.9	4.0±1.1	1.36 (1.24-1.49)	< 0.001
aPTT, s	36±8	37±10	1.02 (1.00-1.03)	0.007
PT (INR)	1.1±0.2	1.1±0.3	1.64 (1.15-2.34)	0.006
Platelet count, ×10^9/L	257±70	263±85	1.001 (1.000-1.002)	0.095
eGFR, mL/min	96±31	80±30	0,98 (0.97-0.99)	< 0.001
Hemoglobin, g/L	143±12	131±15	0.94 (0.93-0.95)	< 0.001
CABG	599 (62)	476 (48)	0.56 (0.47-0.68)	< 0.001
Acute operation	24 (2.5)	64 (6.5)	2.71 (1.68-4.36)	< 0.001
CPB time, min	84±31	106±48	1.015 (1.012-1.018)	< 0.001

Table 6. Univariate risk factors for RBC transfusion (n=1954). Continuous data are shown as mean±SD, categorical data as number (%).

4.2 PROPHYLACTIC TREATMENT WITH FIBRINOGEN CONCENTRATE (II)

Mean preoperative plasma fibrinogen concentration was 3.0±0.4 g/L in the treatment group and 3.0±0.4 in the placebo group (p=0.81). Infusion of fibrinogen concentrate increased plasma fibrinogen concentration by 0.2±0.4 g/L and infusion of placebo decreased the concentration by 0.2±0.4 g/L (p= 0.004 between the groups).

There was no significant difference between the groups in median postoperative bleeding within 12 hours, nor in any other bleeding estimates or transfusions. Five patients in each group bled more than 1000 ml. Variables of postoperative bleeding and transfusions are displayed in Figure 14 and Table 7. Changes of fibrinogen concentration over time are presented in Figure 15.
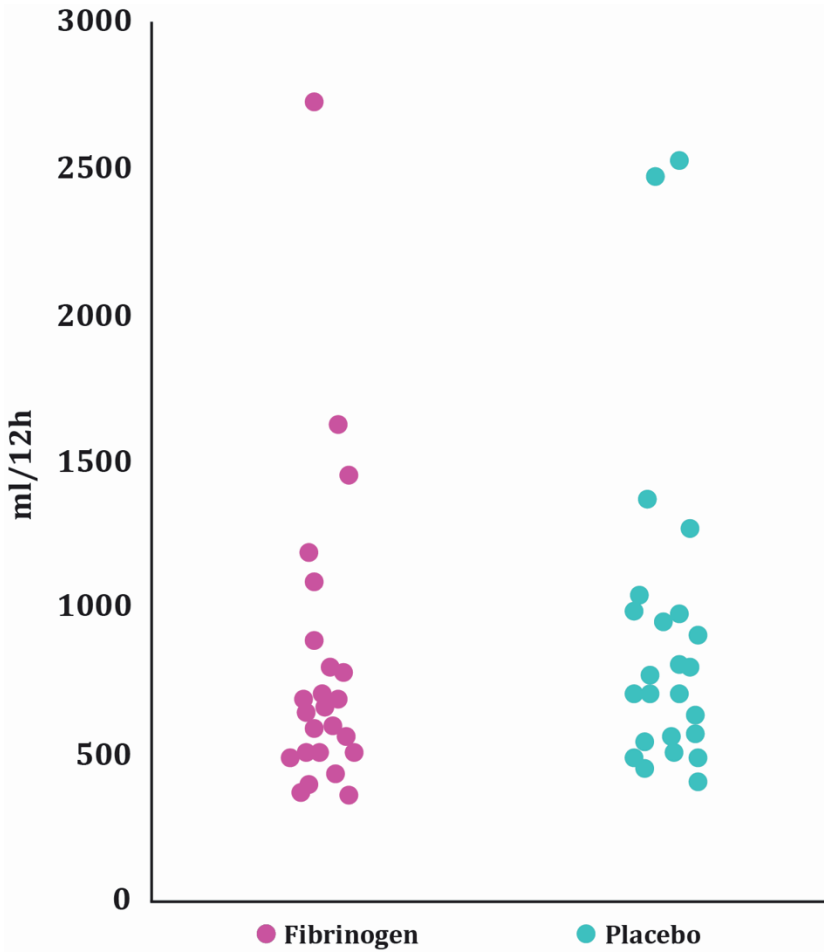
Figure 14. Postoperative bleeding volume in individual subjects. There was no significant difference in median bleeding volume between the groups (p=0.29).

	Fibrinogen (n=24)	Placebo (n=24)	p-value
Total bleeding 12 hours			
Mean	1103 (518)	1272 (588)	0.30
Median	913 (815-1230	1185 (930-1398)	0.18
Re-operation for bleeding	1 (4.2%)	2 (8.4%)	0.55
RBC transfusion			
Number of patients	6 (25.0%)	6 (25.0%)	1.00
Mean (units)	0.63 (1.17)	1.33 (3.11)	0.30
Median (units)	0 (0-1)	0 (0-1)	0.88
Fibrinogen (number)	3 (12%)	7 (29%)	0.55

Table 7. Bleeding and transfusions. Continuous data are shown as mean±SD and median (25th and 75th percentiles), categorical data as number (%).
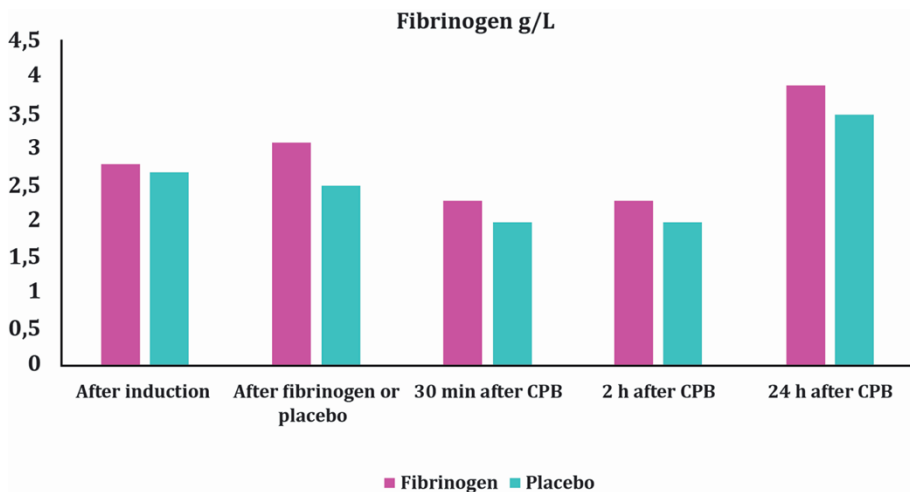

Figure 15. Changes in fibrinogen concentration (g/L) over time.

Aortic clamp time and CPB time were significantly longer and preoperative Hb and Hct were significantly higher in the treatment group than in the placebo group. There were no clinically detectable side effects

of fibrinogen infusion. One patient in the placebo group had signs of postoperative ischemia and heart failure where angiography revealed an occluded vein graft. Fibrinogen concentrate was administered postoperatively outside the study protocol to three patients in the treatment group and to seven in the placebo group.

4.3 FIBRINOGEN ADMINISTRATION AND RISK OF THROMBOEMBOLIC COMPLICATIONS (III)

Patient characteristics, divided into patients who received fibrinogen concentrate and patients who did not, are displayed in Table 8.

One year outcome

After one year, nearly 10% of the included patients had had at least one event included in the composite endpoint and the mortality rate was 4.8%, Table 9. The patients who did receive fibrinogen concentrate did not have a higher risk for thromboembolic complications or death after adjustment for covariates, Table 10, Figure 16.

Variable	Fibrinogen (n = 564)	No fibrinogen (n = 4844)	p Value
Male sex	438 (78%)	3592 (74%)	0.074
Age, years	68±11	67±11	0.14
Body mass index	26±4	27±4	<0.001
Pre-op fibrinogen concentration, g/L	3.2 (2.8-3.8)	3.7 (3.2-4.4)	<0.001
Pre-op hemoglobin, g/L	138±15	136±15	0.005
Pre-op platelet count, ×10^9/L	234±71	253±77	<0.001
Pre-op prothrombin time, INR	1.1 (1.0-1.2)	1.0 (1.0-1.1)	<0.001
Pre-op serum-creatinine, μmol/L	85 (74-98.0)	84.0 (72-98)	0.015
Left ventricular ejection fraction >50%	398 (71%)	3494 (72%)	0.25
Pre-op ASA treatment	391 (73%)	3516 (76%)	0.29
Pre-op dual antiplatelet therapy	240 (43%)	1887 (39%)	0.098
Hypertension	273 (48%)	2531 (52%)	0.084
Atrial fibrillation	75 (13%)	728 (15%)	0.27
Previous myocardial infarction	147 (26%)	1360 (28%)	0.31
Previous stroke	43 (8%)	294 (6%)	0.15
Diabetes	130 (24%)	944 (20%)	0.037
EuroSCORE I	6 (3-8)	5 (3-7)	<0.001
CPB time, min	88 (69-128)	81 (64-107)	<0.001
Surgical procedures			<0.001
Valve surgery	130 (23%)	1082 (22%)	
CABG	273 (48%)	2796 (58%)	
CABG+Valve	114 (20%)	537 (11%)	
Other	47 (9%)	429 (9%)	

Table 8. Patient characteristics, divided into patients who received fibrinogen concentrate and those who did not. Continuous data are shown as mean±SD and median (25th and 75th percentiles), categorical data as number (%).

	No fibrinogen (n = 4844)	Fibrinogen (n = 564)	p Value
Composite endpoint (thromboembolic events or mortality or new revascularization or myocardial infarction)	480 (9.9%)	85 (15%)	<0.001
Mortality	200 (4.1%)	58 (10%)	<0.001
All thromboembolic events	120 (2.5%)	17 (3.0%)	0.44
New revascularization	78 (1.6%)	4 (0.7%)	0.10
Myocordial infarction	93(1.9%)	11 (1.9%)	0.87
Ischaemic stroke	95 (2.0%)	9 (1.6%)	0.55
Deep vein thrombosis	4 (0.1%)	0	-
Pulmonary embolism	13 (0.3%)	7 (1.2%)	<0.001
Mesenterial ischaemia	4 (0.1%)	0	-
Peripheral arterial embolism	4 (0.1%)	1 (0.2%)	0.42
Thrombosis of the portal vein	1	0	-

Table 9. Unadjusted outcome variables at one year after surgery in patients who did and did not receive fibrinogen concentrate perioperatively.

End point	No fibrinogen (n = 4844) n(%)	Fibrinogen (n = 564) n(%)	HR with 95% CI	p Value
Composite endpoint[a]	480 (10%)	85 (15%)	1.56 (1.24-1.97)[b]	<0.001
			1.11 (0.84-1.46)[c]	0.45
Mortality	200 (4%)	58 (10%)	2.58 (1.93-3.46)[d]	<0.001
			1.38 (0.93-2.04)[e]	0.11

Table 10. Association between fibrinogen concentrate administration and composite endpoint and mortality during the first year after surgery. Hazard ratios (HR) based on Cox regression models are presented.[a] composite endpoint [b] unadjusted HR [c] adjusted HR adjusted for age, sex, BMI, diabetes, preoperative antiplatelet therapy, plasma fibrinogen concentration, Hb, PLT count, preoperative serum-creatinine, PT, history of ischemic stroke, EuroSCORE I, CPB time, surgical procedure and total perioperative transfusions [d] unadjusted HR, HR adjusted for see [c].
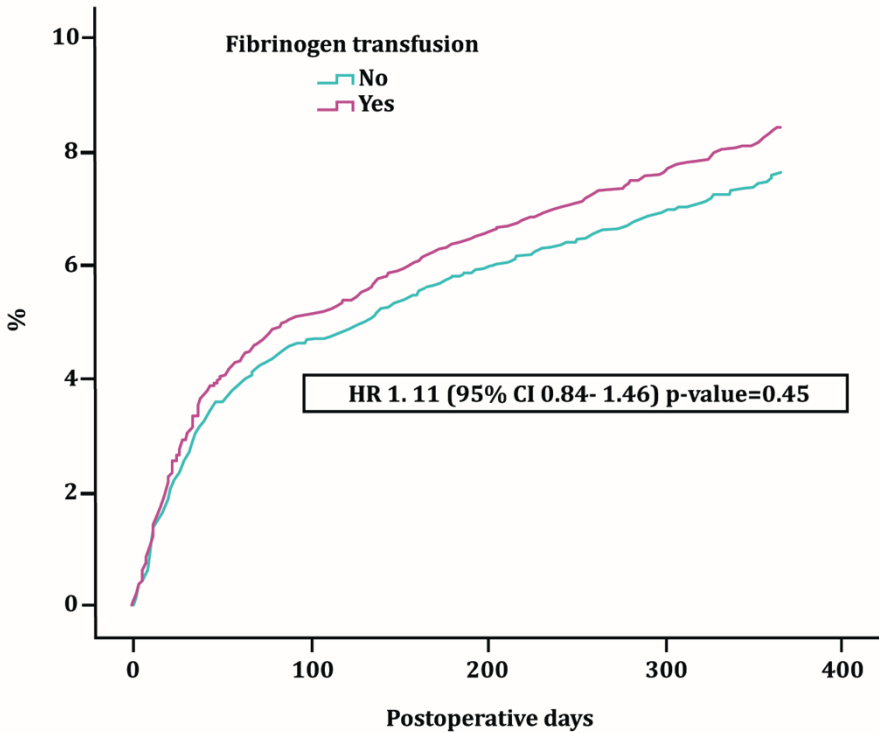
Figure 16. Adjusted incidence of the composite endpoint during the first postoperative year.

30 days outcome

At 30 days, 3.5 % of the patients had had an event included in the composite endpoint and the mortality rate was 2.3%. The patients who did receive fibrinogen concentrate did not have a significantly higher risk of thromboembolic complications or death after adjustment for covariates, Table 11.

End point	No fibrinogen (n = 4844)	Fibrinogen (n = 564)	Odds ratio with 95% CI	p Value
	n(%)	n(%)		
Composite endpoint[a]	151 (3.1%)	40 (7.1%)	2.37 (1.65-3.40)[b]	<0.001
			1.07 (0.64-1.81)[c]	0.79
Mortality	93 (1.9%)	32 (5.7%)	3.07 (2.04-4.64)[d]	<0.001
			1.00 (0.51-1.96)[e]	0.50

Table 11. Associations between fibrinogen concentrate administration and composite endpoint and mortality 30 days after surgery. Odds ratios (OR) based on univariate and multivariable logistic regression models are presented. [a] Composite endpoint [b] unadjusted OR [c] OR adjusted for age, sex, preoperative antiplatelet therapy, preoperative plasma fibrinogen concentration, platelet count, preoperative serum creatinine, prothrombin time, history of ischemic stroke, CPB time, diabetes, total perioperative transfusions, EuroSCORE 1, surgical procedure [d]Unadjusted OR for mortality [e] OR for mortality adjusted for age, sex, preoperative prothrombin time, preoperative fibrinogen concentration, hemoglobin concentration, preoperative platelet count, antiplatelet therapy, preoperative serum creatinine, CPB time, EuroSCORE 1, surgical procedure, total perioperative transfusions.

4.4 FIBRINOGEN SUPPLEMENTATION AND TRANEXAMIC ACID ADMINISTRATION (IV)

Median preoperative plasma fibrinogen concentration was 3.3 (2.9-3.6) g/L. The hemoglobin concentration, platelet count, hematocrit and fibrinogen concentration were significantly reduced at the end of surgery. None of the samples showed signs of fibrinolysis after 30 minutes.

Fibrinogen supplementation, with or without the presence of TXA shortened CT and increased MCF, both preoperatively and postoperatively. Before surgery, fibrinogen supplementation shortened CT somewhat more after TXA administration while there was no difference in effect in relation to TXA administration after surgery, Figure 17. The enhancing effect of fibrinogen supplementation on MCF did not differ significantly before and after TXA administration neither before nor after surgery, Figure 18.
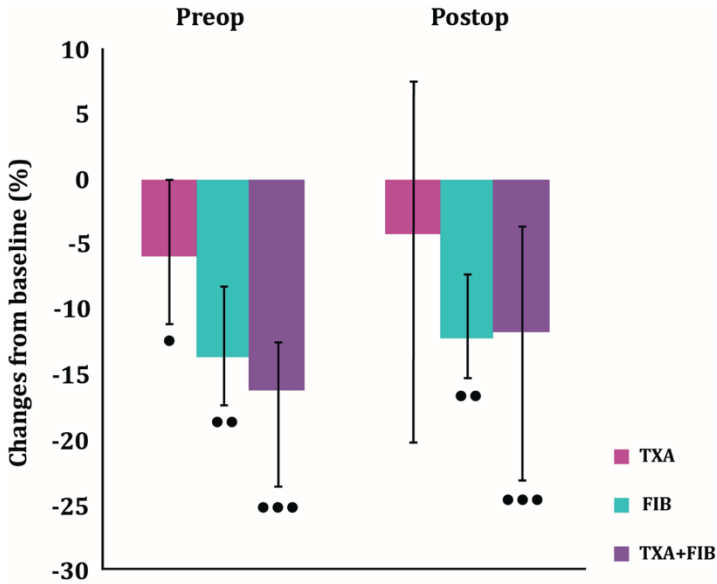
Figure 17. Changes from baseline in CT from after addition of TXA, fibrinogen or both in %. Baseline CT was 72 (70-78) and 107 (81-118) seconds in the pre- and postoperative samples, respectively. Median and 25th and 75th percentiles. • p<0.05, •• p<0.01 ••• p <0.001 vs baseline.
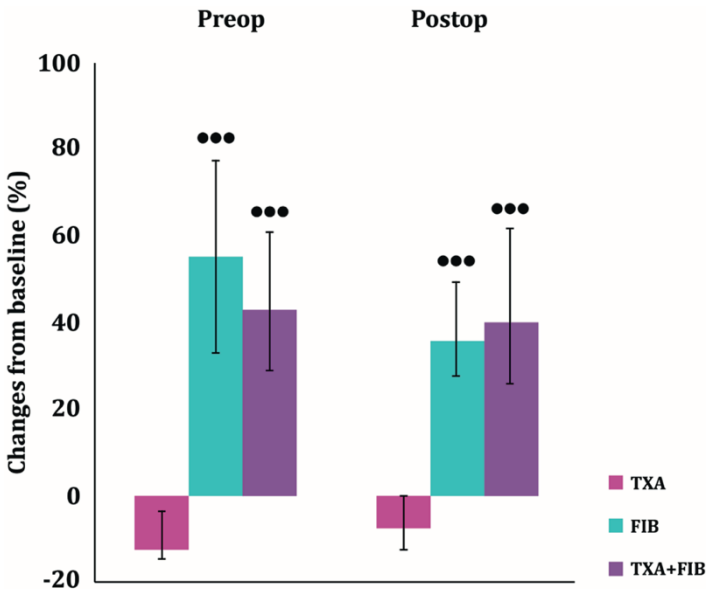

Figure 18. Changes from baseline in MCF after addition of TXA, fibrinogen or both in %. Baseline MCF was 14 (9-15) and 14 (13-15) mm in the pre- and postoperative samples, respectively. Median and 25th and 75th percentiles. •p<0.05, •• p<0.01 ••• p <0.001 vs baseline.

5 DISCUSSION

5.1 FIBRINOGEN CONCENTRATION, BLEEDING AND TRANSFUSIONS (I)

Fibrinogen and its role in hemostasis and bleeding has been thoroughly investigated in the last decade. For many years, a plasma fibrinogen concentration of at least 1.0 g/L was considered to be enough to ensure a stable clot formation not limited by fibrinogen concentration in case of bleeding (21, 113). In study I, the main finding was that preoperative plasma concentration of less than 2.5 g/L was independently associated with increased postoperative bleeding. Fibrinogen levels below the normal reference level are rare in patients without ongoing bleeding and the low prevalence of low fibrinogen levels was confirmed in our cohort. Only 0.4 % of the patients had a fibrinogen concentration below 2.0 g/L. The question is what endogenous fibrinogen concentration that is sufficient for maintaining a stable coagulation process when the system is challenged during major surgical procedures, like cardiac surgery with CPB and/or ongoing bleeding. During CPB, there is a decrease in fibrinogen concentration by approximately 30%, mainly caused by hemodilution during CPB (103, 114). A preoperative level of 2.5 g/L, which in our study occurred in about 5 % of the patients, is thereby reduced to approximately 1.75 g/L, which is under the normal reference range, and on the verge of the level where recent guidelines recommend fibrinogen substitution in case of bleeding (115). Hemodilution also reduces the concentration of other coagulation factors (103). However, supplementation with only fibrinogen concentrate normalized MCF, at least in vitro (114). This indicates that fibrinogen might be a more important limiting factor for whole blood clotting than other coagulation factors and platelets in the case of hemodilution.

In this study, we found a weak (r=-0.17), but statistically significant correlation between preoperative concentration of fibrinogen and postoperative bleeding volume, but far too weak to establish any clinical conclusions. This is in contrast to several other studies (98, 100, 116) and a meta-analysis by Gielen et al (117) which showed stronger correlations. Differences in study populations may explain the variances.

The relationship between low preoperative levels of fibrinogen concentration became more apparent when we instead of overall bleeding volume focused on the risk of excessive bleeding (defined as bleeding

>1000 ml/12 h). The reduction of fibrinogen by 1g/L (one unit), increased the risk of excessive bleeding by 25%, even after correction for other factors that have an impact on postoperative bleeding. Moreover, patients in the group with the lowest levels of fibrinogen, had an almost threefold risk of excessive bleeding compared to the patients with the highest fibrinogen levels. This study thus supports the theory that low plasma fibrinogen concentration is associated with excessive bleeding. But, even if fibrinogen turned out to be an independent predictor of postoperative bleeding, the relative importance of fibrinogen level is difficult to evaluate. The results from the multivariable analyses implicate that other factors such as acute operation, ongoing or recently stopped dual antiplatelet therapy and duration of the operation are more important.

Transfusion of blood products can be lifesaving but it is also related to complications such as acute lung injury, hemolytic reactions, allergic reactions, possible transfer of unknown pathogens and immunomodulation. Over the years, it has been suggested that allogenic blood transfusions lead to increased morbidity and mortality in patients undergoing cardiac surgery. It has in fact been suggested that as little as one or two units of RBCs do increase the mortality risk and severe complications such as infections and ischemic events (118, 119). However, the evidence comes from retrospective observational studies where it is difficult to adjust for the worse condition of patients receiving transfusions. More recent prospective randomized studies comparing liberal and restrictive transfusion protocols have shown somewhat diverging results. In the TITRE-2 study (79) the restrictive transfusion protocol was associated with an increased mortality risk, while in the TRICS study (78) no such association was found. Contrarily in the latter study, older patients seem to benefit from a restrictive transfusion protocol, regarding mortality risk.

In study I, univariate analysis indicated that a high preoperative fibrinogen concentration increased the risk of blood transfusion. However, these patients were older, had lower preoperative hemoglobin levels, were more often female and more likely to be undergoing non-elective surgery. All of these are already known risk factors for transfusions. Hence, after adjustment, there was no longer an association between preoperative fibrinogen levels and transfusions. Other studies have however found associations between low fibrinogen concentration and transfusion requirements (100, 101, 120) while some studies, including study I, have, not found any associations after adjustment for confounding factors (102, 121).

Interestingly, postoperative bleeding volume did not appear to be a risk factor for transfusion. This emphasizes that the decision to transfuse is far more complex than simply substituting a loss of blood. It is therefore a challenge to draw conclusions about bleeding and transfusions, since several of the variables that influence transfusion, are also related to postoperative bleeding volume and low fibrinogen levels.

5.2 TREATMENT WITH FIBRINOGEN CONCENTRATE (II)

Two previous small studies from our group have shown an inverse relationship between the preoperative fibrinogen concentration and the postoperative bleeding volume after cardiac surgery (98, 100). In 2008, a small randomized pilot study was conducted, where patients undergoing CABG surgery were randomized to receive either 2 g of fibrinogen concentrate or nothing before the surgery (105). The study focused on safety aspects including graft patency. The results were in favor of prophylactic fibrinogen supplementation since there was a significant decrease in postoperative bleeding among the patients who had received fibrinogen concentrate. No significant increase in adverse events was detected. Based on these findings, we performed a larger study in study II to further investigate the suggested benefit of prophylactic treatment with fibrinogen concentrate to reduce bleeding and blood transfusions after CABG surgery. However, the results of the pilot study regarding efficacy were not reproduced in the present study. There was no significant difference between the groups regarding postoperative bleeding volume, nor hemoglobin levels, while no safety concerns occurred. The present study had an improved study design (randomized, blinded, placebo controlled) and was carried out on a larger cohort of patients and is thus more valid than the initial pilot study. Hence, the study gives no support for the concept of prophylactic administration of fibrinogen concentrate to low-risk CABG patients with low-normal preoperative fibrinogen levels. This does not exclude that there may be patients who would benefit from prophylactic fibrinogen administration, e.g. those with lower fibrinogen concentrations and/or more complex procedures. In study I, 18% of the patients with a fibrinogen concentration below 2.5 g/L had an excessive postoperative bleeding, when compared to 7% of the patients with a fibrinogen concentration ≥4.6 g/L. In a group consisting of CABG patients only, the difference was even larger 15 % vs 3.5%, i.e. a fourfold increase in bleeding among patients with low fibrinogen levels. These large differences indicate that increasing fibrinogen levels might be

beneficial in patients with really low preoperative fibrinogen concentrations, given the increased mortality risk in patients with excessive bleeding (62, 64).

Guidelines from the European Society of Anesthesiology 2013 comprised a recommendation to consider a prophylactic infusion of fibrinogen in patients with a level <3.8 g/L undergoing CABG, based on our pilot study (122). After study II, this recommendation is now withdrawn. Considering the patient cohort with a level under 2.5 g/L, there might be a rationale for prophylactic treatment, where less than 5% of the patients would be treated (123).

During the past years, a few prospective randomized studies has been carried out with the notion to use fibrinogen concentrate to reduce bleeding complications and transfusion requirements in cardiovascular surgery (124-126). Most of the studies have been focusing on complex cardiac surgery, with a higher risk of bleeding than the elective CABG patients included in our pilot study and in study II. A significant reduction of both postoperative bleeding volume and transfusion of allogenic blood products was observed in a study carried out on higher risk cardiac surgery patients, where fibrinogen concentrate administration was given after weaning of CPB (125). The median dose was 4 g and based on calculated target FIBTEM. Another study, with primary endpoint to reduce intraoperative blood loss in complex cardiac surgery (mean dose 3 g/L, target >2.5g/L) after weaning of CPB and before sternum closure did not result in reduced bleeding volume (126). Hence, the question if the use of fibrinogen concentrate might reduce bleeding induced morbidity and mortality is still to be answered. Currently, the recommendation to use fibrinogen concentrate is restricted to patients with ongoing bleeding and probable or confirmed hypofibrinogenemia (115, 127, 128).

5.3 FIBRINOGEN ADMINISTRATION AND RISK OF COMPLICATIONS (III)

In study III, we compared patients who had received fibrinogen concentrate due to bleeding in the perioperative period, with patients who had not received fibrinogen concentrate. The aim of the study was to investigate if administration of fibrinogen concentrate is associated with increased risk of thromboembolic complications and /or death analyzed

30 days and one year after surgery. A composite endpoint was used and the results showed no increased risk of neither thromboembolic complications nor death within this follow-up time.

Besides its essential effects on hemostasis, elevated fibrinogen levels indicate inflammation and is also a strong marker for cardiovascular disease and mortality. It is well known that increased local aggregation of platelets in atherosclerotic lesions, in which fibrinogen has a major role, is a process that is the beginning of a vaso-occlusive thrombotic event such as myocardial infarction and ischemic stroke. Furthermore, elevated fibrinogen levels are frequently seen in patients with transient ischemic attacks and ischemic stroke (15).

The acute effects on hemostasis and the correlation between elevated plasma concentrations of fibrinogen and cardiovascular disease raise the question whether it is safe to administer fibrinogen as a procoagulant treatment in case of bleeding complications. Further, the potential long-term side effects such as thromboembolic complications when administrating fibrinogen concentrate are not completely known.

Studies focusing on the safety of fibrinogen concentrate administration to reduce perioperative bleeding have shown diverging results. Fassl et al compared two PS-matched groups from an original cohort of 991 cardiac surgery patients in which 20% received fibrinogen concentrate (107). The study did not detect any increased risk of thromboembolic events or mortality within 30 days or at one year. Maeda et al studied a cohort of 1047 patients undergoing thoracic aortic surgery in which 24% of the patients received fibrinogen concentrate or cryoprecipitate (108). In a PS-based analysis, there was no significant difference in thromboembolic events or mortality at 30 days. In contrast to these two studies, Jakobsen et al found that perioperative administration of fibrinogen was an independent risk factor for postoperative stroke and renal failure in a cohort of 1876 patients of whom 10% had received fibrinogen concentrate (109).

The present study, which is markedly larger than the previous ones, confirms the results from Fassl's and Maeda's et al (107, 108). The conflicting results in Jakobsen's study (109) might be explained by the statistical approach since the results were neither adjusted for excessive bleeding, nor transfusion requirements. In study III we adjusted for transfusion requirements while the study by Fassl et al adjusted for the number of transfused blood products and incidence of major bleeding. It

is clear from our analyses that the unadjusted mortality and the incidence of thromboembolic events and death were higher in patients who had received fibrinogen concentrate. The difference between the two groups ceased when a multivariable analysis was done. The present results suggest that the higher unadjusted incidence of mortality and thromboembolic complications which was found in fibrinogen treated patients are likely explained by bleeding complications and not by the actual fibrinogen administration per se.

5.4 EFFECTS OF TRANEXAMIC ACID AND FIBRINOGEN CONCENTRATE (IV)

Little is known about the potential combined effect of TXA and fibrinogen on hemostasis and bleeding in cardiac surgery. Based on a previous in vitro study by He et al (129) in which fibrinogen supplementation combined with TXA counteracted fibrinolysis in platelet poor plasma, we investigated if the global hemostatic effects of fibrinogen concentrate analyzed by thromboelastometry were further enhanced in the presence of TXA. The study was conducted using blood samples from cardiac surgery patients. In contrast to our hypothesis, we did not observe any additional effects on clot stability in the presence of TXA. Fibrinogen supplementation, both with and without TXA, had a significant effect on both clotting time and clot stability. One reason might be that the present study was conducted on whole blood with cardiovascular disease compared to platelet poor plasma from healthy donors in the study by He et al. The effect of patient's preoperative medication such as ASA and different analyzing methods may also be contributing factors.

Improved hemostasis after fibrinogen supplementation has previously been demonstrated in a study by our group, where in vitro fibrinogen administration improved clotting time and clot stability in blood samples from cardiac surgery patients (130). This indicates that fibrinogen per se has a more pronounced effect on clot stability compared to TXA, which would be expected given the different roles of fibrinogen and TXA in the coagulation process. Fibrinogen has a central role in the formation of a clot and substitution of this to diluted blood may therefore stimulate both clot formation and clot stability (131, 132). In comparison, TXA reduces fibrin degradation and has in theory no effect on the actual formation of the clot (133).

TXA as an anti-fibrinolytic drug is used worldwide during cardiac surgery to reduce bleeding and transfusion requirements (81, 134). Fibrinogen is increasingly used to treat ongoing bleeding among these patients, based on studies indicating that fibrinogen concentrate can reduce bleeding caused by low fibrinogen levels, hemodilution and thrombocytopenia (131, 132). In theory, there is a potential risk that the combination of TXA, which inhibits fibrin degradation, and fibrinogen concentrate might be thromboembolic. The results from He et al might suggest this, but the results from the present study do not. Larger in vivo studies analyzing the combined effect of TXA and fibrinogen in a clinical setting could answer if there is any significant clinical effect.

5.5   LIMITATIONS

All four studies were carried out in a single center which raises the question about generalizability. Clinical parameters such as transfusion guidelines, discontinuation of anticoagulation and perioperative drugs may vary between different institutions. Study I and III were both observational studies and there may be inherent limitations such as unrecorded confounding factors and selection bias.

In study I and II, postoperative bleeding volume was the primary endpoint. Bleeding volume is not an ideal endpoint since there are several factors apart from coagulation status that influence postoperative bleeding. Also, unknown confounding factors related to surgery and postoperative intensive care might have an impact on the results. Despite the use of postoperative drainage with container, estimated bleeding volume remains an inconstant measurement. Although other more clinically relevant endpoints such as morbidity and mortality could be more preferable, this would necessitate considerably larger study populations. Several of the variables predicting transfusion were also related to postoperative bleeding volume and low fibrinogen levels, which may obscure the statistical analyses.

In study II, 21% of the patients received fibrinogen concentrate outside the study protocol which is unusually high for routine CABG surgery with low expected bleeding volume. An explanation may be that the included patients did not receive TXA due to a theoretical risk of increased thromboembolic complications when administrating TXA combined with prophylactic fibrinogen concentrate. The patients in study II also had a

significantly larger than normal postoperative bleeding volume (690 ml vs 470 ml) compared to previously reported bleeding volume in Study I, which might be explained by the lack of TXA. Due to low inclusion rate, study II was stopped prematurely after three years. A total of 48 patients were analyzed instead of 50, which was less than the power calculation implied to prove significance. This may in theory cause a risk of a statistical type 2 error.

Study III was a retrospective study. The indication for administering fibrinogen may differ between treating physicians. Neither was it possible to collect reliable information about perioperative myocardial infarctions from the registries. This potential side effect of fibrinogen concentrate administration could therefore not be analyzed. On the other hand, this is the largest cohort study so far analyzing the effects of fibrinogen concentrate administration and potential thromboembolic complications and death. Furthermore, the registries used, NPR and SWEDEHEART have high validity and are carefully monitored (111, 112, 135). A prospective randomized controlled study evaluating fibrinogen concentrate in the setting of postoperative bleeding and using thromboembolic complications as endpoint would be more ideal, but might be ethically questionable given the known effect of fibrinogen on hemostasis and the lack of side effect in study III and other studies (107, 108).

As study IV was an in vitro study, there is indeed the question to which extent the results are applicable in the clinical setting. Thromboelastometry on whole blood was used to measure clot formation and clot lysis, but the potential interactions of blood vessels and vascular endothelium are lacking. Also, the analyses were performed on blood samples from patients without ongoing excessive bleeding and without fibrinolysis. The results may not be applicable to cardiac surgery patients with ongoing bleeding and/or fibrinolysis. In addition, only the FIBTEM-test was performed, and fibrinolysis was only assessed by measuring maximum clot lysis. These are both selected analyses which display coagulation in part and not the global hemostasis in the patient.

6 CONCLUSIONS

I. Preoperative plasma concentration of less than 2.5 g/L is independently associated with increased postoperative bleeding. Preoperative fibrinogen concentration was not associated with RBC transfusion.

II. Preoperative supplementation with fibrinogen concentrate did not reduce postoperative bleeding in CABG patients with low risk of bleeding and normal preoperative fibrinogen concentration.

III. There was no increased risk of thromboembolic complications or death in patients who had received perioperative administration of fibrinogen concentrate.

IV. The enhancing effects of fibrinogen on clot firmness in blood samples from cardiac surgery patients are not further increased in the presence of tranexamic acid.

7 FUTURE PERSPECTIVES

Bleeding and related complications will most probably remain an important issue in cardiac surgery. The quest to find new methods to prevent and handle excessive bleeding will continue. Strategies can include less invasive surgical methods, new treatments to enhance hemostasis in case of bleeding and improved monitoring of perioperative hemostasis. Identifying risk factors for bleeding in cardiac surgery is and will be an ongoing process. In this thesis, the preoperative fibrinogen concentration is identified as a risk factor for excessive bleeding after cardiac surgery, although, the relative importance is difficult to evaluate. Other risk factors are probably more important. Preoperative fibrinogen concentration may therefore be one among many other factors to consider in a preoperative risk evaluation.

The use of fibrinogen concentrate has become an established method to restore and improve hemostasis in case of acquired hypofibrinogenemia due to excessive bleeding. Despite this, the use of fibrinogen concentrate in this setting is in most countries off-label. In contrast, prophylactic administration of fibrinogen concentrate in the absence of ongoing bleeding, especially in low risk procedures, seems not to be of value. Larger, randomized studies would be needed to determine the feasibility of prophylactic fibrinogen concentrate to reduce bleeding and need for blood transfusion in complex, high risk surgery. Also, the timing and dosage of fibrinogen administration would need to be defined.

The safety of fibrinogen use has now been reported in several studies including the present study. Despite this, little is known about the relationship between the actual dose administered to the patient, efficacy and risk of thromboembolic complications. It is in theory possible that larger doses of fibrinogen concentrate may be harmful, and the exact dose in relation to the patient's bodyweight and gender is not known.

We found that a combination of TXA and fibrinogen concentrate had no additive effect compared to fibrinogen alone on clotting time and clot strength when analyzed in vitro. It may be of interest to conduct a trial on cardiac surgery patients with ongoing bleeding since this would add information about the importance of these factors to reduce bleeding. There are also gaps in the evidence regarding how fibrinogen concentrate interacts with already circulating TXA in vivo.

ACKNOWLEDGEMENTS

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