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Fibrinogen and Bleeding in Cardiac Surgery:

Clinical Studies in Coronary Artery Bypass Patients

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To my beloved family

Fibrinogen and Bleeding in Cardiac Surgery:
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Background: Cardiac surgery is accompanied by inflammatory activation and bleeding complications. Fibrinogen is a key factor in the coagulation cascade and can be used to treat ongoing bleeding, but is not well studied as prophylactic treatment to prevent bleeding in patients with normal plasma fibrinogen levels or as a predictive tool to identify patients with increased bleeding risk.

Aims: To investigate the association between biomarkers of inflammation and hemostasis after off pump coronary artery bypass grafting (OPCAB). Further, to study the relationship between plasma fibrinogen concentration and postoperative bleeding and transfusion after on pump coronary artery bypass grafting (CABG). To investigate if prophylactic fibrinogen infusion reduces bleeding and transfusions after CABG. Finally, to study the effect of fibrinogen infusion on markers of coagulation, fibrinolysis and platelet function.

Materials and methods: In study I, biomarkers of inflammation (Il-6, Il-8, PMN-elastase, C3a, sC5b-9) and hemostasis (platelet count, β-thromboglobulin, anti-thrombin, D-dimer and fibrinogen) were measured before and after surgery in 10 OPCAB patients. II: plasma fibrinogen was analyzed the day before surgery in 170 elective CABG patients and related to postoperative bleeding and transfusions. III: 20 patients were randomized to preoperative infusion of 2 grams (g) fibrinogen concentrate or placebo. Side effects, bleeding and transfusions were registered. IV: Biomarkers of coagulation, fibrinolysis and platelet activation in relation to fibrinogen treatment were analyzed in the same patients as in study III.

Results: I: Inflammatory markers did not change during surgery while β-thromboglobulin increased and anti-thrombin and fibrinogen decreased. There were significant correlations between several markers of inflammation and hemostasis. II: Postoperative bleeding volume correlated univariately with preoperative fibrinogen concentration (r = -0.53, p<0.001). Fibrinogen was an independent predictor of postoperative bleeding volume and blood transfusions. III: Infusion of 2g fibrinogen increased plasma levels by 0.6 ± 0.2 g/l and reduced postoperative blood loss by 32%. There were no clinically detectable adverse events of fibrinogen infusion. IV: Fibrinogen infusion induced no or minimal changes in most investigated biomarkers, except D-dimer which was significantly higher 2h after surgery in the fibrinogen group.

Conclusions: There is evidence for an association between the inflammatory response and hemostasis after cardiac surgery. The preoperative fibrinogen concentration is a limiting factor for postoperative hemostasis. Preoperative measurement of fibrinogen provides information about bleeding volume and transfusion requirements after CABG. Prophylactic fibrinogen infusion significantly reduces postoperative bleeding without clinical adverse events. Infusion of 2g fibrinogen to cardiac surgery patients results in no or minimal changes in biomarkers reflecting coagulation and platelet function.

Key words: inflammatory response, CPB, fibrinogen, bleeding, CABG, hemostasis

Original papers

The thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

I)   Aljassim O, Karlsson M, Wiklund L, Jeppsson A, Olsson P, Berglin E

Inflammatory response and platelet activation after off-pump coronary artery bypass surgery

Scand Cardiovasc J 2006; 40: 43-8

II)   Karlsson M, Ternström L, Hyllner M, Baghaei F, Nilsson S, Jeppsson A.

Plasma fibrinogen level, bleeding, and transfusions after on-pump coronary artery bypass grafting surgery: a prospective observational study

Transfusion 2008; 48: 2152-8

III)   Karlsson M, Ternström L, Hyllner M, Baghaei F, Flinck A, Skrtic S, Jeppsson A

Prophylactic fibrinogen infusion reduces bleeding after coronary artery bypass surgery. A prospective randomized pilot study

Thromb Haemost 2009; 102: 137-44

IV)   Karlsson M, Ternström L, Hyllner M, Baghaei F, Skrtic S, Jeppsson A.

Prophylactic fibrinogen infusion in cardiac surgery patients: effects on biomarkers of coagulation, fibrinolysis and platelet function

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Contents

Abbreviations

ACT	activated clotting time
ALAT	alanine-aminotransferase
ANOVA	analysis of variance
AT	antithrombin
APTT	activated partial thromboplastin time
ASAT	aspartate-aminotransferase
AUC	area under the curve
β	beta
CABG	coronary artery bypass grafting
CI	confidence interval
CFT	clot formation time
CPB	cardiopulmonary bypass
CT	computed tomography
CT	clotting time
ECC	extra-corporeal circulation
ECG	electrocardiogram
F	female
FVII	factor seven
Fig.	figure
g	gram
g/L	gram per litre
GP	glycoprotein
h	hours
Hb	hemoglobin
Il	interleukin
IU	international unit (-s)
LMWH	low molecular weight heparin

LVEF	left ventricular ejection fraction
M	male
mL	milliliter
MCF	maximum clot firmness
N	number
ng	nanogram
NSAID	non-steroidal anti-inflammatory drug
OR	odds ratio
OPCAB	off-pump coronary artery bypass (surgery)
%	percent
PE	pulmonary emboli
PF 1&2	prothrombin fragment 1&2
pg	picogram
PLT	platelet(s)
PMN	polymorph nuclear (leukocytes)
PT	prothrombin time
RBC	red blood cells
SD	standard deviation
SIRS	systemic inflammatory response syndrome
TAT	thrombin-antithrombin complex
U	unit (-s)
μg	microgram

1. Introduction

Cardiac surgery as treatment of cardiovascular disease has been routinely performed ever since it was introduced in the 1950s [1]. The majority of operations are performed with the use of cardiopulmonary bypass (CPB) and a heart-lung machine [2], although surgery on the beating heart (without heart-lung machine) is sometimes used as an alternative [3, 4]. Surgery with heart-lung machine is often referred to as <on-pump> while surgery without heart-lung machine is referred to as <off-pump>. The heart-lung machine has been constantly evolved ever since it was first invented and used in 1953 by John and Mary Gibbon [5]. With the discovery and commercial production of the anticoagulant heparin, these two inventions launched the start of the modern era of cardiovascular surgery, followed by a worldwide expansion [6-8]. In Sweden, approximately 7300 open heart surgery procedures were performed in 2008, with an overall 30 day mortality of 2.5 % (Swedish Heart Surgery Registry, annual report 2008). Although constantly evolving, cardiac surgery is still accompanied with a risk of severe complications such as bleeding [9, 10], infections [11, 12], stroke [13, 14], myocardial infarction with heart failure [15, 16], renal insufficiency [17, 18] and pulmonary dysfunction [19, 20]. Some of the pathophysiological causes leading to these complications have been associated with the profound inflammatory reaction and disturbed hemostasis accompanying cardiac surgery.

Cardiac surgery and inflammation

Cardiac surgery with or without CPB induces a systemic inflammatory response, which may contribute to postoperative complications such as bleeding and respiratory failure [21-25]. The contact of blood with the nonendothelial surfaces of the cardiopulmonary bypass circuit and the open wound leads to massive activation of the inflammatory and coagulation systems and release of proinflammatory and procoagulant factors. The onset of the inflammatory reaction is characterized by complement activation followed by activation of different cell types and subsequent release of proinflammatory cytokines, which may lead to tissue injury and organ dysfunction (Fig. 1) [22, 24-26].

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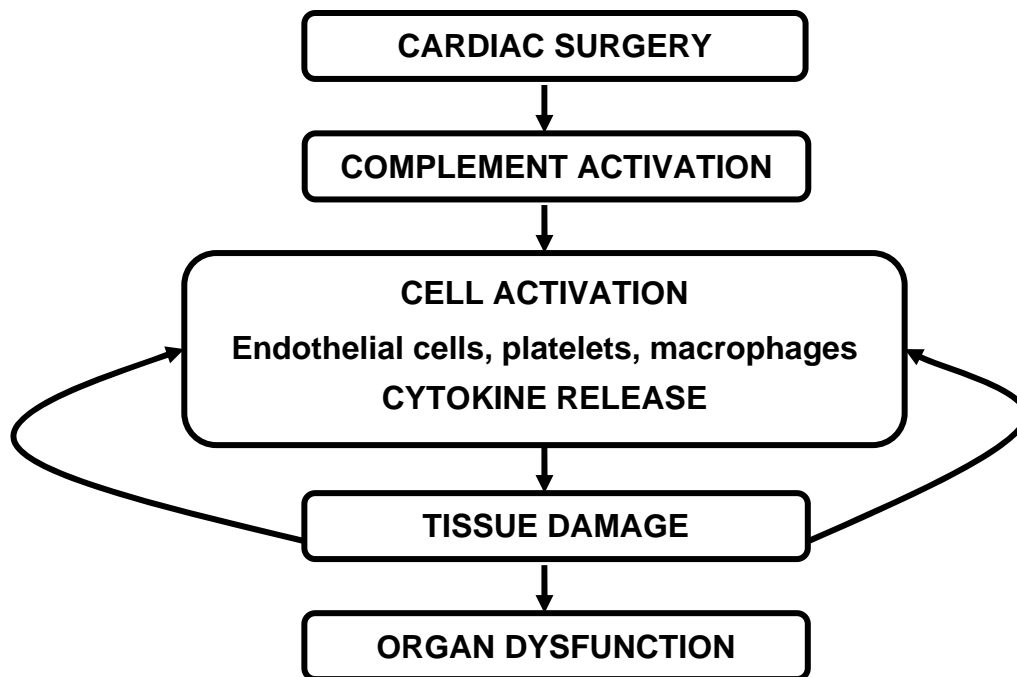
Fig. 1. Cardiac surgery leads to activation of the complement system which activates cells, leading ultimately to tissue damage and various degrees of organ dysfunction. The tissue damage may result in further cell activation and cytokine release.

The complement system consists of plasma proteins and is a part of the human humoral immune system against bacterial and viral infections [27]. The complement factors may be activated by infectious pathogens, but also by trauma such as cardiac surgery and CPB [22, 26]. Complement factor C3 plays a central role in the complement system [22]. The exposure of blood to extracorporeal circuits leads to the activation of C3 and a triggered auto-conversion in a feedback loop to C3a and C3b [28]. While C3a is cleaved off as a split product, C3b cleaves C5 to C5a and C5b. C5b interacts with other complement factors (C6, C7, C8 and C9) to form the terminal sC5b-9 complement complex, also known as the membrane attack complex (MAC) [27]. The anaphylatoxins C3a and C5a are complement split products and act as mediators of inflammatory reactions. Plasma concentrations of complement split products and cytokines, representing the magnitude of the inflammatory reaction, have been related to clinical outcome in pediatric and adult cardiac surgery [22, 29, 30]. However, the inflammatory response is complex and many different factors contribute. The surgical trauma itself is a contributing factor to the inflammatory reaction and complement activation, also in patients

operated without CPB [31]. Ischemia and reperfusion injury of vital organs and endotoxin release may also contribute [32-34].

Cardiac surgery and bleeding

Bleeding is another common and severe complication after cardiac surgery [35]. When surgery is completed, the patient is equipped with chest tubes to drain the thoracic cavity from blood and fluid. In normal cases, postoperative mediastinal drainage averages about 900 milliliter (range 400 – 2200 milliliter) [36]. However, approximately 3-7 % of the patients must undergo an urgent re-exploration because of extensive bleeding from the chest tube drainage, sometimes with concomitant circulatory instability [10, 37-39] Bleeding is therefore a serious complication after cardiac surgery and may result in increased morbidity and mortality [9, 40-43].

Bleeding may be caused by surgical factors or a disturbed hemostasis, or a combination of both. Examples of surgical factors are anastomosis rupture/incompetence and leakage from cannulation sites, which can be surgically corrected. A surgical source of bleeding is found in approximately 50 % of the patients undergoing re-exploration [9, 10, 44]. Disturbed postoperative hemostasis may be related to impaired coagulation, increased fibrinolysis or platelet dysfunction [45-47]. The cause of disturbed hemostasis is often multifactorial. It may be secondary to a surgical bleeding, preoperative medication or the use of CPB (Fig. 2) [48-50]. A surgical bleeding leads to a diminished blood volume and a secondary loss of coagulation factors. Preoperative medication has inhibiting effects on platelet function, e.g. aspirin, clopidogrel, GPIIb/IIIa-blockers, and the coagulation cascade, e.g. low molecular weight heparin and warfarin. In general, a combination of surgical, medical and CPB-related factors causes the alterations in the hemostatic system (Fig. 2).

Cardiac surgery and blood transfusions

A large amount of patients undergoing cardiac surgery receive allogeneic blood product transfusion in the perioperative period. Reported average transfusion rates vary between 10-70 % [51-53]. Cardiothoracic surgery is therefore a major consumer of blood products. However, a minority of patients undergoing cardiac procedures (15% to 20%) consume more than 80% of the blood products transfused at surgery [54].
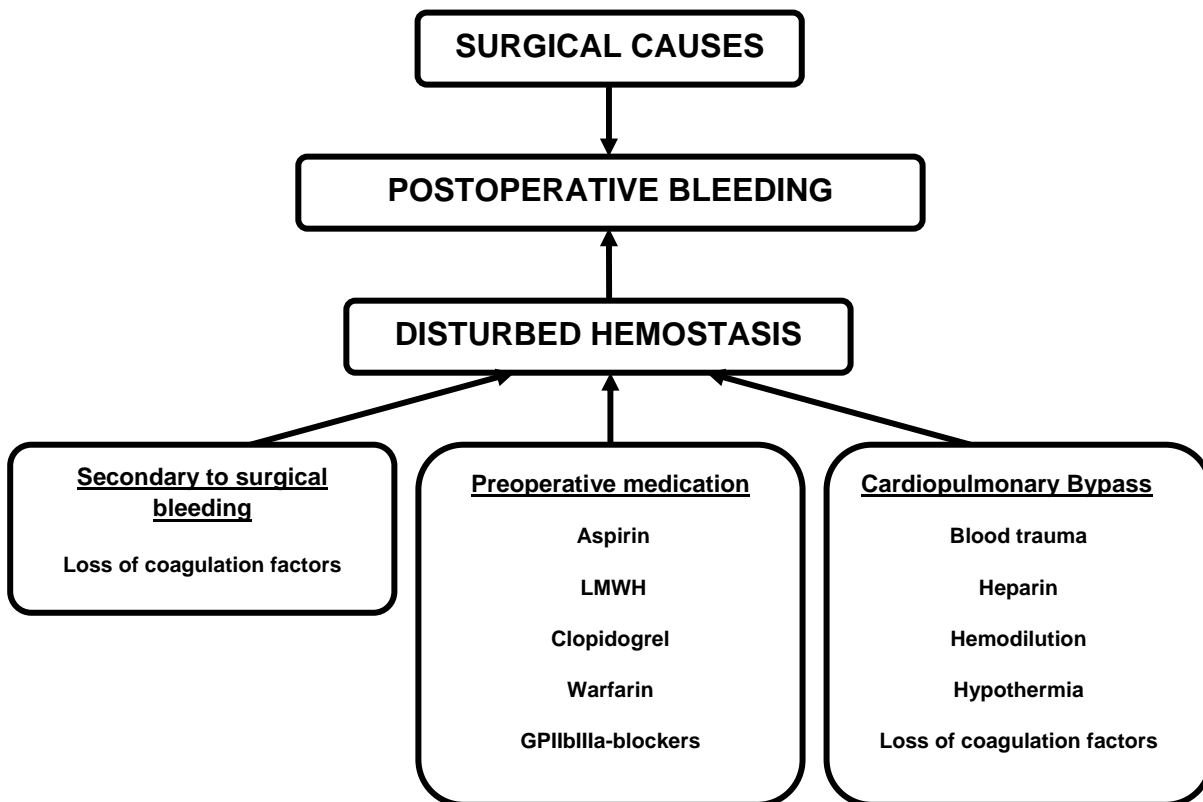
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Secondary to surgical bleeding	Preoperative medication	Cardiopulmonary Bypass
Loss of coagulation factors	Aspirin	Blood trauma
	LMWH	Heparin
	Clopidogrel	Hemodilution
	Warfarin	Hypothermia
	GPIIbIIIa-blockers	Loss of coagulation factors

Fig. 2. Postoperative bleeding may be caused by surgical causes (top) or a disturbed hemostasis (below). A disturbed hemostasis is multifactorial, and may be caused by surgical factors (left), preoperative medication (middle) or the use of CPB (right).

It is known since earlier that blood transfusion is associated with an increased risk of morbidity and mortality, and that it may lead to immunologic reactions and respiratory failure. In addition, transmission of biohazards cannot be completely ruled out [54-56]. Aims to limit the total number of transfused blood products seem to reduce both morbidity and mortality [53, 57, 58]. In a consensus of multiple studies, six variables stand out as important indicators of risk for transfusions in cardiac surgery: advanced age, preoperative anemia or small body size, preoperative antiplatelet or antithrombotic drugs, re-operative or complex procedures, emergency operations and patient comorbidities [54].

Negative changes occur to blood cells when they are stored in a blood bank, and the ability of red blood cells to transport oxygen is diminished [59]. The negative changes that underlie this include a reduction in cell deformability, increased vascular adhesiveness and aggregability, and a reduction in adenosine triphospate (ATP) and levels of 2,3-diphosphoglycerate (2,3-DPG)[59, 60]. Especially the latter one is an important factor in maintaining peripheral tissue

oxygen delivery capacity [59]. In addition, these effects result in altered rheologic properties of the stored red blood cells which may negatively effect their movement in the capillaries [59].

Since blood products used in modern medicine come from human donors, the process of manually assisted donation, handling, testing for infectious agents and storage of blood products, is associated with high costs. The number of available transfusion units in a blood bank may be lacking, and the sustainability is limited [61]. This fact, in combination with transfusion related medical risks of morbidity and mortality, promotes efforts to prevent and by all means reduce perioperative bleeding as well as the use of blood products. A restrictive transfusion protocol may be beneficial not only for the patients' outcome but also for the economy of the healthcare system.

Fibrinogen

Fibrinogen is a central protein in the human coagulation system [62]. Also known as clotting factor I (one), it is a glycoprotein synthesized in liver cells and circulates in plasma at a concentration of 2.0-4.5 g/L [63, 64]. In healthy human adults, about 2-5 g of fibrinogen is synthesized daily and the same amount is catabolized [64]. The plasma half-life of fibrinogen in normal humans has been estimated to be 3-5 days [65]. Fibrinogen is converted in plasma by thrombin into fibrin, which under the influence of factor XIII is formed into a meshwork at the site of tissue damage to minimize blood loss and stimulate tissue repair (Fig. 3) [62, 63]. Fibrinogen is the final clotting factor activated in the coagulation cascade during hemostasis. In primary hemostasis it supports platelet aggregation with formation of a platelet plug, and in secondary hemostasis the formation of an insoluble fibrin clot [66]. Fibrinogen and fibrin also interact with other adhesive glycoproteins, hemostatic factors and blood cells forming a complex system that constitutes the process of hemostasis.
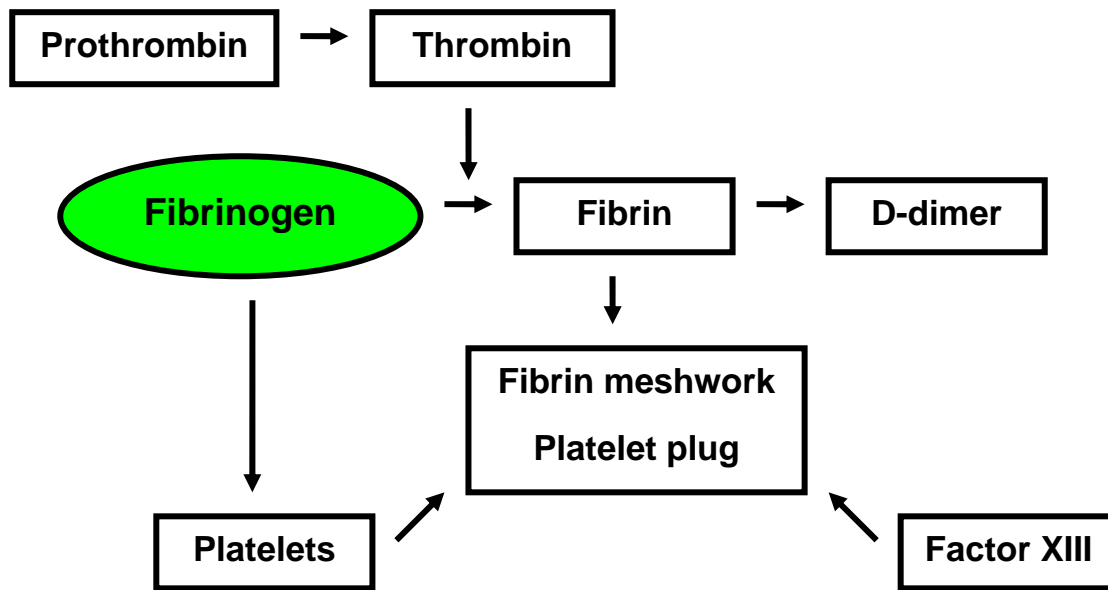
Fig. 3. Fibrinogen is converted in blood plasma by thrombin into fibrin, forming an insoluble fibrin meshwork stabilized by factor XIII. Platelets interact to form a platelet plug.

Treatment and substitution with fibrinogen

In clinical medicine, infusion of fibrinogen concentrate has been used with increasing enthusiasm to treat severe surgical and trauma-related bleeding [67-69], and to substitute hereditary fibrinogen deficiency disorders [70-72]. However, efficacy, dose-responsiveness and potential side effects are little studied in humans. Therapeutic fibrinogen substitution is based on products derived from human plasma, such as fresh-frozen plasma, cryoprecipitate or virally inactivated fibrinogen concentrate [73-75]. Fibrinogen concentrates are manufactured from a pool of plasma donations [76]. The risk of contamination of the donations by pathogens is significantly reduced by selection of donors and through screening of donations by serological and biochemical methods followed by a virus inactivation step including pasteurization [76]. Recent research has focused more on purified fibrinogen concentrate than fibrinogen cryoprecipitate and fresh frozen plasma as a candidate to treat bleeding and correct dilution coagulopathy, both in animal and human experimental models. There are a number of recent publications in which treatment with purified fibrinogen concentrate improves hemostasis in experimental models of induced hemodilution and severe bleeding [77-83].

Study objectives of this thesis

Although extra-corporeal circulation has revolutionized the practice of modern cardiac surgery, it still has several disadvantages. It is associated with a systemic inflammatory response syndrome (SIRS) due to activation of cellular and humoral mediators resulting in complement activation and cytokine release [21, 25]. SIRS initiates a potential prothrombotic stimulus. In addition, the production, release and circulation of microemboli and vasoactive and cytotoxic substances affect almost every organ and tissue within the body [25]. Recognition of this fact has resulted in efforts to attenuate these side effects in order to reduce the risk of postoperative organ dysfunction and risk of bleeding complications.

Studies on patients undergoing off-pump coronary artery bypass grafting surgery (OPCAB) have indicated that avoiding CPB reduces the postoperative systemic inflammatory response compared with conventional on-pump coronary artery bypass grafting (CABG) [84-87], but these findings have been challenged [88]. Regarding clinical outcomes, OPCAB has been associated with less blood loss and need for transfusions, less myocardial injury, and less renal insufficiency compared with conventional CABG [89]. On the other hand, fewer performed grafts and poorer graft patency has also been demonstrated [90]. Compared with on-pump cardiac surgery, the relationship between the inflammatory response and hemostasis during and after off-pump cardiac surgery is less investigated. Therefore, the aim of **Paper I** was to investigate the association between selected biomarkers of inflammatory activity, hemostasis and bleeding after uncomplicated off-pump coronary artery bypass surgery. This was conducted in a prospective, descriptive study.

Since bleeding is a major risk factor for increased morbidity and mortality after cardiac surgery, aims have been taken to in advance predict increased risk of postoperative bleeding in these patients [91-93]. There is at present no biomarker that accurately identifies patients with an increased risk of bleeding. Commonly used preoperative laboratory tests such as activated partial thromboplastin time (APTT), prothrombin time (PT), and platelet count, have been demonstrated to have a low ability to predict postoperative bleeding after cardiac surgery [39, 91, 94-96], although there are conflicting results [97-99]. Clinical studies have reported an inverse correlation between the preoperative [100, 101] and postoperative [37, 91, 92, 102] concentration of fibrinogen in plasma and the volume of bleeding after cardiac surgery with CPB. Other studies have not found a correlation [95, 97, 103]. However, it is difficult to compare studies due to

variations in study design, patient selection and time point for registration of bleeding. The type of surgery also varies, from isolated CABG [92, 100] to various CPB-related procedures [91, 101, 102].

The concept of preoperative identification of patients with an increased risk of severe postoperative bleeding and transfusion of blood products is interesting and offers the possibility to initiate countermeasures. As far as we know, no study has yet investigated if there is an association between the plasma concentration of fibrinogen measured the day before surgery and the amount of postoperative bleeding. In **Paper II**, we therefore designed a prospective descriptive study on patients undergoing isolated first-time CABG in which plasma fibrinogen was measured the day before surgery, and calculated the correlation to bleeding volume during the first 12 postoperative hours. We also investigated if the fibrinogen concentration and selected patient variables were predictive of blood transfusions.

An increasing number of studies indicate that fibrinogen plays a more important role to achieve adequate hemostasis than what previously was thought in patients suffering from major bleeding. Until recently, guidelines have suggested a plasma fibrinogen level of >1 g/L to be efficient for adequate hemostasis [104-107]. However, in some clinical conditions the critical level of fibrinogen may be higher [83]. Estimates of the fibrinogen level by the Clauss-method [108], which is the commonly used coagulation test in which plasma fibrinogen content is determined by the time required for clot formation to occur, may be falsely elevated in bleeding patients due to high levels of fibrin degradation products and the presence of colloids such as gelatin [109].

In situations of traumatic or surgical bleeding in patients without known fibrinogen deficiency disorder, evidence supports a more liberal use of fibrinogen concentrate compared to what previously has been considered [110, 111]. Also, during ongoing bleeding fibrinogen depletion has been described as occurring before depletion of platelets and other coagulation factors [82, 104, 112]. In recent studies, an inverse correlation between the preoperative concentration of fibrinogen in plasma and postoperative bleeding after CABG, even when fibrinogen levels are above the lower reference range, has been demonstrated [100, 101]. This suggests that fibrinogen is a limiting factor for hemostasis after cardiac surgery. We therefore hypothesized, based on these studies and our own experience from paper II, that prophylactic fibrinogen concentrate infusion to patients undergoing CABG might reduce postoperative bleeding. Before the

efficacy and safety of prophylactic fibrinogen infusion can be tested in larger patient populations, feasibility and tolerability needs to be evaluated in smaller patients groups. In theory, infusion of coagulation factors could potentially induce a state of hypercoagulability with an increased risk of thromboembolic events, such as early graft occlusion and myocardial infarction. **In paper III**, we therefore designed a prospective, randomized phase I-II study to assess whether prophylactic infusion of fibrinogen concentrate to CABG patients is feasible. Predefined secondary endpoints were effects of fibrinogen concentrate infusion on bleeding, transfusions, postoperative hemoglobin levels and global hemostasis.

A theoretical contraindication for administration of fibrinogen concentrate is the risk of thromboembolism and myocardial infarction [113, 114]. Studies on the clinical safety of fibrinogen treatment is limited, even in patients with congenital fibrinogen deficiency, but it has so far been reported as effective and without major adverse reactions [64, 114]. A few studies have retrospectively evaluated the safety of fibrinogen concentrate administration to patients with acquired hypofibrinogenemia and on-going bleeding [68, 69, 115], and they could not demonstrate any adverse events related to treatment with fibrinogen infusion. In relation to Paper III, it was the first study ever in which fibrinogen concentrate was administered prophylactically to humans without hereditary or acquired fibrinogen deficiency or as treatment for on-going bleeding. Potential effects on other coagulation factors have so far not been studied. Therefore, a comprehensive test series was applied to determine the effects of fibrinogen concentrate infusion in patients with normal hemostasis. The aim of **Paper IV**, based on the same patient material as in paper III, was to investigate the effect of fibrinogen concentrate infusion on selected biomarkers of coagulation, fibrinolysis and platelet function in CABG patients, without known fibrinogen deficiency disorder or on-going severe bleeding.

2. Aims of the study

1.   To investigate if there are associations between biomarkers of inflammatory activity, hemostasis and bleeding after OPCAB **(Paper I).**

2.   To investigate the relationship between the preoperative plasma fibrinogen concentration and postoperative bleeding volume and transfusion prevalence after CABG **(Paper II).**

3.   To investigate if prophylactic fibrinogen infusion is feasible in CABG patients regarding safety and tolerability **(Paper III).**

4.   To investigate if prophylactic fibrinogen concentrate infusion to CABG patients reduces postoperative bleeding volume and blood transfusion requirements **(Paper III).**

5.   To investigate the effect of fibrinogen concentrate infusion in CABG patients on biomarkers reflecting coagulation, fibrinolysis and platelet function **(Paper IV).**

3. Materials and methods

Patients

The human ethics committee at the Sahlgrenska Academy of University of Gothenburg approved the studies. All patients were included after written informed consent. The studies were performed at the Department of Cardiovascular surgery and Anesthesia at Sahlgrenska University Hospital, Gothenburg, Sweden.

Paper I

Ten patients undergoing OPCAB were included in the study. There were nine men and one woman with a mean age of 65 ± 2 years (Table 1). Exclusion criteria were unstable angina, redo surgery, serum creatinine >130 µmol/L, preoperative NSAID and steroid medication, known bleeding disorder and left ventricular ejection fraction <30% prior to surgery. Anticoagulant treatment with aspirin and clopidogrel was withdrawn at least one week before surgery. Low molecular weight heparin (LMWH) was not administered to any patient before surgery.

Table 1. Patient characteristics study I. Median and range.

Number of patients	10
Age (years)	65 (49-77)
Gender (M/F)	9/1
LVEF (%)	65 (30-85)
Number of anastomoses	1 (1-3)

Key: F=female, LVEF= left ventricular ejection fraction, M=male

Paper II

One-hundred and seventy-five patients undergoing first-time elective CABG with CPB between January and June 2005 or between January and March 2006 were included in a prospective non-interventional observational study (Table 2). Predefined exclusion criteria were emergency CABG, concomitant surgical procedures, known liver or kidney disease, and a surgical source of bleeding at acute re-exploration. Aspirin was not discontinued before

surgery. LMWH was administered until the evening before surgery. Clopidogrel and warfarin was discontinued at least 5 days before surgery. Five patients were excluded from the study due to surgical source of bleeding, leaving 170 patients finally included.

Table 2. Patient characteristics study II. Mean ± SD or number (%).

Number of patients	170
Age (years)	67 ± 9.4
Gender (M/F)	128 (75)
Unstable angina	94 (55)
Aspirin	151 (89)
LMWH	54 (32)
Clopidogrel	52 (30)
Warfarin	9 (5)
ECC time (min)	73 ± 24
Aortic cross clamp time	43 ± 15
Number of anastomoses	3.0 ± 0.8

Key: ECC=extracorporeal circulation, F=female, LMWH=low molecular weight heparin, M=male

Paper III

Twenty patients admitted for elective CABG between September and December 2006 were included after informed written consent. The sample size, 20 patients with complete evaluation randomized to two groups, was determined in agreement with the Regional Research Ethics Committee and the Swedish Medical Products Agency. Patients were eligible if they were scheduled to undergo elective CABG, and had a preoperative plasma fibrinogen concentration of ≤3.8 g/L. Predefined exclusion criteria were known liver or kidney disease, known bleeding disorder and a surgical source of bleeding at acute re-exploration. The patients were preoperatively risk stratified according to the EURO-score system [116]. Patients were randomized to two groups; fibrinogen group and control group (see study design). Aspirin was not discontinued before surgery, while LMWH was administered until the evening before surgery. Clopidogrel and warfarin was discontinued at least 5 days before surgery.

Paper IV

Study IV was performed on the same patient material as in study III. Therefore, the patient material, sample size and exclusion criteria are the same.

Table 3. Patient characteristics study III and IV. Mean ± SD or number (%).

	Fibrinogen group	*Control group*
Number of patients	10	10
Age (years)	66 ± 9	68 ± 8
Gender (M/F)	9/1	9/1
BMI	28 ± 5	26 ± 4
Smokers	0	0
Aspirin	10	9
Clopidogrel	1	2
Euroscore	5.6 ± 2.1	5.6 ± 1.9
Aortic cross clamp time	47 ± 21	44 ± 17
ECC time (min)	73 ± 26	70 ± 24
Number of anastomoses	2.8 ± 0.7	2.9 ± 0.9

Key: BMI=Body Mass Index, ECC=extracorporeal circulation, F=female, M=male

Clinical management

Surgical procedures and anesthesia

Paper I

Anesthesia was induced with remifentanil (0.5-1 µg/kg/min), propofol (1.5-2.5 mg/kg) and pancuronium (0.1 mg/kg) in all patients. The patients received 100 U heparin/kg bodyweight. Activated clotting time was aimed at >200 seconds. The heparin effect was not actively reversed after surgery. The patients were operated as reported previously [117]. In short, body temperature was kept at minimum 36°C with the aid of elevated room temperature and a warming blanket. A generous crystalloid fluid regimen was kept as a means to maintain a sufficient filling of the heart during manipulation and grafting. To achieve a bloodless field, an intracoronary shunt (Flo-Thru-Shunt, Bio-vascular Inc., St. Paul, MN) was used.

Paper II, III and IV

The anesthesia in all patients was induced with 200 to 300 µg of fentanyl and 3 to 5 mg per kg thiopentone followed by 0.1 mg per kg pancuronium, and anesthesia was maintained with sevoflurane. During CPB, anesthesia was maintained with propofol. The patients received 300 U heparin/kg bodyweight in order to maintain an activated clotting time >480 seconds. After completion of CPB, the heparin was reversed by the administration of protamine (1 mg protamine / 100 U of heparin) to an activated clotting time of less than 130 seconds.

The CPB circuit included a membrane oxygenator and roller pumps. Standard nonpulsatile CPB technique with moderate hypothermia (bladder temperature 34-35°C) and hemodilution was used. Cardioprotection was achieved with antegrade cold blood cardioplegia. Weaning off CPB was performed after rewarming to a bladder temperature of at least 36 °C.

In study II, all patients received 2g of tranexamic acid at anesthesia induction and at the end of surgery. Tranexamic acid was not used in study III and IV. Aprotinin was not used in any of the studies.

Study design and analyses

Paper I

Laboratory analysis, bleeding and calculations

Selected biomarkers of inflammation (Il-6, Il-8, PMN-elastase, C3a, and sC5b-9) and hemostasis (platelet count, β-thromboglobulin, antithrombin, D-dimer and fibrinogen) were measured before and immediately after surgery. Preoperative samples were collected just prior to induction of anesthesia. Postoperative blood samples were collected at the end of surgery before closing the chest. Bleeding during the first 18 postoperative hours was registered. Correlations between markers of inflammation, hemostasis and bleeding were calculated.

Paper II

Laboratory analysis

Hemoglobin concentration, platelet count, APTT and PT were analyzed the day before surgery. Plasma fibrinogen concentration was determined according to the method by Clauss [108], with the use of an assay in which excess thrombin is added to diluted, low-fibrinogen-containing plasma to determine the amount of clottable protein (STA-FIB 2, Diagnostica Stago, Asnieres, France). The reference value is 2.0-4.5 g per L. PT was analyzed with a prothrombin complex assay (Owren type, STA-R, SPA 50 Reagent, Diagnostica Stago) and reported as the International Normalized Ratio (INR). APTT was analyzed with a routine assay using APTT-reagent added to platelet poor plasma (STA-R, STA-PTT Automate 5 reagent, Diagnostica Stago).

Calculations

The association between bleeding and transfusion and the following pre- and perioperative variables was investigated: age, sex, body mass index (BMI), number of grafts, unstable angina, extracorporeal circulation time, aortic clamp time, anticoagulation therapy, preoperative plasma fibrinogen concentration, hemoglobin concentration, platelet count, APTT, and PT. Unstable angina was defined according to the Braunwald classification [118].

Bleeding and transfusions

Postoperative bleeding volume was defined as the total amount of chest tube drainage during the first 12 postoperative hours. In case of re-exploration, bleeding volume until re-exploration was registered. The amount of transfused red blood cells, fresh-frozen plasma, and platelets during hospital stay was recorded. The responsible physicians, who prescribed transfusions, were not aware of the preoperative fibrinogen concentration.

Paper III

The study was a prospective randomized controlled study. Patients were randomized to infusion of 2g fibrinogen concentrate (Haemocomplettan®, CSL Behring, Marburg, Germany) or no infusion after arrival to the operating room immediately before surgery. Random assignment was conducted using unmarked envelopes, each containing a card indicating treatment with fibrinogen or control. Primary endpoint was safety with clinical adverse events and graft occlusion assessed by computed tomography (CT) of the heart 3–4 days after surgery. Clinical adverse events were defined as any clinical signs of central or peripheral thromboembolism, respiratory or circulatory failure, or allergic reactions during hospital stay. Predefined secondary endpoints were postoperative bleeding, transfusion of blood products, hemoglobin concentration 24 hours after surgery, and effects of fibrinogen infusion on global hemostasis 2 hours and 24 hours after surgery. Blood samples were collected at baseline (before anesthesia), 15 minutes after completed infusion, and 2 and 24 hours after completed surgery.

Laboratory analysis

APTT, PT, hematocrit, serum creatinine, aspartat-aminotransferase (ASAT), alanine-aminotransferase (ALAT) and hemoglobin were measured with standard clinical methods. Plasma fibrinogen concentration was determined according to the method by Clauss [108].

To assess global hemostasis, dynamic changes in clot formation was analyzed with thromboelastometry (ROTEM®, Pentapharm, Munich, Germany). Technical details have previously been described [119]. ROTEM® analyses on citrated blood were performed in parallel on four channels (INTEM, EXTEM, FIBTEM, and HEPTEM). In each of these channels, the following variables were assessed: clotting time (CT), clot formation time (CFT) and maximum clot firmness (MCF).

Calculations

Plasma concentrations of fibrinogen were adjusted to a standard hematocrit of 40% according to the formula: corrected concentration = measured concentration × (standard hematocrit/measured hematocrit) [120].

Bleeding and transfusions

Postoperative bleeding was defined as the total amount of chest tube drainage after closure of the sternum and during the first 12 postoperative hours. An intensive care nurse blinded to group assignment registered the bleeding every hour. The amount of transfused red blood cells, fresh frozen plasma, and platelets during the hospital stay was recorded. The responsible intensive care unit physicians were not informed of the preoperative fibrinogen concentration or group assignment.

Computed tomography (CT)

All CT examinations were performed with a 64-slice spiral CT scanner (GE Light Speed VCT, GE Medical Systems, Milwaukee, WI, USA). The protocol included an unenhanced scan of the chest to define the scan volume of the contrast-enhanced electrocardiogram (ECG)-gated study. ECG-gated tube current modulation was used and ECG-correlated image reconstruction was performed and sent to a workstation for further post-processing and evaluation of graft patency by an experienced thoracic radiologist blinded to patient group. A bypass graft was considered to be patent if it was filled with contrast that could be followed through the anastomotic site.

Paper IV

Blood samples were collected at baseline (before anesthesia), fifteen minutes after completed infusion, and 2 and 24 hours after surgery. Control patients did not receive any placebo infusion, but the second measurement is referred to as "after infusion" in both groups. The following biomarkers reflecting coagulation were analyzed at the four time points: fibrinogen, APTT, Activated Clotting Time (ACT), PT, antithrombin (AT), thrombin-antithrombin complex (TAT), prothrombin fragment 1&2 (PF 1&2) and modified rotational thromboelastometry (Rotem®). Plasma D-dimer was analyzed as a marker of fibrin degradation and fibrinolysis. Platelet count and platelet impedance aggregometry (Multiplate®) were analyzed as a measure of platelet function.

For APTT, PT, hematocrit, hemoglobin, fibrinogen and thromboelastometry, see above. ACT was measured with a Hemochron Jr Signature +[®] point of care device (ITC, Edison, New Jersey, USA). Antithrombin was measured by STA-R[®] using the STA[®]-Stachrom[®]AT III reagent (Diagnostica Stago, Asnieres, France). Reference range is 0.80 – 1.20 kIU/L. TAT and PF 1&2 were measured using ELISA methods with the iEMS Reader with Enzygnost* TAT micro reagent, reference range 1.0 – 4.1 µg/L, and Enzygnost*F1&2 (monoclonal) reagent, reference range 70-230 pmol/L, respectively (both Dade Behring AB, Skärholmen, Sweden). D-dimer was analyzed with a Latex Agglutination method by STA-R[®] using STA[®]-Liatest[®]D-DI reagent, reference interval ≤0.5 mg/L (Diagnostica Stago, Asnieres, France).

Dynamic changes in clot formation were analyzed with thromboelastometry as described in Paper III. Platelet impedance aggregometry was analyzed with a Multiplate[®] platelet function analyzer (Dynabyte Medical, Munich, Germany), which analyses platelet aggregability in whole blood after addition of different platelet receptor agonists [122]. In the present study, adenosine diphosphate (ADP) was used as agonist with a concentration of 6.4 µmol/L. Increase in impedance is calculated as aggregation units x minutes (AU x min) and reported as area under the curve (AUC).

Statistics

Paper I

The non-parametric Wilcoxon's paired test was used to compare pre- and postoperative values within the group. Correlation was analyzed with Spearman Rank Sum Test. Statistical significance was defined as p<0.05. All results are expressed as median and range.

Paper II

Results are expressed as mean ± standard deviation (SD) or number and percent (%). Statistical significance was defined as a p-value <0.05. Simple linear regression was used to analyze the relationship between hematologic and demographic data and the volume of postoperative bleeding. Multiple linear regression analysis using forward selection was then used to identify factors independently associated with bleeding volume. Group comparisons were performed with two-sample t-tests for continuous data and with chi-square tests for categorical data. Independent predictors for transfusion were analyzed with multiple logistic regression.

Paper III

Data are presented as mean ± SD. A p-value <0.05 was considered statistically significant. Group comparisons were made with the Student's t-test (continuous variables) or Fisher's exact test (categorical variables). Changes from baseline within a group were analyzed with paired t-test. For group comparisons of variables analyzed at more than one occasion, ANOVA for repeated measurements was used.

Paper IV

Data are presented as mean ± SD. A p-value <0.05 was considered statistically significant. Group comparisons of continuous variables were made with Student's t-test. Intragroup changes from baseline were analyzed with paired t-test. For group comparisons of variables analyzed at more than one occasion, ANOVA for repeated measurements was used. Correlations were analyzed with Pearson's test.

4. Results

"Inflammatory response and platelet activation after off-pump coronary artery bypass surgery" (Paper I)

General

All patients had an uncomplicated postoperative course and were discharged from hospital within 7 days. Median postoperative bleeding during the first 18 hours was 900 (190 – 940) ml.

Inflammatory and hemostatic biomarkers

The inflammatory biomarkers, C3a, sC5b-9, IL-6, IL-8 and PMN-elastase, did not change significantly during surgery compared to the preoperative levels. The hemostatic biomarkers antithrombin and fibrinogen levels decreased (from 0.82 IU/mL (0.75-1.05) to 0.80 (0.68-0.97), p = 0.017 and from 2.46 g/L (1.85-3.90) to 2.36 (1.65-3.57), p = 0.012, respectively) and β-thromboglobulin increased significantly (from 21.7 IU/mL (11.5-62.9) to 33.9 (20.7-115.8), p = 0.007) after surgery. D-dimer and platelet count did not differ significantly (p = 0.51 and p = 0.11, respectively).

Correlation between inflammatory response, hemostasis and bleeding

There were significant postoperative correlations between PMN-elastase and β-thromboglobulin (r = 0.82, p = 0.004, Fig 4), between PMN-elastase and fibrinogen (r = 0.69, p = 0.028) and between C3a and β-thromboglobulin (r = 0.71, p = 0.022, Fig 5). In addition, there were significant inverse correlations between postoperative bleeding and pre- and postoperative fibrinogen levels (r = -0.76, p = 0.011, Fig 6 and r = -0.84, p = 0.002, Fig 7, respectively), between bleeding and postoperative PMN-elastase (r = -0.75, p = 0.012, Fig 8), and between bleeding and β-thromboglobulin (r = -0.66, p = 0.037, Fig 9). IL-6, IL-8, C3a and sC5b-9 concentrations did not correlate to bleeding or PMN-elastase.
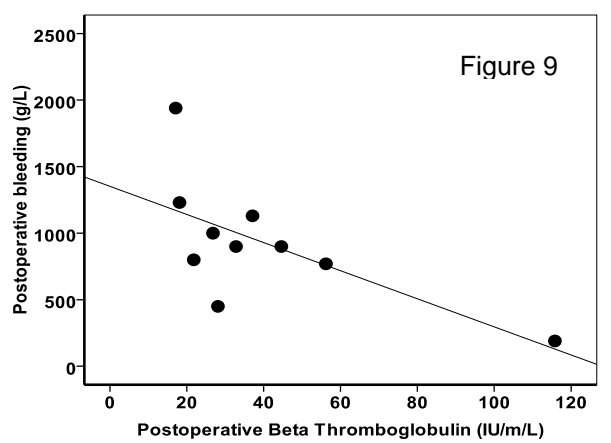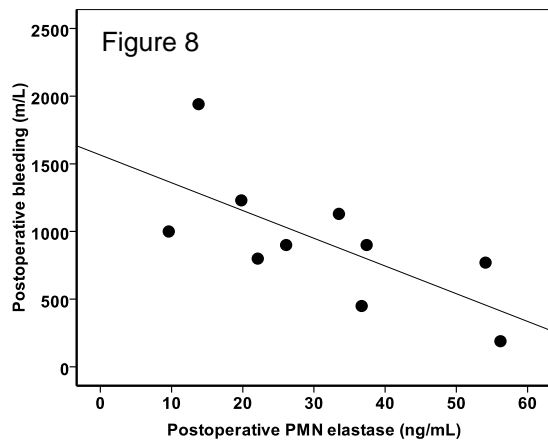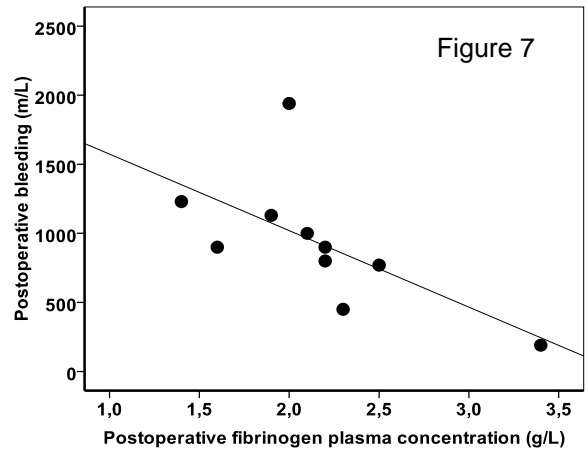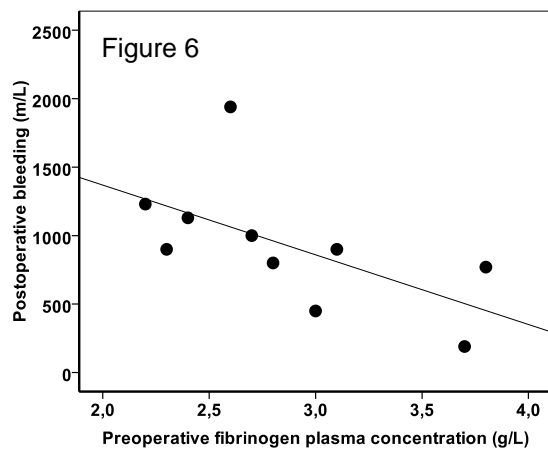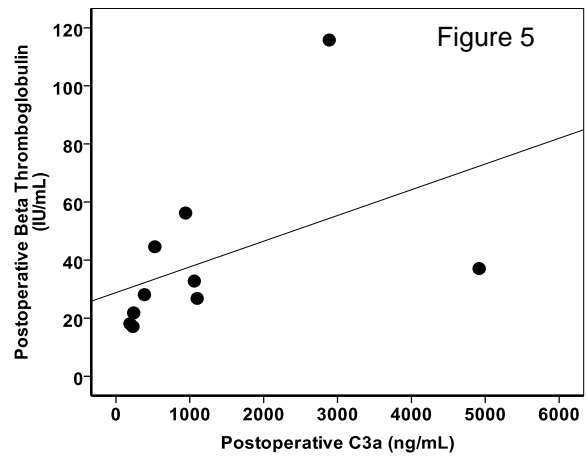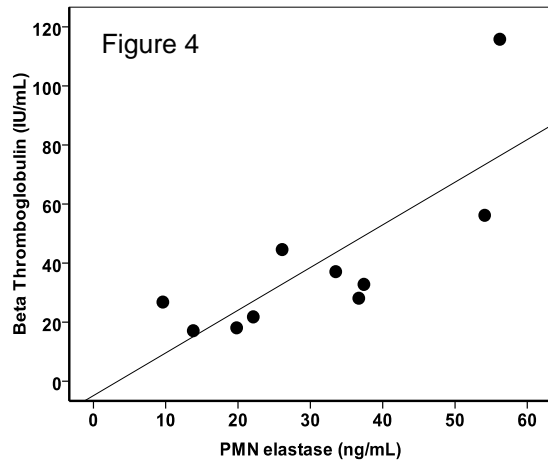
Figure 4-9. Correlations between biomarkers of inflammatory response, hemostasis and postoperative bleeding.

"Plasma fibrinogen level, bleeding, and transfusion after on-pump coronary artery bypass grafting surgery: a prospective observational study." (Paper II)

General

One patient with a postoperative myocardial infarction died of multiorgan failure 9 days after surgery. Four of the 170 patients (2.3 %) were re-explored within 12 hours due to diffuse postoperative bleeding. Median postoperative bleeding was 360 mL per 12 hours, range = 110 - 2085 mL, and 29 patients (17%) were transfused with blood products.

Laboratory variables

Mean fibrinogen plasma concentration was 4.2 ± 0.9 g/L (range $2.4 - 8.1$ g/L). 116 patients (68%) had concentrations within the normal range ($2.0 - 4.5$ g/L), and the remaining 54 patients (32%) had higher concentrations.

The mean preoperative platelet count, APTT and PT values were all within the normal range, except for six patients who had elevated PT (INR $1.3 - 1.9$). The mean preoperative Hb concentration was 140 ± 14 g/L.

Associations between pre- and postoperative variables, bleeding and transfusions

There were significant inverse correlations between postoperative bleeding and preoperative fibrinogen concentration ($r = -0.53$, $p < 0.001$, Fig. 10), preoperative platelet count ($r = -0.26$, $p < 0.001$, Fig. 11), and preoperative Hb concentration ($r = -0.25$, $p = 0.001$). Neither APTT nor PT or preoperative anticoagulation correlated to bleeding. In multivariate testing, preoperative fibrinogen concentration was the only factor independently associated with postoperative bleeding volume ($r = -0.53$, $p < 0.001$).
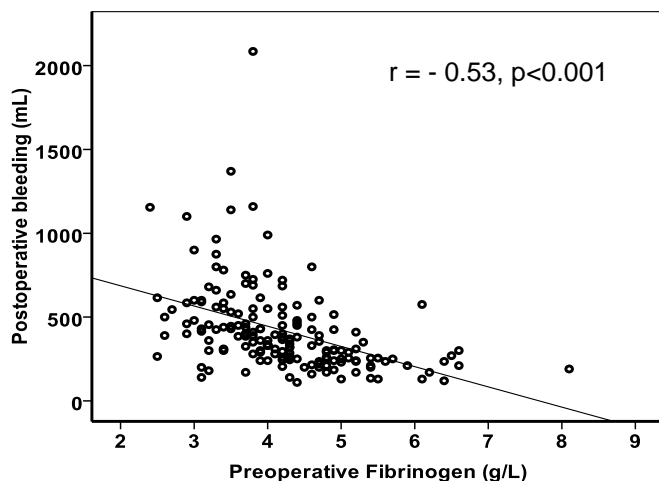
Fig 10. Correlation between the preoperative fibrinogen concentration and postoperative bleeding.
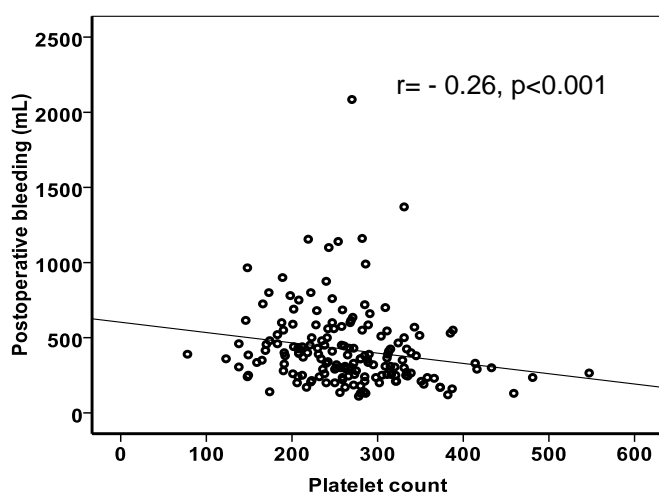

Fig 11. Correlation between the preoperative platelet count and postoperative bleeding.

Significant independent predictors of transfusion in a logistic regression model were preoperative fibrinogen concentration (OR 2.0, 95% confidence interval (CI) 1.1 - 3.7 per 1 g/L decrease, p = 0.027), female sex (OR 5.0, 95% CI 1.8 - 14.7, p = 0.002), and aortic cross-clamp time (OR 1.03, 95% CI 1.01 - 1.06 per minute, p = 0.013). The absolute risks of transfusion for men and women with different preoperative fibrinogen concentrations are displayed in the figure below (Fig 12).

Risk of transfusion (%)
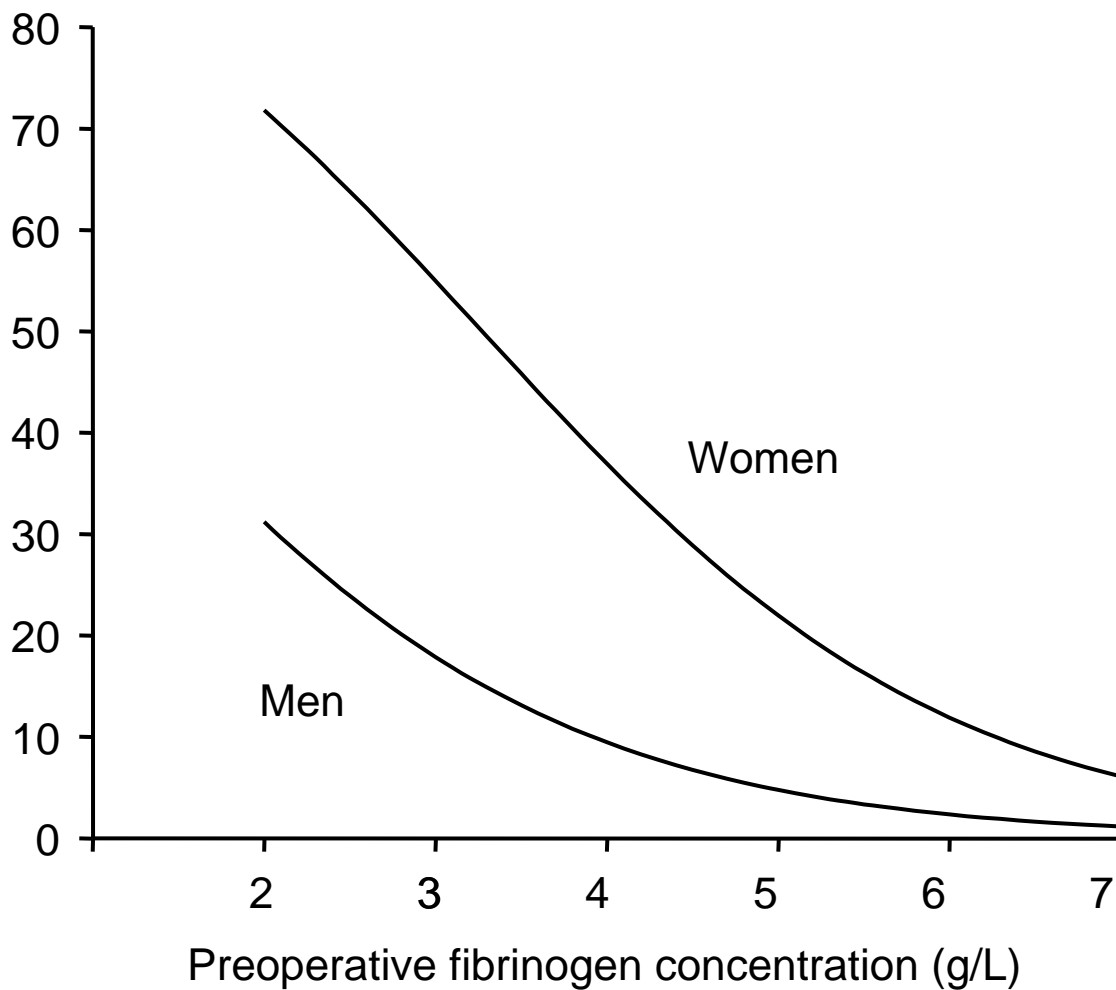
Figure 12. Absolute risk for transfusion of blood products in men and women with different preoperative fibrinogen plasma concentrations.

33

"Prophylactic fibrinogen infusion reduces bleeding after coronary artery bypass surgery. A prospective randomized pilot study" (Paper III)

General

All patients had an uncomplicated postoperative course and were discharged from the clinic within 7 days.

Fibrinogen

Mean preoperative plasma fibrinogen concentration was 2.9 ± 0.2 g/l. Infusion of 2g fibrinogen concentrate increased plasma fibrinogen concentration by 0.6 ± 0.2 g/l (range 0.4 - 1.1 g/l), while fibrinogen levels remained unchanged in the control group ($p=0.002$ between groups, Fig. 13). Plasma fibrinogen concentrations did not differ significantly between the groups 2 h and 24 h after surgery.
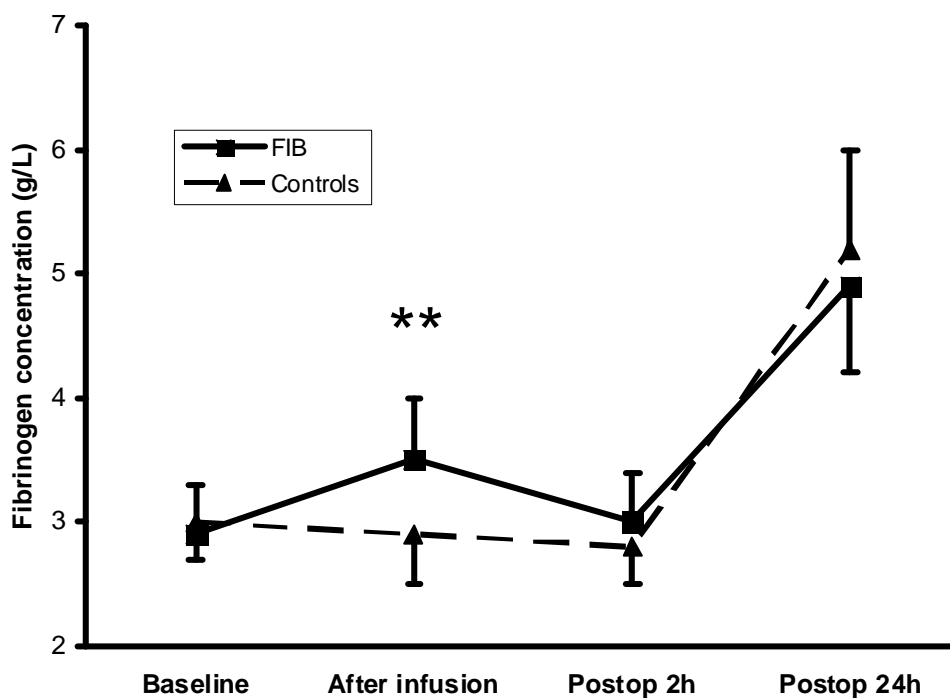
*Figure 13. Fibrinogen concentration before surgery (baseline), after infusion and 2h and 24h after surgery in the Fibrinogen (FIB) group and in the control group. ** = p=0.002 between groups.*

Safety: adverse events and early graft occlusion

There were no clinically detectable adverse events in the fibrinogen group. One patient in the control group had a perioperative myocardial infarction diagnosed with ECG and Troponin-T elevation. On CT-scan, there was one vein graft occlusion in the fibrinogen group and none in the control group. CT also detected accidentally a subclinical peripheral pulmonary embolus (PE) in one patient in the fibrinogen group.

Bleeding, transfusions and hemoglobin levels

Fibrinogen infusion reduced postoperative bleeding by 32% (565 ± 150 vs. 830 ± 268 ml/12h, p=0.010). Individual amounts of postoperative bleeding are given in Fig. 14. Blood hemoglobin concentration was significantly higher 24 h after surgery in the fibrinogen group (110 ± 12 vs. 98 ± 8 g/l, p=0.018, Fig. 15). One patient in the fibrinogen group and three patients in the control group received blood transfusions (p=0.29).
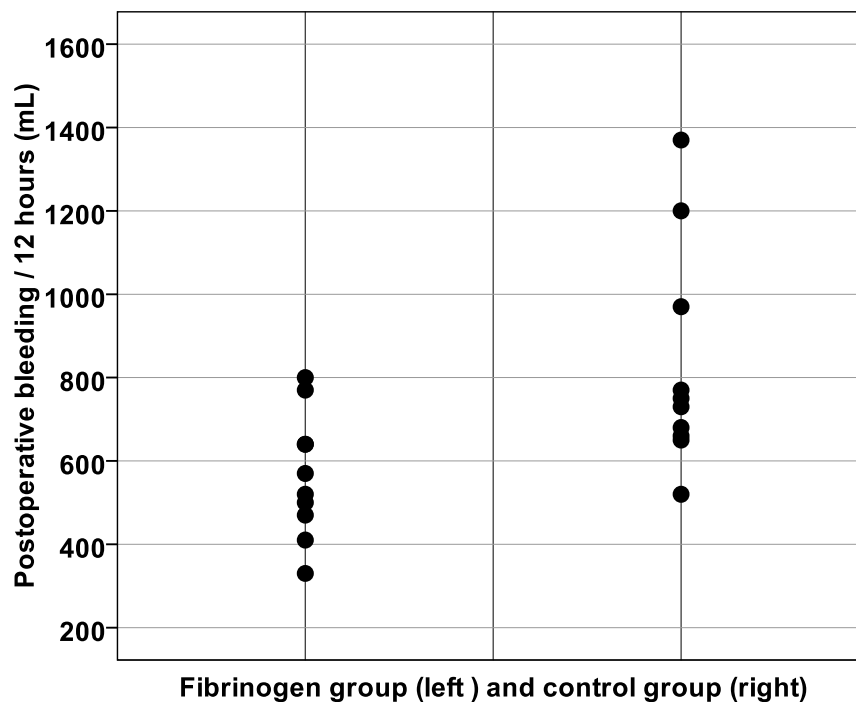

Figure 14. Individual amounts of postoperative bleeding in the Fibrinogen group (left) and in the control group (right) (p=0.010). There was a mean reduction in postoperative bleeding between the groups of 32%.
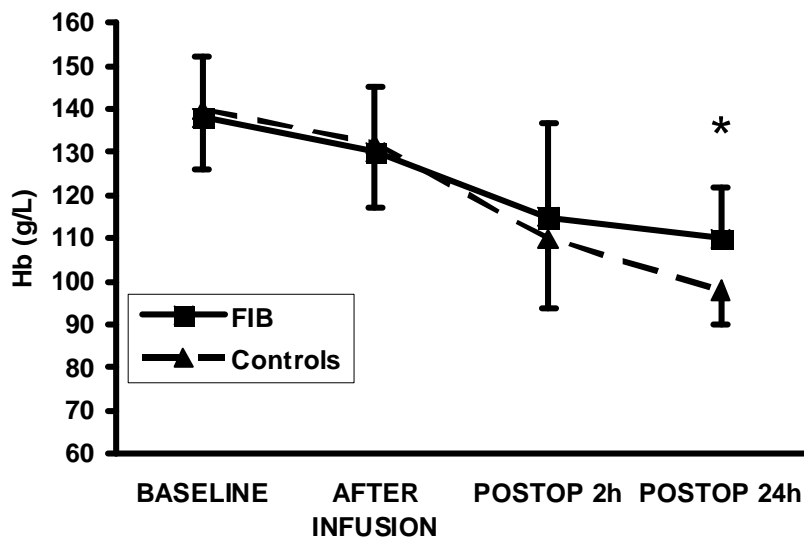
*Figure 15. Hemoglobin concentration before surgery, after infusion, and 2h and 24h after surgery in the Fibrinogen (FIB) group and in the control group. * = p=0.18*

Effects of fibrinogen infusion on global hemostasis after CABG

There were no statistically significant differences between the groups in APTT or PT. Also, there were no significant differences in thromboelastometry values between the groups at any time point.

*"**Prophylactic fibrinogen infusion in cardiac surgery patients: effects on biomarkers of coagulation, fibrinolysis and platelet function" (Paper IV)***

Immediate effects of fibrinogen infusion

Fifteen minutes after infusion, there was a slight but statistically significant decline in plasma levels of antithrombin in the fibrinogen group (-0.02 ± 0.02 kIU/L vs. +0.01 ± 0.03 kIU/L (control group), p=0.012 between the groups), while no other variables differed between the two groups.

Postoperative effects of fibrinogen

D-dimer levels were significantly elevated 2 hours after surgery in both groups (Fig. 16). None of the other biomarkers reflecting coagulation and platelet function differed significantly between the groups.
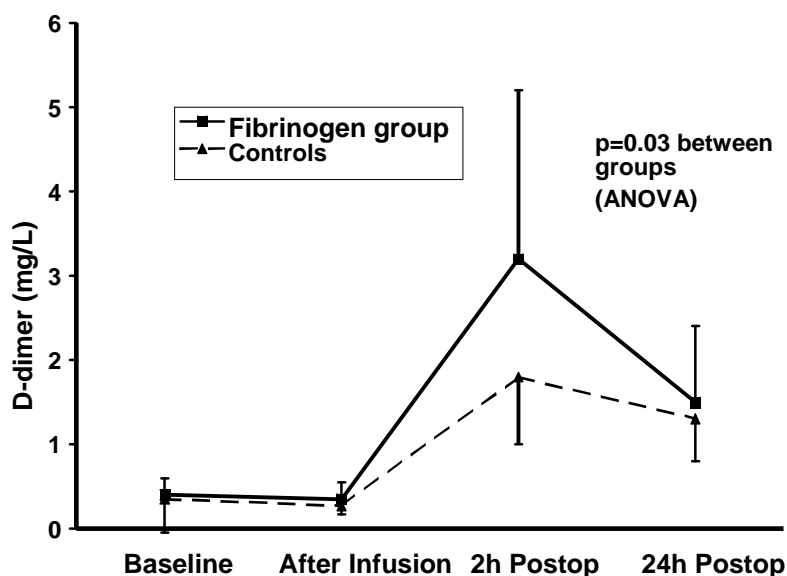

Figure 16. Plasma concentrations of D-dimer in the Fibrinogen group and in the control group. There was a significant difference 2 hours after surgery.

5. Discussion

Cardiac surgery induces a systemic inflammatory response [22, 23], which may contribute to organ dysfunction and bleeding complications [22, 24]. The inflammatory response is complex in its nature and a number of different factors may contribute [22-24]. Historically, the inflammatory response was generally attributed to the use of CPB, but in recent years a number of other factors such as the operative trauma per se, regional ischemia/reperfusion injury and endotoxin release have been suggested to be of importance [22, 24, 123-125]. There are indications that OPCAB reduces the postoperative systemic inflammatory response compared with conventional on-pump CABG [84-87], but these findings have been challenged [88].

The relationship between inflammatory response, hemostasis and bleeding after cardiac surgery has been extensively studied but is still not fully understood [22-25, 126]. There is, however, evidence for such an association. Rinder et al. has described adhesion of leukocytes to platelets during CPB [127]. Inhibition of sC5b-9 decreased formation of leukocyte/platelet complexes and attenuated the reduction in platelet count during simulated CPB [128]. Since the effects of surgery without CPB is less studied compared to surgery with CPB, we aimed at characterizing the relationship between inflammation and hemostasis after cardiac surgery by investigating the potential correlation between selected markers of inflammation, hemostasis and bleeding after OPCAB.

Our results support a relationship between inflammatory and hemostatic activation after surgery since temporal correlations between two markers of inflammation, PMN-elastase and C3a, and the platelet activity marker β-thromboglobulin were demonstrated (Fig. 4 and 5). β-thromboglobulin is a platelet specific protein which is released from granulae upon activation [129], while circulating concentrations of PMN-elastase provides a measure of neutrophil granulation which may cause tissue damage [130]. Three different mechanisms may explain the association. First, it could be a cause/effect relationship, i.e. that pro inflammatory mediators such as complement proteins and cytokines directly activate platelets. A second explanation would be that platelet-derived thrombin activates PMNs, and a final explanation would be a common pathway for activation of both platelets and cells releasing inflammatory mediators. The present study cannot discriminate between these mechanisms, but it is plausible that

measures primarily directed to modulate inflammatory response may also impact platelet activity.

Platelet activation and inflammation vs. bleeding

The complex effect of cardiac surgery, with subsequent inflammatory reaction on platelets, must not necessarily be negative with an increased risk of bleeding. On the contrary, the present results suggest a beneficial effect since there was an inverse correlation between β-thromboglobulin and postoperative bleeding (Fig. 9), i.e. higher β-thromboglobulin levels were associated with a lower degree of bleeding. In addition, we found an inverse correlation between PMN-elastase and bleeding (Fig. 8), indicating that patients with a more pronounced postoperative inflammatory response, measured as PMN-concentration, actually bleed less than those with an attenuated response. This is in contrast to what has been reported previously. Despotis et al found evidence for an association between postoperative bleeding and increased inflammation [131]. In this study, inflammation was defined as white blood cell count changes in percent.

Regarding effects on clinical outcomes, previous studies have shown ambiguous results. The use of an antibody as a pharmacologic C5-complement suppression has been shown to reduce postoperative bleeding [132, 133]. Approaches to optimize the biocompatibility of the CPB circuit, which is known to cause inflammation, has not been able to demonstrate any effect on bleeding [134, 135]. In a recent interventional study, it was concluded that pharmacologic treatment by indomethacin, an immune modulator inhibiting cyklooxygenase, decreased complement activation consumption during CPB, but did not significantly reduce bleeding [136]. In the same study, ketoconazole, a lipoxygenase and thromboxane A2 synthetase inhibitor, reduced postoperative bleeding by limiting coagulation abnormalities but did not significantly affect complement activation. In a few studies, steroid treatment before surgery has been shown to reduce levels of IL-6, Il-8 and TNF-alpha, however, without effect on clinical outcomes [137-140]. It has been reported that corticosteroid treatment improves perioperative hemodynamics, but without relation to inflammatory markers [141]. The results from the present study do not support active administration of e.g. steroid treatment to attenuate the inflammatory response in order to accomplish reduced postoperative bleeding. Inflammatory response and platelet activation may instead be beneficial in terms of hemostasis and

bleeding, at least up to a certain level. However, the complexity of this issue is further illustrated by the inverse relation between PMN-elastase and bleeding in the present study, while no other markers of inflammatory activation and postoperative bleeding was detected.

Although still very complex, the results of paper I give further evidence for an association between the inflammatory response and hemostatic factors after cardiac surgery. An interesting finding was the strong relationship between pre– and postoperative fibrinogen levels and the amount of postoperative bleeding in these patients, (Fig. 6 and 7), despite the fact that all patients had fibrinogen levels within the normal range (2.0 – 4.5 g/L). This has by our knowledge not been reported before in OPCAB patients. Even though the patient material is limited, this suggests that preoperative fibrinogen analysis might provide information about risk of increased postoperative bleeding.

Relationship between plasma fibrinogen, bleeding and transfusions

The result that fibrinogen correlates to bleeding in paper I was followed up by a prospective, non-interventional, observational study in paper II. A larger patient cohort including 170 CABG patients was studied between January and June 2005 and January and March 2006. The primary endpoint was to investigate if the correlation between plasma fibrinogen measured the day before surgery and the amount of postoperative bleeding could be confirmed. The reason why fibrinogen was measured the day before surgery was the hypothesis that low fibrinogen levels might predict risk of increased bleeding and need for blood transfusions. Secondary aims were to investigate possible associations between patient variables, bleeding and transfusions.

The main finding was that preoperative plasma fibrinogen concentration was an independent predictor of postoperative bleeding and blood transfusions after CABG. No patient had fibrinogen levels below normal reference range of 2.5 g/L. As in paper I, we could confirm the result that the concentration of fibrinogen in plasma correlated to the amount of postoperative bleeding volume ($r = -0.53$, $p < 0.001$, Fig. 10). The results give further evidence that the fibrinogen concentration, even within the normal range, is a limiting factor for postoperative hemostasis, and that it may be used as a biomarker to identify patients with an increased risk of severe bleeding and transfusions after cardiac surgery.

As mentioned in the introduction, some previous studies have demonstrated a correlation to postoperative bleeding and transfusions [37, 91, 92, 100-102], while others have not [95, 97, 103]. In studies which demonstrated a correlation, fibrinogen was measured either preoperatively just before start of surgery [100, 101], or after completed surgery [37, 91, 92, 102]. However, it is difficult to compare the studies due to variations in blood sampling, type of surgical procedures, patient selection and time point for registration of bleeding.

To our knowledge, the present study is the first and largest so far and the only one in which fibrinogen is measured the day before surgery. It was designed in a prospective manner, exclusively to investigate the association between preoperatively measured plasma fibrinogen and the amount of postoperative bleeding and transfusions of blood products. We standardized the design by measuring fibrinogen the day before surgery instead of after arrival in the operating theatre. This regimen was chosen since fibrinogen concentration at time of surgery may be affected by preoperative fluid therapy. Another reason for this was to test the hypothesis that early identification of patients with an increased risk of postoperative bleeding may offer the possibility to initiate countermeasures.

Another important question raised by this investigation is the level of plasma fibrinogen required to maintain optimal hemostasis in the perioperative period. It has for a long time been considered that a laboratory plasma level of >1 g/L is sufficient for appropriate hemostasis [111]. In accordance, most transfusion algorithms in general surgery and trauma do not treat low fibrinogen levels unless they are <1 − 1.5 g/L, which is below the normal values of 2 − 4.5 g/L [106, 142]. However, the present results indicate that this level may not be sufficient when the hemostatic system is challenged by cardiac surgery and the use of CPB. The results suggest that plasma fibrinogen concentration is a limiting factor for postoperative hemostasis, and that fibrinogen levels in the lower normal range may be too low to ensure an appropriate coagulation during and after major surgical procedures. Our results are supported by two recent studies. In patients suffering from post partum bleeding, fibrinogen levels <2 g/L was 100 % predictive of severe postpartum hemorrhage, and a fibrinogen plasma concentration of 4 g/L was suggested to be the lowest transfusion trigger level, in order to maintain adequate hemostasis [110]. An in-vitro dilution model simulating coagulopathy after acute loss of two blood volumes demonstrated that clot formation is optimized only after increasing fibrinogen concentration to above 2 g/L [83].

In the present study, patients with a plasma concentration of approximately >5 g/L generally bled less compared with patients with lower values (Fig. 10). The relationship is not straight linear, but supports previous studies about the importance of an adequate fibrinogen concentration in relation to bleeding tendency [83, 110]. In addition, the risk of being transfused varied in relation to the preoperative plasma fibrinogen level (Fig. 12). Altogether, the results give further evidence that the plasma fibrinogen concentration is a limiting factor for postoperative hemostasis and that fibrinogen levels in the lower normal range may be too low to ensure an appropriate coagulation during and after major surgical procedures.

There are several potential reasons why fibrinogen levels were associated with bleeding in the present study. Fibrinogen supplementation in animal experiments and in-vitro studies has been shown to counteract dilutional coagulopathy, impaired hemostasis caused by platelet deficiency, and to improve laboratory clot formation tests measured by ROTEM® thromboelastometry [77-80, 83, 113, 143-145]. Therefore, it is possible that the relationship between fibrinogen and bleeding is mediated by such effects, since hemodilution and platelet consumption have been suggested to be two important factors in the pathogenesis of severe bleeding after cardiac surgery [35, 48, 146].

There was also a significant inverse correlation between platelet count and bleeding, but this correlation was less pronounced (r = -0.26, p <0.001, Fig. 11). PT and APTT did not correlate to bleeding. In multivariate testing, fibrinogen was the only independent predictor among these coagulation biomarkers. We therefore speculate that measurement of the preoperative fibrinogen level provides more information about potential bleeding risk, than the standard coagulation screening tests used today.

Sex is a well known risk factor for transfusion [146, 147], which was confirmed in the present study. However, women did not bleed more compared with men. One explanation may be lower basal hemoglobin levels and a smaller total blood volume in women, resulting in a more pronounced hemodilution during and after CPB, with subsequently more women reaching the transfusion trigger. The association between preoperative fibrinogen concentration and absolute risk of transfusion is given in Fig. 12. The absolute risk of transfusion was moderate in the study group of elective CABG patients, with only 17 percent of the patients receiving any transfusion. However, Fig. 12 shows that the risk of transfusion varies considerably between patients. In a woman with a preoperative fibrinogen

concentration of 3.0 g/L, the risk is markedly higher than for a man with a fibrinogen concentration of 5.0 g/L (58% vs. 6%).

Effects of fibrinogen infusion on postoperative bleeding and transfusions

Based on the results in paper II, which demonstrated a strong inverse correlation between the preoperative concentration of fibrinogen in plasma and the amount of postoperative bleeding after CABG surgery, we hypothesized that prophylactic fibrinogen concentrate infusion before CABG could reduce postoperative bleeding. However, infusion of coagulation factors before or during a surgical procedure may, at least in theory, induce a state of hypercoagulability with increased risk of thromboembolic events, such as early graft occlusion and myocardial infarction. To investigate this, a prospective, randomized phase I-II pilot study was designed. Primary endpoint was clinically detectable adverse effects and graft occlusion assessed by multi-slice computed tomography. Secondary endpoints were effects of fibrinogen concentrate infusion on bleeding, transfusions, postoperative hemoglobin levels and global hemostasis. This study was a clinical attempt to investigate the feasibility of prophylactic fibrinogen infusion in patients undergoing cardiac surgery with CPB, and with normal plasma coagulation. None of the patients suffered from any preoperative fibrinogen deficiency.

The results show that prophylactic fibrinogen infusion reduced postoperative bleeding by approximately 30 % (Fig. 14), and maintained postoperative hemoglobin at a higher level in patients who were randomized to fibrinogen infusion compared with the control group (Fig. 15). There were also less transfused patients in the fibrinogen group, n 1/10, compared with controls, n 3/10, but the difference was not statistically significant.

We could not detect any clinical adverse effects that could be directly related to the fibrinogen infusion. Computer tomography discovered coincidentally one subclinical small peripheral PE in one of the patients in the fibrinogen group. However, this finding is not necessarily related to the fibrinogen infusion since the reported prevalence of clinically detectable PE in patients undergoing CABG with CPB is 0.4 - 9.5 % (mean 3.4 %), while the prevalence of subclinical PE, i.e. when the patient is asymptomatic, is not known [148-150]. However, in patients undergoing OPCAB, the prevalence of subclinical PE was recently reported to be as high as

25 % [151]. The prevalence of subclinical PE in the present study, 10 % in the fibrinogen group, is therefore probably what could be expected in the current study population. There was also one early vein graft occlusion in the fibrinogen group, resulting in an occlusion rate of 3.7 % in this group. The prevalence of early graft occlusion after CABG has previously been reported to be 3.9 % after five days [152], and 12 % in early follow up (mean 0.9 month) after surgery [153]. Therefore, there is no certain evidence of an increased occlusion rate in the present study, not overall nor in the fibrinogen group.

The significant reduction in bleeding volume further supports the hypothesis from paper II that plasma fibrinogen concentration is a limiting factor for postoperative hemostasis, and that fibrinogen levels in the lower normal range may be too low to ensure an appropriate coagulation during and after cardiac surgery. As mentioned previously, this finding is supported by recent clinical [68, 73, 110, 154, 155] and laboratory studies [78-81, 83, 143, 144]. Despite this, the mechanism behind reduced bleeding, and the relationship between fibrinogen and bleeding is not clarified. Plasma fibrinogen was no longer different between the groups two hours after surgery. This may be interpreted as stimulated fibrin generation leading to an improved hemostasis. Fibrinogen also interacts with platelets by binding to GPIIb/IIIa receptors leading to a stabilized fibrin network, rapidly forming an active seal at the site of vascular damage (Fig. 3) [156]. This mechanism may also have contributed to improved hemostasis and reduced bleeding.

There is a few but increasing number of prospective studies on humans. Rahe-Meyer et al investigated patients operated for aortic valve replacement or aortic aneurysm. Fibrinogen was given prophylactically following a blood transfusion algorithm, which resulted in reduced transfusion requirements and postoperative bleeding compared with patients treated primarily with blood products alone [157]. Similar results and study design was seen in patients operated for thoracoabdominal aneurysms [158]. Fibrinogen has also been shown to improve clot formation in patients with dilutional coagulopathy during orthopedic surgery [154], and in children undergoing craniosynostosis surgery [73]. When it comes to retrospective studies, administration of fibrinogen concentrate has been shown to significantly reduce bleeding and blood transfusions in patients with low to normal fibrinogen levels treated for massive hemorrhage related to trauma, surgery and obstetric complications [68]. Similar results were demonstrated by Weinkove et al [115].

One important objective in this study was safety since artificially stimulated coagulation, at least theoretically, may lead to an increased risk of harmful hypercoagulability. In addition to assessing clinical symptoms and graft patency, we were interested in the effects of fibrinogen concentrate infusion on global hemostasis assessed by thromboelastometry. There was moderately impaired global hemostasis in both groups 2 hours after surgery, but no significant differences were found between the groups. As mentioned earlier, fibrinogen infusion statistically increased plasma fibrinogen by 0.6 ± 0.2 g/L. The reason why this was not seen on thromboelastometry variables, FIBTEM in particular, may be several. One possible explanation is that the increase of fibrinogen by 0.6 g/L was too discrete or that the FIBTEM test was not sensitive enough. Another possible explanation may be that the difference was concealed, since thromboelastometry measurements were not performed intra-operatively when the difference in plasma fibrinogen concentration was likely still significant between the groups.

The global hemostasis had almost normalized 24 h after the operation. The lack of changes in thromboelastometry variables between the two groups may not necessarily be interpreted as lack of effect of the fibrinogen infusion. Instead, the absence of unwanted hypercoagulability was concomitant with desired clinical effects. However, the study is underpowered to make any robust conclusions about safety and efficacy, and the current results are not sufficient to propose that prophylactic fibrinogen infusion should currently be used to prevent bleeding in any cohort of CABG patients.

Effects of fibrinogen on coagulation, fibrinolysis and platelet function

The major finding in paper III was that prophylactic treatment with fibrinogen concentrate was feasible and significantly decreased postoperative blood loss after CABG. Postoperative hemoglobin was maintained at a higher level, without evidence of an increased incidence of thrombo-embolic events, such as graft occlusion, myocardial infarction and PE. As mentioned previously, this was the first time fibrinogen concentrate was administered to patients without fibrinogen deficiency or on-going severe bleeding, and therefore, we found it essential from a safety point-of-view to thoroughly monitor the effect of fibrinogen concentrate on markers of coagulation, fibrinolysis and platelet function.

In addition to conventional markers of hemostasis and fibrinolysis, we used impedance aggregometry (Multiplate®) to assess platelet function and rotational whole blood thromboelastometry (ROTEM®) to analyze global hemostasis. As primary aim, we were interested in investigating immediate effects of fibrinogen measured 15 minutes after completed infusion, before the on-start of surgery. As secondary aim, we studied postoperative effects measured 2 and 24 hours after completed surgery.

Regarding the primary aim, fibrinogen concentrate infusion induced no or minimal changes in biomarkers of coagulation, fibrinolysis and platelet function. Antithrombin, an anti-coagulant factor in human blood plasma which is usually decreased during and after cardiopulmonary bypass [159], was marginally but significantly decreased in the fibrinogen group compared with the control group. However, both measurements were made before heparinazation and the biological meaning of this variation is likely irrelevant. TAT and PF 1&2, molecular markers of thrombin activity and generation [159, 160], were discretely elevated in both groups, somewhat more markedly among controls, but there was no significant difference between the groups. Discrete variations in TAT and PF 1&2 might be due to sampling technique and the analysis itself rather than increased thrombin generation [160]. Since the differences were minor, this cannot be ruled out. Also, no major surgical trauma had yet occurred that would stimulate thrombin generation to a larger extent.

Our second aim was to compare the fibrinogen group and the control group in relation to completed surgery. In this analysis, we used measurements after infusion as baseline and compared this to measurements 2 and 24 hours after surgery. The elevation in plasma fibrinogen after infusion was no longer significantly different between the groups 2 and 24 hours after surgery. Plasma concentrations of D-dimer were, as expected [161], increased after surgery compared with the preoperative levels (Fig. 16). Furthermore, D-dimer was significantly higher in the fibrinogen group 2 hours after surgery compared with the control group (p = 0.03, Fig 16). The results indirectly indicate an increased fibrin generation in the fibrinogen group during the preceding period of time, i. e. during surgery, which may be partly responsible for the reduced postoperative blood loss in this group. Also, the fact that no or minimal changes in biomarkers were seen in the present study may indicate that infusion of fibrinogen, even in a low dose of 2g, has a clinical effect without unwanted signs of harmful hypercoagulability.

Clinical implications

The fact that the plasma fibrinogen concentration measured the day before surgery correlates to postoperative bleeding is very interesting in a clinical point of view. Fibrinogen is often considered merely an acute phase reactant or a marker for risk of thrombo-embolism or cardiovascular disease, rather than a potential pro-hemostatic factor [111, 162]. Since fibrinogen is the most abundant protein in the hemostatic system, (>2.5 g/L, compared with 145 mg/L for all other coagulation factors together [111]), the overall hemostatic capacity was thought not to depend on minor changes in the plasma concentration of fibrinogen [111]. An increasing number of studies suggest that the plasma fibrinogen concentration is probably more important for maintaining sufficient hemostasis caused by hemodilution and/or bleeding than previously recognized, especially since fibrinogen seems to be the coagulation factor which first reaches critically low plasma levels [68, 104]. Our results further support the importance of adequate plasma fibrinogen levels, since patients with higher levels in general bleed less. Measuring the plasma concentration the day before surgery offers the possibility to initiate countermeasures. This was investigated in paper III, which showed reduced bleeding and less transfusions among patients who received fibrinogen prophylactically. However, larger and placebo controlled studies are warranted to investigate this closer. It is noteworthy that there was a significant difference in bleeding volume between the groups, despite the fact that there were only 10 patients in each group.

One may speculate if it is worth the effort of reducing transfusions of blood products by the prophylactic administration of another human derived plasma product such as fibrinogen. However, virally inactivated fibrinogen concentrate seems to be associated with less negative side effects compared with red blood cells, plasma, platelets and cryoprecipitate [68, 69, 113]. Accumulating evidence suggest that transfusion of allogeneic blood products, such as red blood cells, fresh frozen plasma, platelets and cryoprecipitate, are associated with increased risk of morbidity and mortality [54-56, 163, 164]. Management of traumatic and perioperative bleeding with plasma derived or recombinant coagulation factor concentrate, such as fibrinogen and FVII, may therefore reduce transfusion of allogeneic blood products. It may also be beneficial to use fibrinogen early in the perioperative period since this has been shown to significantly reduce bleeding and the need for allogeneic blood transfusions [157].

Limitations

There are limitations in these studies that should be mentioned. In paper I, the inflammatory variables were not altered significantly by the surgical procedure. The study design does not allow any conclusions about the magnitude of the inflammatory response after OPCAB surgery since the single postoperative measurement was performed immediately after surgery, and some of the investigated inflammatory mediators have a markedly later peak concentration [135]. Furthermore, the investigation was performed in low risk patients and only a few markers of inflammation and hemostasis were analyzed. Both the inflammatory response and hemostasis after cardiac surgery are extremely complex processes, and other markers and mediators may react differently. In addition, the limited number of patients included infers a risk of a statistical type II error.

Paper II does not prove causality, even if the results suggest that fibrinogen per se is a limiting factor for postoperative hemostasis. The results may be caused by other factors that vary in correlation with fibrinogen. This study is a single-center experience. Transfusion thresholds, hematologic practice, use of thromboelastometry, discontinuation of anticoagulants, and the use of perioperative antifibrinolytics may not reflect worldwide practice, and the generalizability of our results needs thus to be proven in larger multicenter studies.

In paper III, it should be noted that this is a pilot study focusing on feasibility and tolerability of fibrinogen prophylaxis. The study is underpowered to make any robust conclusions about safety and efficacy and the current results are thus not sufficient to propose that prophylactic fibrinogen infusion currently should be used to prevent bleeding in any cohort of CABG patients. It should also be noted that the preoperative fibrinogen level alone is insufficient to determine whether a patient is at high risk for bleeding complications after cardiac surgery. Other factors such as patient characteristics, preoperative medication, the magnitude of the operative trauma and preoperative hemoglobin and platelet levels may be equally or more important. Other limitations include the absence of placebo-treatment in the control group and that factor XIII activity was not analyzed before surgery.

Paper IV has the same main limitation as paper III, with a low number of study participants, n=20. A statistical type II error can therefore not be ruled out. It is possible that changes in hemostatic factors would have been detected in a larger number of patients. The study size

was limited as a safety precaution since this was the first study on the subject, and the authorities restricted the allowed number of participants. Another limitation may be the number and choice of biomarkers, where other biomarkers may have yielded a different result. However, the current markers are common in clinical practice. Furthermore, the biomarkers were not analyzed during surgery. Since there was a difference in postoperative bleeding between the groups, one may speculate that there may be differences in the hemostatic variables during surgery, which were not detected by the pre- and postoperative measurements. It is also possible that a higher dose of fibrinogen in paper III would have generated detectable differences by thromboelastometry. The used dose of 2g, corresponding to 18-34 mg/kg bodyweight, is rather small. In patients with congenital afibrinogenemia, a recommended starting dose of 70 mg/kg bodyweight raised the plasma fibrinogen level by 1 g/L, which may be easier to detect with thromboelastometry [64].

6. Summary

1. There are associations between the inflammatory response, hemostasis and bleeding after OPCAB (**Paper I**).

2. The preoperative fibrinogen plasma concentration correlates independently to bleeding volume and transfusion prevalence after CABG (**Paper II**).

3. Prophylactic fibrinogen concentrate infusion in CABG patients seems feasible without clinically detectable side effects **(Paper III)**.

4. Prophylactic fibrinogen concentrate infusion in CABG patients significantly reduces postoperative bleeding **(Paper III)**.

5. Prophylactic fibrinogen concentrate infusion in CABG patients results in no or minimal changes in biomarkers reflecting coagulation, fibrinolysis and platelet function **(Paper IV)**.

7. Acknowledgements

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Sammanfattning på svenska
Blödning i samband med hjärtkirurgi

Hjärtkirurgi med användning av hjärtlungmaskin innebär att det aktuella ingreppet utförs på stillastående hjärta, vilket är vanligt vid operationer på kranskärl, hjärtklaffar och vid hjärt- och lungtransplantationer. Ingreppet medför dessvärre en kraftig inflammatorisk aktivering i blodet och en stor risk för blödningskomplikationer, som kan uppstå under och efter operationen. Kranskärlsoperationer kan även utföras utan hjärtlungmaskin på slående hjärta, men är inte lika vanligt förekommande.

Studiernas mål

I det första delarbetet görs en beskrivande studie över sambandet mellan inflammatoriska ämnen i blodet, koagulationsmarkörer och mängden blödning hos patienter som kranskärlsopereras utan hjärtlungmaskin. I delarbete II studeras sambandet mellan plasmakoncentrationen av fibrinogen och mängden postoperativ blödning samt behov av blodtransfusioner. Syftet är att kartlägga fibrinogenets eventuella möjlighet att prediktera risk för blödning och blodtransfusioner. I delarbete III studeras huruvida förebyggande behandling med fibrinogenkoncentrat innan kranskärlskirurgi är säkert att genomföra, samt om blödningsmängd och transfusionsbehov reduceras av behandlingen. Hypotesen är att substitution med fibrinogen är gynnsamt till hjärtopererade patienter. I delarbete IV kartläggs effekterna av fibrinogenbehandling på utvalda markörer för koagulation, fibrinolys och trombocytfunktion.

Material och metoder

I delarbete I studerades 10 elektiva patienter som genomgick kranskärlskirurgi utan hjärtlungmaskin (OPCAB). Utvalda markörer för inflammation och hemostas mättes före och efter kirurgi. I delarbete II studerades 170 elektiva patienter som genomgick kranskärlskirurgi med användning av hjärtlungmaskin. Plasmakoncentrationen av fibrinogen mättes dagen innan kirurgi. Postoperativ blödning och antalet blodtransfusioner registrerades. I delarbete III studerades 20 elektiva kranskärlspatienter som randomiserades till 2 grupper där 10 patienter fick behandling med 2g fibrinogenkoncentrat innan kirurgi, och den andra gruppen fick ingenting. Blödning och blodtransfusioner samt förekomst av graftocklusion studerades. I

delarbete IV studerades samma patientmaterial med avseende på effekter av fibrinogenbehandling på markörer för koagulation, fibrinolys och trombocytfunktion.

Resultat

I delarbete I fann vi signifikanta korrelationer mellan bland annat inflammationsmarkörerna PMN-elastase och C3a, och hemostasmarkören β-thromboglobulin. Ett omvänt samband mellan plasmakoncentrationerna av pre- och postoperativt fibrinogen, postoperativt β-thromboglobulin och postoperativt PMN-elastase, och mängden postoperativ blödning förelåg. I delarbete II fann vi ett signifikant, inverst samband ($r=-0.53$, $p<0.001$) mellan plasmakoncentrationen av fibrinogen och mängden postoperativ blödning. Fibrinogen var enda oberoende prediktor för postoperativ blödning. Fibrinogen, kvinnligt kön och aortaklamp-tid var oberoende prediktorer för att bli blodtransfunderad. I delarbete III fann vi inga kliniskt detekterbara biverkningar. Postoperativ blödning reducerades med 32 %. I delarbete IV fann vi inga större skillnader i markörer för koagulation, fibrinolys och trombocytfunktion mellan grupperna, varken direkt efter fibrinogeninfusion eller efter avslutad kirurgi. En förhöjd nivå av D-dimer påvisades i fibrinogengruppen jämfört med kontroller.

Slutsatser

Det finns evidens för ett samband mellan inflammatorisk aktivering och hemostas efter hjärtkirurgi. Inflammation samt aktiverade trombocyter förefaller i detta sammanhang inte vara av ondo, utan kan bidra till minskad postoperativ blödning. Plasmakoncentrationen av fibrinogen är en begränsande faktor för postoperativ hemostas och dess preoperativa nivå förefaller kunna prediktera risk för blödning och blodtransfusioner efter hjärtkirurgi. Förebyggande behandling med fibrinogenkoncentrat minskar den postoperativa blödningsvolymen signifikant efter kranskärlskirurgi, utan tecken på postoperativ hyperkoagulabilitet eller kliniskt detekterbara biverkningar. Effekterna av fibrinogen på markörer för koagulation, fibrinolys och trombocytfunktion är marginella.