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APROTININ FOR REDUCTION OF BLEEDING AND TRANSFUSIONS IN PATIENTS ON CLOPIDOGREL UNDERGOING URGENT CORONARY SURGERY

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Aprotinin for reduction of bleeding and transfusions in patients on clopidogrel undergoing urgent coronary surgery

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“Always look on the bright side of life”

Monty Python; “Life of Brian”

To
Anna, Linnea, and Bosse

ABSTRACT (ENG)

Background: An increased proportion of patients with acute coronary syndrome undergo coronary surgery while treated with clopidogrel, an irreversible platelet ADP-receptor inhibitor. Clopidogrel in combination with aspirin is known to augment bleeding, transfusion requirements, and reoperation rates after coronary surgery. Aprotinin, a protease inhibitor, has been approved for use in cardiac surgery to reduce bleeding. Studies on the safety of aprotinin in coronary surgery have given conflicting results. The aim was to investigate whether or not intraoperative use of aprotinin decreases bleeding and number of transfusions after coronary surgery in patients treated with clopidogrel <5 days before surgery. The possible link between perioperative aprotinin treatment and renal dysfunction in patients undergoing first time coronary surgery with a high risk of bleeding was also studied. Finally, we studied the adenosinediphosphate mediated platelet aggregation before and after administration of aprotinin in patients on clopidogrel.

Methods: I. We retrospectively reviewed the medical records of all consecutive patients, with preoperative clopidogrel exposure <5 days before surgery, who underwent urgent coronary surgery at our institution during 1 year (n=33). 18 patients received a full-dose aprotinin, 15 patients served as a control group. **II.** 75 consecutive patients with unstable angina, administered clopidogrel <5 days before coronary surgery, were randomized to full-dose aprotinin (n=37) or saline (n =38). **III.** In a matched cohort study, 200 patients receiving high-dose aprotinin were compared with 200 patients receiving tranexamic acid during primary isolated coronary surgery. **IV.** 15 clopidogrel-treated patients with acute coronary syndrome undergoing coronary surgery were studied. ADP-mediated platelet aggregation and platelet count ratio (%) were measured before and after a bolus dose of aprotinin.

Results: I. Mean postoperative bleeding was 710 mL (95%CI:560-860) in the aprotinin group vs. 1210 mL (95%CI:860-1550) in the control group (p=0.004). The aprotinin group received fewer transfusions of packed red blood cells (0.9 U, 95%CI:0.1-1.7, vs. 2.7 U, 95% CI:1.4-4.1; p=0.01), platelets (0.1 U, 95%CI:0-0.3, vs. 0.6 U, 0.2-0.9; p=0.02), and fewer blood product units (1.1 U, 95%CI:0.1-2.0, vs. 3.7 U, 95%CI:2.1-5.4; p=0.002). There were 3 reoperations for bleeding, all in the control group (p = 0.05). **II.** Postoperative bleeding was 760±350 mL in aprotinin-treated patients versus 1200±570 mL (P<0.001) in control. During the hospital stay, patients in the aprotinin group received 1.2±1.5 and 0.1±0.4 U of erythrocytes and platelets, respectively, versus 2.8±3.2 (P=0.02) and 0.9±1.4 (P=0.002) units in the control. In the aprotinin group, 53% of patients received transfusions, whereas 79% of controls were exposed to blood products (p=0.02). **III.** No significant differences were found in fractional change in creatinine clearance (-11% vs. -12%, medians, p=0.75) or any other assessments of postoperative renal function between the tranexamic acid and the aprotinin group. Patients in the aprotinin group received fewer transfusions (48% vs. 60.5%, p=0.02), fewer units of packed red blood cells (2.0 U vs. 1.4 U, p=0.02) and plasma (1.3 U vs. 0.5 U, p<0.001), but more platelets (0.1 U vs. 0.2 U, p=0.02). **IV.** Aprotinin induced an increased aggregation in eleven of fifteen patients (73%), and a decrease was registered in two patients (13%). The median (25th/75th percentile) adenosinediphosphate mediated platelet aggregation before and after aprotinin, was 84% (76/91) and 94% (86/97, p<0.01). Clopidogrel non-responders with >90% aggregation (n=4) had a median aggregation of 94.5% (91.5/97.5) versus 82% (73/87, p<0.01) in the responders (n=11). The median increase in platelet aggregation after aprotinin was 8% (5/20) in the responders versus 0% (-5.25/3, p<0.01) in the non-responders.

Conclusions: Full-dose aprotinin reduces bleeding, transfusion requirements of packed red blood cells, platelets, and total number of blood units in patients on clopidogrel undergoing urgent CABG. Perioperative aprotinin treatment during primary coronary surgery in patient on clopidogrel treatment is not associated with impaired renal function postoperatively in comparison with patients receiving tranexamic acid. The use of aprotinin reduces the overall transfusions rate to a greater extent than tranexamic acid. Aprotinin reduces the antiplatelet effect of clopidogrel. This effect is restricted to patients with a platelet inhibition of ≥ 10%.

Key words: Acute coronary syndrome, aprotinin, clopidogrel, platelets, surgery, tranexamic acid, transfusions.

ABSTRACT (SWE)

Aprotinin minskar blödning och antal transfusioner hos clopidogrelbehandlande patienter som genomgår akut koronarkirurgi

Patienter med instabil kranskärlssjukdom behandlas rutinmässigt med de trombocythämmande läkemedlen clopidogrel och aspirin för att minska risken för akut hjärtinfarkt. Clopidogrelbehandlade patienter som genomgår akut kranskärlskirurgi har en ökad risk för blödning och transfusioner.

Delarbete I

I en retrospektiv studie omfattande 33 konsekutiva patienter som exponerats för clopidogrel mindre än fem dygn före coronarkirurgi, behandlades 18 patienter med full dos aprotinin intraoperativt medan 15 patienter utgjorde en kontrollgrupp. De två grupperna var jämförbara med avseende på demografiska data. Medelvärdet för den postoperativa blödningen var 710 ml (95% CI 560-860) i aprotiningruppen mot 1210 ml (95% CI 860-1550) i kontrollgruppen (p=0.004). Aprotiningruppen erhöll färre transfusioner av erytrocytkoncentrat (0.9 enh 95% CI 0.1-1.7 enh mot 2.7 enh 95% CI 1.4-4.1 enh; p=0.01), trombocyter (0.1 enh 95% CI 0-0.3 enh mot 0.6 enh 95% CI 0.2-0.9 enh; p=0.02), och färre enheter blodprodukter totalt (1.1 enh 95% CI 0.1-2.0 enh mot 3.7 enh 95% CI 2.1-5.4 enh; p=0.002).

Delarbete II

Studien omfattade 75 konsekutiva patienter med instabil angina som behandlats med clopidogrel inom fem dygn före koronarkirurgi. Med en dubbelblind design, randomiserades patienterna till behandling med aprotinin (n =37) eller koksaltlösning (n =38). Demografiska data skilde sig inte signifikant mellan grupperna. Postoperativ blödning var 760±350 ml (medel±SD) i aprotiningruppen mot 1200±570 ml (p<0.001) i kontrollgruppen. Patienterna i aprotiningruppen erhöll 1.2±1.5 enheter erytrocytkoncentrat och 0.1±0.4 enheter trombocyter mot 2.8±3.2 enheter erytrocytkoncentrat (p=0.02) och 0.9±1.4 enheter trombocyter (p=0.002) i kontrollgruppen. I aprotiningruppen fick totalt 53 % av patienterna transfusioner mot 79 % i kontrollgruppen (p=0.02).

Delarbete III

I en matchad kohortstudie jämfördes 200 patienter som behandlats med fulldos aprotinin med 200 patienter som behandlats med tranexamsyra vid primär och isolerad kranskärlskirurgi. Patienterna matchades för ålder, kön och diagnosen akut koronart syndrom. Det primära målet var att studera relativ ändring i kreatininclearance. I andra hand studerades mortalitet, stroke, reoperation pga. blödning och transfusionsbehov. Grupperna var jämförbara beträffande demografiska data, förutom högre prevalens av trekärlssjukdom och preoperativ infarkt i aprotiningruppen. Inga signifikanta skillnader påvisades mellan grupperna beträffande fraktionell ändring av kreatininclearance (-11% mot -12%, median, p=0.75) eller någon annan njurfunktionsparameter. Komplikationsfrekvensen var jämförbar mellan grupperna med avseende på: tidig mortalitet (3.5% mot 4.5%, p=0.80), stroke (1.5% mot 2%, p=1.0), reoperation pga. blödning (3.5% mot 2.5%, p=0.77), och 5-årsöverlevnad (87% mot 84%, p=0.17). Patienterna i aprotiningruppen fick färre transfusioner totalt (48% mot 60.5%, p=0.02), färre enheter erytrocytkoncentrat (2.0 mot 1.4, p=0.02) och plasma (1.3 mot 0.5, p<0.001), men fler enheter trombocyter (0.1 mot 0.2, p=0.02).

Delarbete IV

Femton patienter med akut koronärt syndrom inkluderades i studien. Alla patienter var behandlade med clopidogrel inom 5 dygn före kranskärlsoperation. Den ADP-medierade trombocyt-aggregationskvoten (%) mättes före och efter en bolusdos aprotinin ($2x10^6$ KIE). Aprotinin inducerade en ökad trombocytaggregation hos 11 patienter (73%), och en minskning hos 2 patienter (13%). Den ADP-medierade trombocytaggregation var 84% (76/91, median och 25:te/75:te percentilen) före och 94% (86/97, p<0.01) efter aprotinin. Patienter med en aggregation över 90% (n=4), s.k. icke-responders, hade

en median aggregation på 94.5% (91.5/97.5) mot 82% (73/87, $p<0.01$) hos responders (n=11). Medianökning av trombocytaggregation efter aprotinin var 8% (5/20) hos responders mot 0% (-5.25/3, $p<0.01$) hos icke-responders.

Slutsatser

1) Aprotinin reducerar blödning, transfusionsbehov av erytrocytkoncentrat, trombocyter och det totala antalet blodenheter hos clopidogrelbehandlade patienter som genomgår akut kranskärlskirurgi. 2) Intraoperativ behandling med aprotinin vid primär kranskärlskirurgi hos clopidogrelbehandlande patienter kan ej kopplas till försämrad postoperativ njurfunktion vid jämförelse med patienter som fick tranexamsyra. 3) Aprotininbehandling medförde att det totala antalet transfusioner blev lägre än om tranexamsyra användes. 4) Aprotinin minskar den trombocythämmande effekten av clopidogrel. Detta påvisades endast hos patienter vars trombocyter var hämmade med mer än 10%.

CONTENTS

LIST OF ABBREVIATIONS

ACC	American College of Cardiology
ACS	Acute coronary syndrome
ACT	Activated clotting time
ADP	Adenosinediphosphate
AHA	American Heart Association
BMI	Body mass index
CABG	Coronary artery bypass grafting
CI	Confidence interval
CPB	Cardiopulmonary bypass
Cr	Creatinine
CrCl	Creatinine clearance
ECC	Extra corporeal circulation
ECG	Electrocardiography
EDTA	Ethylenediaminetetraacetic acid
EF	Ejection fraction
GP	Glycoprotein
ICU	Intensive care unit
KIU	Kallikrein inhibiting units
LMWH	Low molecular weight heparin
NSTEMI	Non-ST elevation myocardial infarction
PAR	Protease activated receptor
PCI	Percutaneous coronary intervention
PRBC	Packed red blood cells
RR	Relative risk
SD	Standard deviation
TXA	Tranexamic acid
TEE	Transesophageal echocardiography
tPA	Tissue plasminogen activator
UA	Unstable angina

LIST OF ORIGINAL ARTICLES

This thesis is based on the following papers that are referred to by their roman numerals I-IV in the text:

I **Lindvall G**, Sartipy U, van der Linden J.
Aprotinin reduces bleeding and blood product use inpatients treated with clopidogrel before coronary artery bypass grafting.
Ann Thorac Surg 2005; 80:922–7.

II van der Linden J**, Lindvall G**, Sartipy U.
Aprotinin decreases postoperative bleeding and number of transfusions in patients on clopidogrel undergoing coronary artery bypass graft surgery: a double-blind, placebo-controlled, randomized clinical trial.
Circulation 2005; 112:I276-80.

III **Lindvall G**, Sartipy U, Ivert T, van der Linden J.
Aprotinin is not associated with postoperative renal impairment after primary coronary surgery.
Ann Thorac Surg 2008; 86:13-9.

IV **Lindvall G**, Sartipy U, Bjessmo S, Svenarud, Lindvall B, van der Linden J.
Aprotinin reduces the antiplatelet effect of clopidogrel.
Submitted

INTRODUCTION

The majority of patients undergoing cardiac surgery receive blood transfusions. Yet, only 15-20% of the patients consume more than 80% of the transfused blood products. In this context one should appreciate that blood is a scarce resource with risks and benefits. When deciding whether the patient should receive blood products the physician in charge must strictly consider if the advantages of a transfusion including a possible improved tissue oxygenation outweigh potential hazards such as transmission of blood-related disease or induction of adverse reactions. Thus, efforts should be made to identify patients undergoing cardiac surgery that have a high-risk for bleeding, and in those patients undertake preventive actions that may minimize blood loss and transfusion needs.

Platelets play a critical role in the hemostasis mechanism by their formation of the primary hemostatic plug as well as by their contribution to the clotting cascade. Activated platelets not only synthesize and release a platelet-activating lipid, thromboxane-A2, but they also release ADP and serotonin. They expose receptors for fibrinogen in the form of activated GP IIb/IIIa, which represents the final common pathway leading to the aggregation of platelets. Activated platelets also promote the generation of thrombin—the most potent of all platelet agonists. Thrombin acts predominantly via PAR-1 and PAR-4, expressed on platelets, whereby PAR-1 is more potent [1]. Finally, thrombin generated by the coagulation cascade transforms fibrinogen to fibrin, which stabilizes the thrombus. Thrombus formation initiated by endothelial lesions in a coronary artery may set off an ACS with the risk of myocardial infarction and ultimately death. Aspirin irreversibly blocks the formation of thromboxane-A2 in platelets, which inhibits platelet aggregation, making it a useful tool for reducing the incidence of thrombus formation in patients with coronary artery disease. Particularly, in patients with ACS there is a continuous activation of platelets and an aggravated clot formation [2,3]. Platelets are thus critically important in the acute setting of PCI and CABG, where both platelet count and function are affected. Notably, antiplatelet and antithrombin therapy has been demonstrated to reduce the risk of cardiac events in patients presenting with ACS. During the last decade, clopidogrel given together with aspirin has become standard therapy in these patients [4].

Clopidogrel is a thienopyridine derivate, the active metabolite of which is short-lived. It

selectively and irreversibly inhibits the ADP-$P2Y_{12}$ receptor on the surface of platelets. This receptor is important in platelet aggregation, the cross-linking of platelets by fibrin, and in platelet-leukocyte aggregation that can trigger an inflammatory response in endothelial cells [5]. The dose-dependent inhibition of platelets results in decreased aggregation after ADP-release by blocking activation of the GPIIb/IIIa pathway. Clopidogrel is mainly metabolized in the liver, and is activated via cytochrome P450. The plasma elimination half life is approximately eight hours. The active metabolite forms disulfide bridges between cysteine residues on the $P2Y_{12}$ receptor, which irreversibly modifies the receptor site and inhibits ADP-dependent platelet activation and aggregation. A loading dose of approximately 300 mg induces a maximal platelet inhibition within 2 to 5 hours. However, a steady state is first achieved after 3 to 7 days of therapy with 75 mg/d, resulting in a platelet inhibition of approximately 50%. $P2Y_{12}$ receptor inhibition by clopidogrel is thought to be irreversible, thus affecting aggregation during the the whole lifespan of platelets (5–10 days). When treatment with clopidogrel is stopped platelet function will recover after approximately 5-7 days [6-8]. Thus, from the time of drug discontinuation, restoration of normal hemostasis is dependent on the introduction of new platelets into the circulation.

It is thought that non-responsiveness, or resistance, to clopidogrel may at least partly explain the occurrence of adverse ischemic events [9-11]. This resistance is due to several mechanisms. Conventional methods for analysis of platelet function are time consuming, operator dependent and lack standardization. Validated point of care methods are needed to detect clopidogrel resistance on a routine basis. However, even when clopidogrel resistance is detected its appropriate treatment remains to be determined.

After PCI, long-term clopidogrel therapy significantly reduces the risk of adverse ischemic events [12,13]. Thus, many ACS patients are treated with clopidogrel, in addition to aspirin, and, possibly, LMWH. In the CURE study, the combination of clopidogrel and aspirin was superior to aspirin alone for patients hospitalized with non-ST-elevation ACS [4]. For patients with ACS requiring CABG surgery during the initial hospital stay (530 placebo and 485 clopidogrel patients), the incidence of CV death, MI, or stroke before the operation was 4.7% for placebo and 2.9% for clopidogrel patients (relative risk 0.56; 95% CI 0.29-1.08) [14]. Even more important, fewer patients proceeded to revascularization during the initial hospitalization in the blinded clopidogrel group than in the placebo group (20.7% v 22.6%; RR, 0.92; $p = 0.03$). Thus, in accordance with the overall findings of the CURE study, the

addition of clopidogrel in patients with ACS not only reduced the incidence of major cerebrovascular events, but it also reduced the number of patients who needed revascularization during the initial hospitalization. Moreover, the absolute benefit of clopidogrel in patients with ACS increased with increasing Thrombolysis In Myocardial Infarction (TIMI) risk score [15]. This indicates that although all categories of patients with ACS will benefit from clopidogrel, the greatest absolute benefit will be in high-risk patients (i.e., those needing urgent coronary intervention, including CABG). An invasive approach is the preferred strategy in patients with ACS and signs of ischemia on ECG or raised levels of biochemical markers of myocardial damage [16]. Since clopidogrel is often given before angiography and PCI, the patient may later be referred to surgery with the additional handicap of an irreversible platelet inhibition for 5-7 days. When clopidogrel was discontinued <5 days before surgery the drawback of this treatment was increased perioperative bleeding and higher transfusion- and reoperation rates in patients with ACS [17-20]. A meta-analysis by Purkayastha et al. revealed that patients on clopidogrel undergoing coronary surgery had a mean increase in blood loss of 324 ml, a three fold increase in ventilation requirement, a five fold increase in overall transfusion risk, a seven fold increase in the odds of re-exploration, and 50% increase in adverse events [21]. Re-exploration due to bleeding may not only lengthen the hospital stay, but has also been associated with an increase in mortality [22]. Thus, the surgical team is facing the question whether the patient should have coronary surgery delayed for 5 days at the risk of acute ischemic events, or should be operated upon earlier at the risk of increased bleeding and morbidity. To complicate things further, the clinical response to clopidogrel varies greatly according to platelet aggregometry, which may influence the volume of bleeding in patients undergoing coronary surgery while on clopidogrel. As an example, Chen et al. found that clopidogrel-induced preoperative platelet dysfunction, measured as ADP aggregometry response <40%, identified all but 1 case of severe coagulopathy requiring multiple transfusions of platelets and PRBC after CABG [18].

Aprotinin is a naturally occurring serine protease inhibitor that reduces surgical blood loss and the need for perioperative blood transfusion. It is extracted from bovine lung tissue and inhibits serine proteases by forming reversible complexes. Aprotinin is considered to be an anti-fibrinolytic modulator of coagulation (pro- and anticoagulant), a modulator of the inflammatory cascade and a platelet protectant. Two important proteases inhibited by aprotinin are plasmin and kallikrein, whence plasmin is the final enzyme in the fibrinolytic pathway. Kallikrein is a serine proteinase that cleaves kininogens to form kinins (e.g.

bradykinin) and also activates blood coagulation factors XII, VII and plasminogen. Other proteases that interact with aprotinin include trypsin, chymotrypsin, thrombin, activated protein C, elastase and tPA. The enzymatic activity of aprotinin is expressed in KIU, with 1 KIU equivalent to the amount of aprotinin that decreases the activity of 2 biological kallikrein units by 50%, and 1 mg of the drug is equivalent to 7143 KIU. In cardiac surgery the usual i.v. dosing consists of 2 x 10^6 KIU as a loading dose, followed by 5 x 10^5 KIU/h during surgery, and 2 x 10^6 KIU in the CPB circuit prime. Lower-dosing regimens are not considered to provide a full anti-inflammatory effect. By inhibiting thrombin activation of PAR-1 on platelet activation during CPB, aprotinin reduces the activation and depletion of platelets during CPB. This allows platelets to retain their function perioperatively. By interacting with kallikrein at higher doses, aprotinin inhibits the intrinsic pathway of coagulation, possibly decreasing over-consumption of coagulation products during CPB. Aprotinin prolongs partial thromboplastin time and whole-blood celite ACT. Thus, kaolin-based ACTs are much less affected than celite-based ACTs. After glomerular filtration, aprotinin is actively reabsorbed and stored in the proximal tubules, where it is metabolized. Approximately 90% of aprotinin is excreted after 24 h [1].

Aprotinin has successfully been used in cardiac surgery to reduce overall bleeding and transfusion requirements in patients including those exposed to aspirin [23-27]. Aprotinin is appealing in cardiac surgery as it not only reduces overall bleeding and transfusion requirements but also appears to preserve platelet function during CPB [28,29]. Moreover, in animals, aprotinin has been shown to shorten prolonged bleeding induced by clopidogrel [30]. Aprotinin appears to be of particular interest in patients treated with clopidogrel and aspirin who undergo urgent or acute coronary surgery. This may partly be due to aprotinin´s additional inhibitory effect on the inflammatory cascade [31] and to platelet-protective properties, which may help to preserve platelet function after CPB [32,33]. However, the effect of aprotinin on platelet function in patients on clopidogrel undergoing CABG is still unclear.

Contradicting earlier randomized studies, including three meta-analyses [34-36], two large observational studies by Mangano et al. [37,38] indicated that, when compared with lysine analogs or a control group, perioperative treatment with aprotinin significantly increased the risk of renal, cardiac or cerebral events, as well as mortality. The authors concluded that “continued use of aprotinin is not prudent” and that “lysine analogs are safe

alternatives". However, these findings have been contradicted by large observational studies by other investigators [39-41] except for the finding that the use of aprotinin in CABG may be associated with renal dysfunction [42]. In addition, two large more recent observational studies in patients undergoing CABG have again indicated an increased mortality after aprotinin exposure [43,44]. Eventually, a recent large randomized trial evaluating aprotinin versus lysine analogs in patients categorized as "high risk cardiac surgery patients" observed an increased mortality in those patients receiving aprotinin [45].

AIMS OF THE THESIS

The aims of this thesis were:

- to determine whether or not aprotinin decreases bleeding and transfusion requirements in patients undergoing urgent or acute CABG and treated with clopidogrel less than 5 days before surgery.
- to investigate the possible association between perioperative aprotinin treatment and renal dysfunction in patients undergoing first time coronary surgery compared with TXA treatment.
- to investigate whether aprotinin influences ADP-mediated platelet aggregation in patients with varying degrees of response to clopidogrel.

PATIENTS AND METHODS

All patients underwent primary isolated coronary artery bypass grafting, except for one patient in *Study II*. This patient underwent concomitant mitral valve repair, since routine intraoperative TEE identified a preoperatively unknown significant mitral regurgitation. Preoperative patient characteristics and perioperative and postoperative data were collected from patient records, our institution's database, and in *Study III* additionally from the national Swedish Cardiac Surgery Register.

Patients were defined as having diabetes if treated with insulin or oral hypoglycemic agents, and as having hypertension if treated with antihypertensive medication. Left ventricular EF was assessed by preoperative contrast ventriculography or echocardiography and was categorized as normal (>49%), reduced (30-49%), or severely reduced (<30%). Peripheral vascular disease was defined as a history of exertional claudication, prior revascularization, or both, to the legs. Prior stroke was defined as history of stroke regardless of residual neurological deficit. The patients were classified as having ACS if chest pain at rest on admittance to the hospital or new onset or accelerated angina within four weeks of the operation.

All patients had an electrocardiogram on the day before surgery, the first day after surgery, before discharge, and additionally at the discretion of the attending physician. Serum levels of troponin-T were followed preoperatively, including the day before surgery, and postoperatively on day 1.

Study I

The medical records of 33 patients who underwent urgent and acute on-pump CABG operations at the Huddinge site of Karolinska University Hospital (former Huddinge Hospital) between July 2001 and July 2002 were reviewed. Preoperative patient characteristics and perioperative and postoperative data were collected from patient records and our institution's database. During the postoperative period, patients received transfusions of packed red blood cells, platelets, and plasma at the discretion of the surgeon or the intensive care unit (ICU) physician. Number and type of transfusions given during surgery, post-operatively, and during the total hospital stay were recorded, as well as use of aspirin, clopidogrel, and LMWH during the 5 days before surgery. Postoperative (< 30 days) survival data were collected from the Total Register of the Swedish Population, Statistics Sweden. There was no loss to follow-up. Hemoglobin concentration was measured the day before surgery, after induction of

anesthesia, every 30 minutes during CPB, at end of surgery, at arrival to the ICU, every 2 hours during the first 10 hours in the ICU, postoperatively day 2 and 4, and additionally at the discretion of the attending physician.

Since bleeding in patients on clopidogrel undergoing CABG became a clinical problem, some of the surgeons were persuaded by one of the anesthetists (JvdL) to use aprotinin, while others adhered to the policy of the department. The final decision to administer aprotinin was at the discretion of the operating surgeon. There were altogether 9 surgeons involved. Tranexamic acid was given at the discretion of the surgeon after reversal of heparin with protamine sulphate if clots were absent in the wound or in the chest tubes.

Study II

Seventy-five consecutive patients with unstable angina unsuitable for PCI and planned for urgent isolated CABG at the Huddinge site of Karolinska University Hospital were included. Surgery was scheduled for the next available session. Before the start of surgery, patients were randomized to full-dose aprotinin intraoperatively or an equal volume of saline solution. Random assignment was conducted using unmarked envelopes, each containing a card indicating treatment with aprotinin or placebo. A nurse, assigned to another department in our hospital, was responsible for the preparation of placebo and treatment solutions, which were identical in appearance and packing. Thus, neither patients nor staff were aware of treatment assignment. After induction of anesthesia, patients received a 1-mL test dose of aprotinin or placebo. If no adverse reaction occurred, either the full dose aprotinin regime or an equal volume of placebo was administered. Preoperative use of aspirin and LMWH within 24 hours before surgery, as well as the number of hours elapsing between the last intake of clopidogrel and start of surgery, were recorded. Tranexamic acid (range 0 – 6 g IV) was given at the discretion of the anesthetist, if excessive drainage without clots was observed after reversal of heparin with protamine sulfate and transfusion of platelets.

Study III

At the Huddinge site of Karolinska University Hospital we identified 209 patients treated with high-dose aprotinin during isolated primary CABG performed because of ACS during 2001 through 2003. In all cases, aprotinin had been administered to reduce perioperative blood loss in patients for whom clopidogrel treatment had been stopped less than 5 days before surgery. This cohort was matched according to age, sex, and presence of

ACS, to patients having isolated primary CABG at the Solna site of Karolinska University Hospital (former Karolinska Hospital) where not aprotinin but TXA was used during this period. The pool of patients available for matching at the latter hospital consisted of 1809 patients. Three elderly female patients with ACS in the aprotinin group had to be excluded since it was not possible to find a suitable match. So were two patients with incomplete personal identification numbers and four with dialysis-dependent renal failure, leaving 200 patients in each group. All aprotinin treated patients from *Study II* were also included in *Study III* except one, who underwent concomitant mitral plasty. The primary outcome measure was fractional change in CrCl. Additional outcome measures included early mortality within 30 days of the operation; postoperative stroke, defined as focal neurologic deficit persisting more than 72 hours; and postoperative atrial fibrillation, defined as a new onset of atrial fibrillation or flutter in a patient without history of chronic or intermittent atrial fibrillation. Data were collected by reviewing patients' records, hospital databases, and the national Swedish Cardiac Surgery Register. Patient data were prospectively entered into the databases at the time of hospital discharge. Follow-up of mortality was performed by linking each subject's unique Swedish personal identification number to data from the Total Register of the Swedish Population, Statistics Sweden. Thus, all patients could either be assigned to a date of death or identified as being alive on July 18, 2007.

All patients in the aprotinin group received the full Hammersmith aprotinin regimen [46-48], and CPB was conducted with a roller pump perfusion system. In the TXA group, all patients received a bolus of 4 g TXA intravenously before start of surgery, and CPB was accomplished with a centrifugal pump.

Study IV

Fifteen consecutive patients with ACS scheduled for CABG at the Solna site of Karolinska University Hospital were included in the study. At the time of the study we still routinely administered aprotinin according to the Hammersmith regime to patients on clopidogrel undergoing CABG [46-48]. Blood samples were drawn from the radial arterial cannula before and after administration of a bolus of 2 million KIU just before start of surgery.

Clopidogrel dosage

All patients, except for the control group of *Study III*, had their last intake of clopidogrel less than 5 days before surgery after having been given a loading dose of 300 mg (*Study I-III*) or 300 to 600 mg (*Study IV*) of clopidogrel orally, followed by 75 mg daily. In addition, patients were usually on oral aspirin, 75 mg/day, and subcutaneous LMWH. Aspirin and LMWH treatment was never stopped before surgery.

Aprotinin dosage

After induction of anesthesia, patients received a 1-mL test dose of aprotinin. If no adverse reaction occurred, patients were given the full Hammersmith regime of aprotinin, consisting of 2 x 10^6 KIU before start of surgery, 2 x 10^6 KIU in the CPB prime, and 0.5 x 10^6 KIU/h during surgery [46-48].

Surgical, anesthetic and CPB management

Operations were performed through a standard midline sternotomy, the left internal thoracic artery was harvested whenever possible as a pedicle and used as an in situ graft. The saphenous vein and the radial artery were harvested when needed. CPB was instituted in a routinely fashion and CABG was then conducted using saphenous and arterial grafts. An off-pump procedure was preferred, if intraoperative epiaortic ultrasound or palpation of the ascending aorta revealed severe atherosclerosis. Two 32 French chest tubes (Argyle, Tyco Health Care, Tullamore, Ireland), inserted through separate skin incisions, were positioned in the left pleura and mediastinum, respectively, and connected to the vein reservoir from the CPB circuit at a negative pressure of 15 cm H_2O.

Anesthetic and CPB management was standardized for all patients. CPB was performed with a flow rate of 2.4 L/m^2 or more at 34°C, through a hollow fiber membrane oxygenator (Dideco Simplex D708; Dideco, Mirandola, Italy). The CPB circuit was primed with Ringer's acetate and 300 mL of mannitol 10%. Antegrade or retrograde cold blood cardioplegia, or both, was applied. Anticoagulation was achieved with sodium heparin (400 IU/kg) intravenously and 7,500 IU in the CPB prime, and monitored with a kaolin-activated device (Hemotec; Medtronic, Englewood, Colorado). The activated clotting time was maintained above 400 seconds. At completion of CPB, heparin was reversed with protamine sulfate at a

1:1–1:3 ratio. In addition, if activated clotting time remained greater than 140 s, 100 mg protamine was administered. ACE-inhibitors were omitted at the day of surgery.

Evaluation of renal impairment

Serum creatinin was evaluated pre- and postoperatively at day 1 in *Study I*, whereas the peak postoperative values were compared with preoperative values in *Study II and IV*. In *Study III and IV* CrCl was calculated from serum creatinine applying the equation of Cockroft and Gault [49]. In *Study III* creatinine was routinely measured preoperatively, day 1, 2, and 4 or 5 postoperatively, and more frequently if creatinine was abnormal. The postoperative calculation of CrCl was based on the highest postoperative creatinine level. Primary outcome measure was ΔCrCl% calculated as follows: ([peak postoperative_CrCl - preoperative_CrCl] / preoperative_CrCl) x100. Secondary outcome measures of postoperative renal function were defined and calculated as follows: absolute change in CrCl (ΔCrCl) as CrCl_postoperative - CrCl_preoperative; absolute change in Cr (ΔCr) as Cr_postoperative - Cr_preoperative; fractional change in Cr (ΔCr%) as ([Cr_postoperative - Cr_preoperative]/ Cr_preoperative) x100; and renal dysfunction as a 50% increase in creatinine.

Bleeding and transfusion management

The intraoperative volume of bleeding was estimated from the intraoperative net volume in the suction reservoirs and the net weight of the surgical dressings. Chest tube output was measured at hourly intervals in the ICU and mediastinal drains were removed when blood loss was less than 100 ml over 4 hours. During the postoperative period, patients received transfusions of PRBC, platelets, and plasma at the discretion of the surgeon or the intensivist. PRBC was given at an arterial hemoglobin of <70 g/L during CPB and <85 g/L after CPB, except in patients with major ongoing hemorrhage; plasma was given if >2 U of PRBC was given; and platelets were given if bleeding was excessive and clots were missing after the reversal of heparin with protamine. Transfusions of any blood products were recorded during the operation, postoperatively until the next morning, and every postoperative day until discharge. In *Study I and II*, patients were auto-transfused hourly during the first 4 hours in the ICU if the drain output exceeded 100 mL per hour. Auto-transfusion was avoided if hemolysis was present i.e. if the urine was discolored.

Functional platelet count *(Study IV)*

Platelet counts were determined in two steps immediately after sampling of 5 ml fresh whole blood, first in an EDTA tube, and then in a tube containing 20 μM ADP (Plateletworks Helena Lab, Beaumont, USA). Each sample was analyzed in a cell-counter (ABX Micros 60, Diamond Diagnostics, Holliston, MA, USA). A simple formula was used to calculate the grade of inhibition before and after the patient received aprotinin (%-inhibition = [ADP platelet count/EDTA platelet count] x 100). In principal, when aggregation occurs, the functional platelets aggregate to clumps that cannot be counted because of their increased size. Functional platelets will aggregate maximally after ADP-stimulation resulting in a platelet count close to zero, which corresponds to 0% inhibition (=100 % aggregation) [50].

ETHICS

The local Ethical Committee approved all studies. Informed consent was obtained from involved patients when judged appropriate by the local Ethical Committee.

STATISTICAL ANALYSIS

Results were expressed as mean±standard deviation (SD), or median and $25^{th}/75^{th}$ percentiles or range. Student's t-test was used to compare continuous variables between groups, and non-parametric tests including the Mann-Whitney U-test were used if data were found to be not normally distributed after testing for normality with the Kolmogorov–Smirnov test. Chi-square and Fisher´s Exact Test were used for categorical variables. Data were analyzed with SPSS statistical program (Statistical Package for the Social Science, SPSS Inc., Chicago, Illinois). Differences were considered significant at a probability level of $p<0.05$.

Study III

Quantile regression was used to estimate and compare the median in the continuous outcomes of renal function (absolute and fractional change in Cr and CrCl) because the distributions were not symmetrical. Quantile regression is a robust statistical method that makes no assumptions about the distribution of the outcome variable. Standard errors and confidence intervals for the regression coefficients were obtained by generating 500 bootstrap samples. Multivariable analysis was performed to adjust for the difference in cardiac

morbidity (prior myocardial infarction and ejection fraction) between groups by assessing outcomes of renal function by logistic or quantile regression. A two-way analysis of variance (ANOVA) was used to study the effect of number of transfusions on ΔCrCl% by treatment group (aprotinin or TA). A separate analysis was made for PRBC, plasma, and platelets. Number of transfusions was categorized as follows: 0, 1, 2, 3, 4, 5, and ≥ 6 units of PRBC; 0, 1, 2, 3, 4, and ≥ 5 units of plasma, and 0, 1, and ≥ 2 units of platelets. Cumulative survival rates are presented as Kaplan–Meier estimates. Differences between survival curves were analyzed by using the log-rank test. Statistical analyses were performed using SPSS and STATA 10 (StataCorp LP, College Station, TX).

RESULTS

Study I

Eighteen patients received a full dose regimen of aprotinin intraoperatively, whereas 15 patients were not treated with aprotinin (control group). None of the patients died during the 30-day study period. Baseline characteristics and operative data of the two groups are summarized in Table 1.

Table 1
Baseline Characteristics and Operative Data (mean with 95% CI, n)

	Aprotinin group (*n=18*)	**Control group** (*n=15*)	p
Male/female	12/6	12/3	0.44
Age (years)	64.2 (58.6-69.8)	66.4 (59.3-73.5)	0.49
Last clopidogrel intake before surgery			
< 24 hrs	10	7	0.62
≥ 24 < 48 hrs	2	0	0.19
≥ 48 < 72 hrs	3	3	0.81
≥ 72 < 96 hrs	1	2	0.45
≥ 96 < 120 hrs	2	3	0.49
Aspirin	15	13	0.67
LMWH	16	13	0.89
Creatinine (μmol/L)	77 (68-86)	92 (67-117)	0.23
Hemoglobin (g/L)	131 (122-140)	132 (124-139)	0.94
CRP (mg/L)	12 (0.7-24)	14 (3.0-31)	0.89
Operative risk evaluation			
Euroscore	4.6 (3.2-6.0)	6.0 (4.4-7.6)	0.20
Higgins	2.1 (1.1-3.2)	3.7 (1.8-5.6)	0.22
Parsonnet	8.3 (4.1-12.5)	8.5 (3.5-13.4)	1.00
No. of grafts	3.9 (3.6-4.3)	3.7 (3.1-4.3)	0.59
Saphenous vein	2.7 (2.2-3.2)	2.3 (1.7-3.0)	0.37
Left internal thoracic/Radial artery	1.3 (1.0-1.6)	1.4 (1.0-1.8)	0.70
Surgery (minutes)	196 (185-208)	247 (202-292)	0.05
CPB (minutes)	84 (75-94)	93 (72-114)	0.68
Aortic cross clamping (minutes)	53 (45-61)	51 (38-63)	0.50

LMWH = Low-Molecular Weight Heparin; CPB = Cardiopulmonary Bypass.

No statistically significant differences were found for clinical parameters, including duration of CPB and cross-clamping, and number of distal anastomoses. The exception was duration of surgery, which in average was almost one hour longer in the control group (p=0.05). The mean time between the last administration of clopidogrel and surgery was 1.1 days (95% CI: 0.7-2.5) in the aprotinin group and 1.6 days (95% CI: 0.7-2.5) in the control group (p=0.38). Postoperative data as well as bleeding and transfusion requirements are

summarized in Table 2 and 3, respectively.

Table 2
Postoperative Data (mean and 95% CI, or n)

	Aprotinin group (*n=18*)	**Control group** (*n=15*)	p
Early mortality	0	0	1.0
Reoperation	0	3	0.05
Creatine kinase-MB, post op day 1 (µg/L)	45 (16-74)	47 (21-73)	0.96
Troponin-T, post op day 1 (µg/L)	0.54 (0.33-0.75)	0.94 (0.54-1.34)	0.05
Time to extubation (hrs)	7.0 (4.9-9.1)	10 (6.9-13.1)	0.25
Hemoglobin at discharge (g/L)	101 (96-106)	103 (97-109)	0.64
Length of ICU stay (hrs)	19 (17-21)	44 (9-79)	0.01
Length of hospital stay (days)	5.8 (5.5-6.2)	7.6 (6.0-9.3)	0.04
Creatinine post op day 1 (µmol/L)	101 (88-115)	113 (76-149)	0.73
Stroke	0	2	0.12
Q-wave infarction	0	0	1.0
Atrial fibrillation	4	7	0.14
Tranexamic acid (n)	3	7	0.03
Tranexamic acid (g)	2 (2-2)	2.6 (1.7-3.5)	0.33

ICU=Intensive Care Unit

Patients in the aprotinin group stayed a significantly shorter time in the ICU and in the hospital. No reoperations for bleeding occurred in aprotinin group versus 3 in the control group (p=0.05). On the first postoperative day troponin-T levels were lower in the aprotinin group (p=0.05). Hemolysis was not present in any of the patients. Two patients in the control group versus 6 patients in the aprotinin group did not receive auto-transfusion since the drainage output was <100 mL per hour (p=0.12).

The quantity of bleeding in the operation room and ICU as well as the total volume of bleeding is shown in Figure 1. Total bleeding and bleeding in the ICU was significantly less in the aprotinin group. Moreover, significantly fewer transfusions of PRBC, platelets and total number of units of blood products were required in the aprotinin group. On average more than three times as many units of blood products were given in the control group as compared with the aprotinin group. As a result 80% of the patients in the control group received blood products during their hospital stay versus 39% in the aprotinin group (p=0.004). The total use of different blood products is depicted in Figure 2.

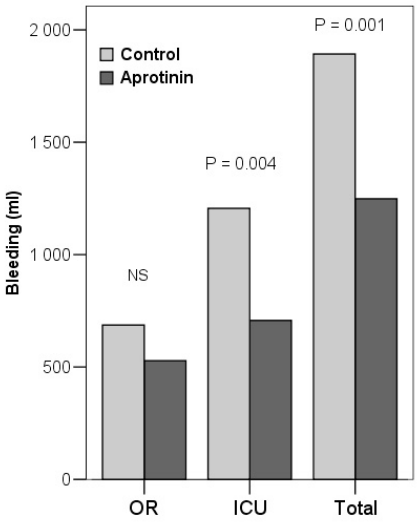

Figure 1. Average bleeding (mL) in the operating room (OR), intensive care unit (ICU) and total bleeding in the OR and ICU (Total) in the aprotinin and control groups.

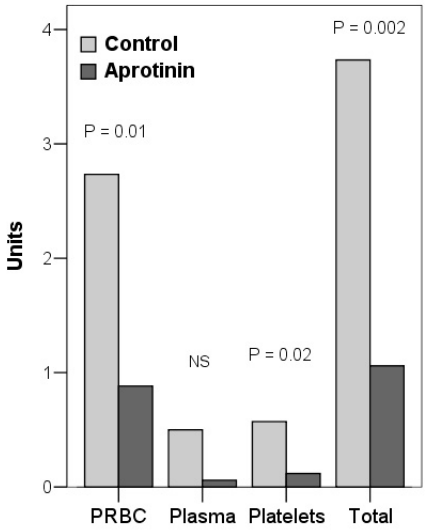

Figure 2. Average blood product use (Units) in the aprotinin and control groups. PRBC=Packed red blood cells.

Table 3

Bleeding and Transfusions (mean and 95% CI)

	Aprotinin group (n=18)	**Control group** (n=15)	p
Bleeding (mL)			
Operating Room	530 (420-630)	690 (470-910)	0.44
Intensive Care Unit	710 (560-860)	1210 (860-1550)	0.004
Total	1250 (1090-1410)	1890 (1550-2230)	0.001
Autotransfusion (mL)	270 (120-430)	510 (300-710)	0.05
Transfusions (Units), Operating Room			
PRBC	0.4 (0-1.0)	0.5 (0-1.1)	0.60
Plasma	0.1 (0-0.2)	0	0.36
Platelets*	0.1 (0-0.2)	0.2 (0-0.4)	0.21
Transfusions (Units), Intensive Care Unit			
PRBC	0.3 (0-0.6)	1.5 (0.3-2.6)	0.03
Plasma	0	0.5 (0-1.0)	0.02
Platelets*	0.1 (0-0.2)	0.4 (0-0.7)	0.09
Transfusions (Units), Ward			
PRBC	0.2 (0.1-0.5)	0.7 (0.1-1.4)	0.13
Transfusions (Units), Total			
PRBC	0.9 (0.1-1.7)	2.7 (1.4-4.1)	0.01
Plasma	0.1 (0-0.2)	0.5 (0-1.0)	0.08
Platelets*	0.1 (0-0.3)	0.6 (0.2-0.9)	0.02
Total blood products (Units)	1.1 (0.1-2.0)	3.7 (2.1-5.4)	0.002

PRBC = Packed Red Blood Cells, *=1 Unit is equal to 500 ml from 6 donors

Furthermore, we analyzed patients not receiving tranexamic acid. In the aprotinin group (n=15) bleeding in the operating room, in the ICU, and total bleeding was 520 mL (95% CI: 410-630 mL), 670 mL (95% CI: 520-820 mL) and 1190 (95% CI: 1020-1350 mL), respectively, as compared with 810 mL (95% CI: 420-1200 mL, p=0.18), 1180 mL (95% CI: 650-1700 mL, p=0.02) and 1990 mL (95% CI: 1420-2560 mL, p<0.01) in the control group (n=8). Total number of PRBC, plasma, platelets and transfusions was 0.8 U (95% CI: 0-1.6 U), 0 U (95% CI: 0-0 U), 0.1 U (95% CI: 0-0.2 U), 0.9 U (95% CI: 0.1-0.6 U), respectively, in the aprotinin group, and 1.9 U (95% CI: 0.4-3.4 U, p=0.08), 0.4 U (95% CI: 0-1 U, p=0.06), 0.3 U (95% CI: 0-0.6 U, p=0.25), 2.5 U (95% CI: 0.4-4.6 U, p=0.05), respectively, in the control group.

Study II

Baseline characteristics and operative data are listed in Table 4. Thirty-eight patients were administered saline and 37 were administered aprotinin. All patients were first time cardiac surgery patients and were treated with clopidogrel <5 days before surgery.

Table 4
Baseline characteristics and operative data (mean±SD)

	Saline n=38	**Aprotinin** n=37	P
Sex (M/F)	66%	84%	0.08
Age (yrs)	68.3±10	66.4±10	0.51
Length (cm)	174±11	176±8	0.51
Weight (kg)	84.2±16	80.9±13	0.32
Euroscore	5.5±2.9	5.2±3.0	0.65
Earlier cardiac surgery	0%	0%	1.0
Hypertension	50%	41%	0.41
Diabetes mellitus*	21%	19%	0.82
Hours without clopidogrel preoperatively	54.4±27	58.0±28	0.86
LMWH sc < 24h before surgery	82%	73%	0.38
Aspirin < 24h before surgery	100%	95%	0.15
Proximal anastomoses (N)	1.3±0.5	1.5±0.6	0.27
Distal anastomoses (N)	3.7±1.0	3.6±1.0	0.79
Left internal thoracic artery	100%	100%	1.0
Operation (min)	200±53	192±48	0.55
ECC (min)	84±29	80±30	0.85
OPCAB	8%	8%	0.97
Total dose of heparin IU iv†	37000±7900	36200±6500	0.96
Total dose of protamine mg iv	480±96	460±95	0.39

*=Treated with insulin or oral antidiabetics, LMWH=Low-molecular-weight Heparin, ECC=Extra corporeal circulation, OPCAB=Off-pump coronary artery bypass †=not including 7500 IU (75mg) in the CPB-prime.

The last oral dose of clopidogrel was taken 54.4±27 and 58.0±28 hours before start of surgery in the control and treatment groups, respectively (p=0.86). Corresponding medians, were 51 hours (25/75-percentiles 31/75, ranges 5-120) and 50 hours (25/75-percentiles 30/82, ranges 25-102) hours, respectively (p=0.86). Almost all patients also received aspirin and LMWH within 24 hours of surgery with no significant differences between the groups. Three patients in each group underwent off pump CABG due to severe arteriosclerosis of the ascending aorta. Routine intraoperative transesophageal echocardiography identified one patient with a preoperatively unknown significant mitral regurgitation in the aprotinin group and this patient underwent concomitant mitral valve repair.

Table 5
Bleeding and transfusions (mean±SD)

	Saline N=38	**Aprotinin** n=37	p
Hemoglobin (g/L)			
Preoperatively <24h	137±14	131±17	0.08
Lowest during ECC	88±14	87±14	0.60
At discharge	105±12	110±12	0.12
Transfusion of units - PRBC			
Intraoperatively	0.5±1.4	0.3±0.7	0.63
Postoperatively <24h	1.6±1.9	0.8±1.3	0.04
Total hospital stay	2.8±3.2	1.2±1.5	0.02
Transfusion of units - Plasma			
Intraoperatively	0.1±0.5	0.0±0.0	0.09
Postoperatively <24h	1.6±1.9	0.4±1.1	0.08
Total hospital stay	1.0±1.9	0.4±1.1	0.12
Transfusion of units - Platelets*			
Intraoperatively	0.1±0.36	0.0±0.0	0.17
Postoperatively <24h	0.8±1.3	0.1±0.4	0.002
Total hospital stay	0.9±1.4	0.1±0.4	0.002
Total number of units transfused	4.8±5.7	1.8±2.3	0.02
Patients receiving transfusions			
PRBC	71%	47%	0.04
Plasma	32%	17%	0.13
Platelets	42%	11%	0.003
Total	79%	53%	0.02

PRBC= Packed red blood cells, *= 1 Unit is to 500 ml from 6 donors

No significant differences between the groups were observed regarding pre-, intra-, and postoperative hemoglobin levels (Table 5). Total postoperative bleeding was 1200±570 ml in placebo versus 760±350 ml in the aprotinin group (p<0.001, Figure 3), a 37% reduction compared with placebo. Control subjects received significantly more units of PRBC and platelets during the first 24 postoperative hours, as well as the total hospital stay (Figure 4).

Thus, more than twice as many units of blood products were given to controls (4.8±5.7) as compared with aprotinin-treated patients (1.8±2.3, p=0.02). Seventy-nine percent of patients in placebo received blood transfusions during the hospital stay versus 53% in the aprotinin group (p=0.02). Fifty-five percent of patients treated with saline received 1.8±1.8 g tranexamic acid, as compared with 25% of aprotinin-treated patients (p=0.008) receiving 0.5±0.9 g (p=0.001).

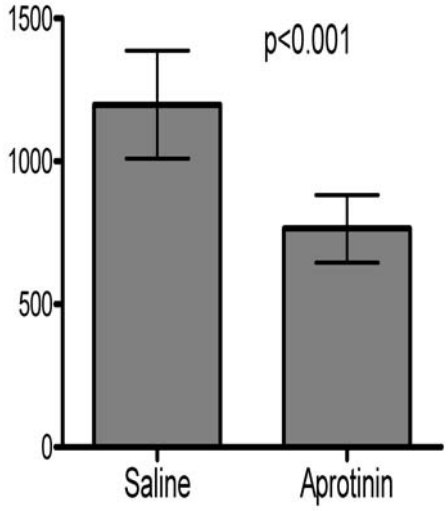

Figure 3. The mean (95% CI) postoperative bleeding in patients undergoing CABG and randomized to treatment with aprotinin or saline.

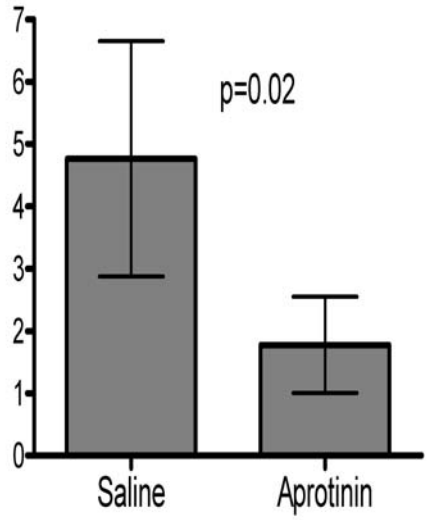

Figure 4. Incidence The mean (95% CI) number of transfusions during the total hospital stay in patients undergoing CABG and randomized to treatment with aprotinin or saline.

Clinical outcomes are depicted in Table 6. One patient in the control group died on postoperative day 25 primarily because of postoperative mediastinitis. Three deaths occurred in the treatment group. One patient got atrial fibrillation, a stroke on postoperative day 2, and died on postoperative day 8. A second patient suffered a postoperative stroke and died on postoperative day 13. The third patient had a myocardial infarction in the operating room and died. This patient was excluded from the postoperative analysis. None of the patients who died had received TXA. The aprotinin group had significantly higher preoperative troponin-T values than the controls (p=0.02), but did not show a rise in values on postoperative day 1 (p=0.47). In contrast, troponin-T levels in the controls increased significantly on postoperative day 1 (p<0.001). Thus, the change in troponin-T of the two groups' differed significantly (p=0.003).

Table 6
Clinical outcomes (mean±SD)

	Saline n=38	**Aprotinin** n=37	p
Stroke	2	3	0.60
AMI (new Q-wave)	0	0	1.0
Re-exploration (Bleeding)	5	1	0.10
Creatinine (µmol/L) day 0	85±17	90±22	0.35
Creatinine (µmol/L) peak* - day 0	12±24	30±56	0.28
Troponin-T (microgram/liter) day 0	0.30±0.6	0.94±2.2	0.02
Troponin-T (microgram/liter) day 1 - day 0	0.49±0.5	0.01±1.8	0.003
Hospital stay (postoperative days)	7.2±3.9	6.4±1.5	0.56
Mortality ≤ 30 days postoperatively	1	3	0.28

AMI=Acute myocardial infarction, *= peak value during the hospital stay

Study III

Preoperative patient characteristics are shown in Table 7. The groups were matched for age, sex, and presence of ACS. Other baseline characteristics were well balanced between the groups with the exception of number of diseased coronary vessels and history of myocardial infarction.

Table 7
Preoperative data (mean or number of patients and standard deviation or percentages) in 200 aprotinin treated patients compared with 200 matched patients not receiving aprotinin undergoing primary CABG.

	Tranexamic acid		**Aprotinin**		
	Mean or n	*SD or %*	*Mean or n*	*SD or %*	*p*
Age (years)*	66.8	9.9	66.8	9.9	0.95
Female sex*	46	23	46	23	1.0
Acute coronary syndrome*	147	73.5	147	73.5	1.0
BMI (kg/m²)	26.5	3.5	27.1	4.5	0.45
Hypertension	149	74.5	158	79	0.34
Diabetes mellitus	49	24.5	54	27	0.65
History of stroke	9	4.5	15	7.5	0.29
Smoking habits					
Never	47	23.5	57	28.5	0.30
Former smoker	64	32	65	32.5	1.0
Current smoker	35	17.5	47	23.5	0.17
COPD	24	12	20	10	0.63
Peripheral vascular disease	12	6	9	4.5	0.66
History of myocardial infarction	118	59	148	74	0.002
Significant coronary lesions					
1-vessel	11	5.5	2	1	0.02
2-vessel	33	16.5	13	6.5	0.003
3-vessel	156	78	185	92.5	<0.001
Left main stem	61	30.5	62	31	1.0
Left ventricular EF					
EF >0.49	132	66	119	59.5	0.22
EF 0.30-0.49	54	27	73	36.5	0.05
EF <0.30	14	7	8	4	0.27

* Patients were matched for these factors. BMI = body mass index, COPD = chronic obstructive pulmonary disease, EF = ejection fraction.

Patients in the aprotinin group had more often triple vessel disease and a history of myocardial infarction. In the aprotinin group, 40.5% of the patients had a reduced or severely reduced left ventricular ejections fraction, compared with 34% in the TXA group, but the difference did not reach statistical significance. Thus, the only observed dissimilarity between the groups was a higher preoperative cardiac morbidity in the aprotinin group.

Perioperative data are shown in Table 8. As expected there were more grafted vessels in the aprotinin group, since triple vessel disease was more common in this group. The left internal thoracic artery was also more frequently used in the aprotinin group. Duration of CPB and aortic cross-clamping were similar in both groups.

Table 8
Perioperative data (mean or number of patients and standard deviation or percentages) in 200 aprotinin treated patients compared with 200 matched patients not receiving aprotinin undergoing primary CABG.

	Tranexamic acid		**Aprotinin**		
	Mean or n	*SD or %*	*Mean or n*	*SD or %*	*p*
No. of distal anastomoses	3.3	1.0	3.9	0.8	<0.001
LITA used	190	95	199	99.5	0.01
ECC (minutes)	80	27	76	26	0.19
XCL (minutes)	47	18	43	17	0.09

ECC = extra corporeal circulation, LITA = left internal thoracic artery, XCL = cross-clamp time.

Table 9
Renal outcome in 200 aprotinin treated patients compared with 200 matched patients not receiving aprotinin undergoing primary CABG.

	Tranexamic acid		**Aprotinin**		
	Mean (SD)	*Median (95%CI)*	*Mean (SD)*	*Median (95%CI)*	*P*
Serum creatinine, preop (µmol/L)	94 (32)	88 (85-91)	89 (23)	87 (83-91)	0.71
Serum creatinine, postop (µmol/L)	117 (57)	101 (96-106)	117 (78)	99 (94-104)	0.57
Absolute change in serum creatinine (µmol/L)	22 (41)	12 (8.1-16)	28 (73)	12 (8.5-15)	1.0
Fractional change in serum creatinine* (%)	25 (44)	13 (8.0-18)	31 (80)	14 (9.3-19)	0.74
Creatinine clearance, preop (mL/min)	79 (28)	78 (72-84)	84 (30)	80 (76-84)	0.59
Creatinine clearance, postop (mL/min)	68 (27)	66 (60-71)	72 (32)	69 (65-72)	0.35
Absolute change in creatinine clearance (mL/min)	-11 (15)	-8.1 ([-11]-[-5.7])	-12 (15)	-10.4 ([-12]-[-7.5])	0.17
Fractional change in creatinine clearance** (%)	-14 (20)	-11 ([-15]-[-7.5])	-15 (20)	-12 ([-16]-[-8.6])	0.75

*ΔCr%=((Cr_postop – Cr_preop)/Cr_preop) x 100, **ΔCrCl%=((CrCl_postop – CrCl_preop)/CrCl_preop) x 100

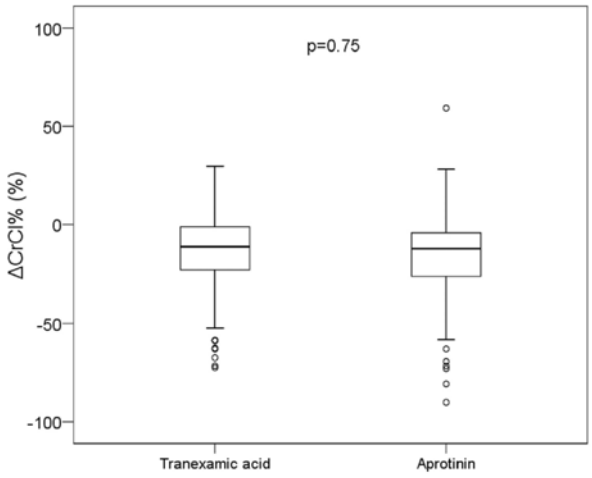

Figure 5. *Boxplot of fractional change in median (±95%CI) creatinine clearance (ΔCrCl%) in 200 aprotinin treated patients compared with 200 matched patients receiving tranexamic acid undergoing primary CABG.*

Postoperative renal function measurements are shown in Table 9. There was no significant difference in the primary outcome measure, ΔCrCl% (Figure 5), between the TXA and the aprotinin group (-11% vs. -12%, medians, p=0.75). Early mortality, stroke, reoperation for bleeding, and renal dysfunction were similar in both groups as shown in Table 10.

Table 10
Secondary outcomes (mean or number of patients and standard deviation or percentages) in 200 aprotinin treated patients compared with 200 matched patients not receiving aprotinin undergoing primary CABG.

	Tranexamic acid		**Aprotinin**		
	n	*%*	*n*	*%*	*P*
Early mortality	7	3.5	9	4.5	0.80
Stroke postoperatively	3	1.5	4	2	1.0
Atrial fibrillation	70	35	59	29.5	0.28
Reoperation for bleeding	7	3.5	5	2.5	0.77
Transfusion, any	121	60.5	96	48	0.02
Renal dysfunction*	31	15.5	31	15.5	1.0
Composite endpoint *Any of the following: Early mortality, stroke postoperatively, reoperation for bleeding, renal dysfunction* postoperatively, any transfusion.*	130	65	111	55.5	0.07
Composite endpoint except transfusions *Any of the following: Early mortality, stroke postoperatively, reoperation for bleeding, renal dysfunction* postoperatively.*	42	21	42	21	1.0

*Renal dysfunction was defined as >50% increase in serum creatinine compared to preoperatively.

In the TXA group 29 patients (14.5%), versus 30 patients (15%, p=1.0) in the aprotinin group, had a postoperative peak Cr above 150 μmol/L (>2mg/dL). One patient in each group

(0.5%) had acute renal failure, namely required dialysis, postoperatively. Patients in the aprotinin group received fewer units of PRBC (2.0 versus 1.4, p=0.02) and plasma (1.3 versus 0.5, p<0.001), but more units of platelets (0.1 versus 0.2, p=0.02) as shown in Table 11.

Table 11
Transfusions in 200 aprotinin treated patients compared with 200 matched patients not receiving aprotinin undergoing primary coronary artery bypass surgery.

	Tranexamic acid		**Aprotinin**		
Transfusions	*Mean or n*	*SD or %*	*Mean or n*	*SD or %*	*P*
Packed red blood cells (Units)	2.0	2.6	1.4	2.0	0.02
0	85	42.5	111	55.5	0.01
1-3	70	35	57	28.5	0.20
4-6	31	15.5	28	14	0.78
>6	14	7	4	2	0.03
Plasma (Units)	1.3	3.1	0.5	1.3	<0.001
0	131	65.5	168	84	<0.001
1-2	41	20.5	19	9.5	0.003
3-4	15	7.5	7	3.5	0.12
>4	13	6.5	6	3	0.16
Platelets (Units)	0.1	0.4	0.2	0.6	0.02
0	188	94	174	87	0.03
1	7	3.5	20	10	0.02
2	4	2	3	1.5	1.0
>2	1	0.5	3	1.5	0.62

The two-way ANOVA showed a non-significant main effect of treatment group (p=0.74) and a significant main effect of number of transfusions of PRBC (p=0.004) on ΔCrCl%. There was a non-significant interaction between treatment group and number of transfusions of PRBC (p=0.62). There was also a non-significant main effect of treatment group (p=0.60) and a significant main effect of number of transfusions of platelets (p=0.02) on the fractional change in creatinine clearance. The interaction between treatment group and number of transfusions of platelets was not significant (p=0.96). Neither the main effect of treatment group (p=0.10) nor the number of transfusions of plasma (p=0.15) was significantly associated with ΔCrCl%. These results indicate that an increasing number of transfusions of both PRBC and platelets, but not aprotinin treatment, were associated with impaired postoperative renal function.

The composite endpoint of early mortality, postoperative stroke, reoperation for bleeding, postoperative renal dysfunction occurred in 42 (21%) patients in the TXA group and

in 42 patients (21%) in the aprotinin group (p=1.0). Patients in the aprotinin group were significantly less likely to receive transfusion (48% versus 60.5%, p=0.02).

After adjustment for previous myocardial infarction and baseline ejection fraction by logistic regression and quantile regression, as appropriate, the different renal outcomes remained unchanged.

The cumulative follow-up was 1829 patient-years and median follow-up was 4.7 years. Overall 5-year survival was 87% in the TXA group and 84% in the aprotinin group (p=0.17). There was no loss to follow-up.

Study IV

The median (25th/75th percentile) ADP mediated platelet aggregation before and after aprotinin, was 84% (76/91) and 94% (86/97, p<0.01). As depicted in Figure 6, aprotinin induced an increased aggregation in eleven of fifteen patients (73%), whereas a decrease was registered in two patients (13%). EDTA platelet counts before (207±42) and after aprotinin (196±51) did not differ significantly (p=0.125).

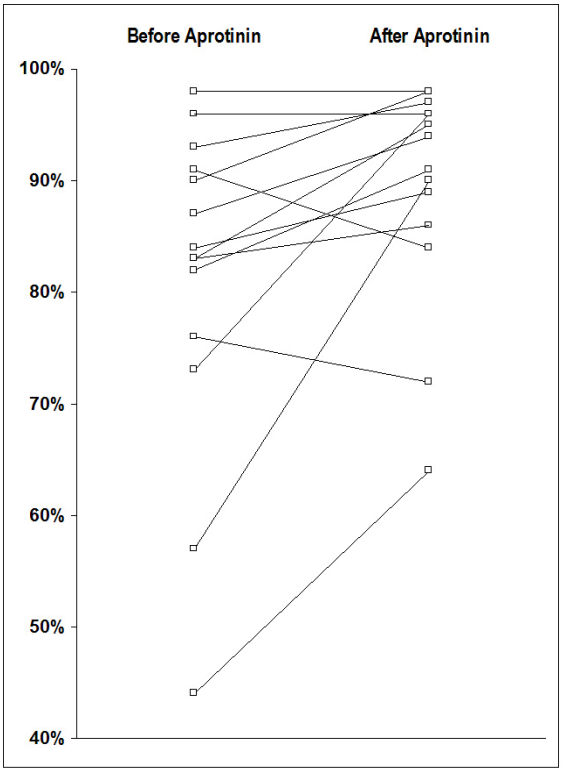

Figure 6. Platelet aggregation after ADP stimulation (platelet count ratio, %) before and after a bolus of $2x10^6$ KIU of aprotinin in 15 patients with ACS on clopidogrel undergoing primary CABG. Two patients have overlapping values before (90%) and after aprotinin.

When applying the cut-off limit of <10% inhibition (>90% aggregation) for clopidogrel non-response to ADP four out of 15 patients were classified as non-responders [9-11,51,52].

Table 11
Clinical data (mean ± SD, median and 25th/75th percentiles, or percentages) in 15 patients with ACS on clopidogrel undergoing primary CABG.

	Mean or (n)	SD	Median	25th/75th percentiles	P
Age (years)	61.8	11.6	64	51/67	
Female gender (n)	(3)				
BMI (kg/m²)	27.2	4.0	27	24.5/30.5	
Duration between the last oral intake of clopidogrel and start of surgery (hours)	63.7	28	72	29.5/78	
Pre-A Hemoglobin (Hbg/L)	131	13	129	122/140	
Post-A Hemoglobin (Hbg/L)	114	14	115	110/124	0.001
Pre-A Hematocrit (%)	38.2	3.8	38	35/41	
Post-A Hematocrit (%)	32.5	4.5	32	31/36	0.001
Pre-A platelet count EDTA (x 10^9/L)	207	42	210	185/240	
Post-A platelet count EDTA (x 10^9/L)	196	51	199	174/219	0.125
Pre-A platelet count ADP (x 10^9/L)	36.8	31.7	27	15/42	
Post-A platelet count ADP (x 10^9/L)	18.0	16.0	12	7/27	0.005
Difference pre-A platelet count (EDTA-ADP) (x 10^9/L)	170	50.6	174	138/198	
Difference post-A platelet count (EDTA-ADP) (x 10^9/L)	178	57.0	177	137/208	0.31
Pre-A platelet aggregation (%)	82	85	84	76/91	
Post-A platelet aggregation (%)	89.9	90.0	94	86/97	0.009
Intra-operative bleeding (ml)	485	350	450	250/580	
Post-operative drainage output (ml)	645	389	500	390/950	
Total bleeding (ml)	1130	600	950	760/1300	
Packed red blood cells (units)	0.73	1.2	0	0/2	
Plasma (units)	0.53	1.2	0	0/0	
Platelets (units)	0.33	0.62	0	0/1	
Postoperative CK-MB (µg/L), day 1	22.4	27	15	7/26	
Postoperative ASAT (µcat/L), day 1	1.30	0.97	0.91	0.8/1.8	
Preoperative s-Creatinine (µmol/L)	95.0	32.8	90	69/119	
Postoperative s-Creatinine (µmol/L), maximum	114	49	99	79/125	0.001
Preoperative CrCl (ml/min)	85.3	25.3	93	66/101	
Postoperative CrCl (ml/min)	74.1	26	72	56/98	0.001
ΔCrCl%	-14.1	10.3	-14.1	-24/-8	0.001

P before and after aprotinin or when applicable. A=Aprotinin, BMI = body mass index, Diff.= Difference, CrCl = Creatinine clearance calculated from serum creatinine applying the equation of Cockroft and Gault [49]. ΔCrCl% = Fractional change in creatinine clearance calculated as: ((peak postoperative_CrCl – preoperative_CrCl) / preoperative_CrCl) x 100.

The nonresponders had a median aggregation of 94.5% (91.5/97.5, 25th/75th percentile) versus 82% (73/87, p<0.01) in the responders. The median increase in platelet aggregation after aprotinin was 8% (5/20) in the responders versus 0% (-5.25/3, p<0.01) in the non-responders. The median duration after the last intake of clopidogrel until start of surgery was very similar for non-responders and responders, 72 h (40/76) versus 74 h (24/98, p=0.75).

Preoperative patient characteristics are shown in Table 12. The mean last oral intake of clopidogrel was 63.7±28 hours before surgery (range 24-103 hours). The mean age was 61.8±12 years and twelve of the fifteen patients were men. One patient underwent a reoperation due to bleeding. Median time in the intensive care unit until extubation was three hours (2/6, 25th/75th percentile). According to myocardial injury markers one patient suffered a perioperative myocardial infarction on the first postoperative day with a CKMB of 110 μg/L and ASAT of 2.63 μcat/L. Interestingly, this patient was one of only two patients with an increased platelet inhibition after a bolus of aprotinin (from 9% to 16%). None of the patients suffered a clinically evident stroke during hospital stay, nor did any patient need postoperative dialysis. The mean ΔCrCl% was -14.1±10.3%. Furthermore, the mean postoperative drainage output was 645±389 ml and 73% (11/15) of the patients were neither given PRBC or platelets, and 80% (12/15) were not given plasma after the operation. Postoperatively, one patient received 1.5 g TXA i.v. and three patients received desmopressin 0.3 μg/kg i.v.

GENERAL DISCUSSION

The major findings of this thesis, including a randomized double-blind placebo-controlled trial, are that intraoperative aprotinin decreases postoperative bleeding and transfusion requirements in patients undergoing CABG and treated with clopidogrel <5 days before surgery. This thesis did not find any evidence that aprotinin negatively influences renal function when compared with TXA in patients undergoing primary CABG. However, aprotinin reduced the overall transfusions rate to a greater extent than TXA. Moreover, the exact interaction between clopidogrel and aprotinin remains unknown, although our results suggest an influence of aprotinin on the level of ADP-receptors of platelets.

PLATELETS AND CLOPIDOGREL

Platelets play a critical role in the hemostasis mechanism by their formation of the primary hemostatic plug as well as by their contribution to the clotting cascade. There is a continuous activation of platelets and an aggravated clot formation related to the unstable condition of patients with ACS.

To prevent coronary thrombosis clopidogrel has become the gold standard for treatment of ACS, in addition to aspirin and low molecular weight heparin [4,53,54]. These oral drugs are nowadays administered even before admission to hospital, as well as before it is decided whether the individual patient needs acute PCI or coronary surgery. Clopidogrel selectively inhibits platelet aggregation by reducing ADP-mediated activation. However, given the long half-life of clopidogrel [17-20], this approach augments the risk of excessive perioperative bleeding in ACS patients undergoing urgent coronary surgery. Conversely, if surgery is postponed the necessary 5-7 days, there is probably an increased risk of ischemic events before revascularization due to time delay and rebound hyperaggregation after stopping clopidogrel [46,55]. The great variability of platelet recovery should be kept in mind because speedy recovery may result in the patient ending up with more reactive new platelets. In a small randomized clinical trial Akowuha et al. [46] tested the strategy of continuing clopidogrel before coronary artery surgery. Patients undergoing urgent CABG surgery for ACS were randomly assigned to remain on aspirin and clopidogrel therapy until surgery while receiving aprotinin intraoperatively, whereas the control group received the placebo for 5 days before and placebo infusions during surgery. Postoperative blood loss was significantly greater in the placebo group than in the treatment group (702 ± 120 mL versus 446 ± 62 mL,

p = 0.004). Patients in the placebo group also required more blood transfusions (1 ± 0.3 U versus 0.3 ± 0.2 U, p = 0.03). Noticeably, 3 patients in the placebo group, compared with none in the treatment group, suffered an MI (p = 0.07). Thus, the strategy of combining continued aspirin and clopidogrel therapy with intraoperative aprotinin treatment reduces postoperative blood loss and transfusion requirements, prevents delay of surgical treatment, and may prevent major adverse cardiac events before surgery.

The individual response to clopidogrel varies within a wide range. This is due to various mechanisms including genetic (receptor polymorphism [56]), clinical (poor compliance and absorption including dosage, loading, decreased bioavaibility, drug interactions such as with proton inhibitors [57], ACS, diabetes mellitus/insulin resistance, elevated BMI), and cellular (up-regulation of the $P2Y_{12}$ and P2Y-independent pathways, accelerated platelet turnover factors [58]). The surgical dilemma may get even worse in the future if platelet inhibition is optimized in low- or non-responders. That may well be the case if the degree of platelet inhibition is routinely analyzed with a valid point of care method that narrows the variation after individualization of drug and dosage or if new more effective drugs, i.e. prasugrel, are used [59]. An editorial in Circulation [60] has highlighted the problem of clopidogrel resistance and therapy failure, since approximately 5-15% of clopidogrel treated patients appear to be non-responders. Other reviewers have reported a prevalence of non-responsiveness among patients with cardiovascular disease between 4% and 34% [61].

Several new methods for platelet function analysis are available to evaluate the response to clopidogrel medication [62]. Thus, future studies may help to individualize antiplatelet treatment and identify patients requiring urgent surgery who may benefit the most from use of intraoperative aprotinin. Screening of patients on clopidogrel before coronary surgery may identify patients with adequate inhibition of platelets, thus allowing selection of patients for aprotinin treatment. Consequently, clopidogrel non- or low-responders should be excluded from aprotinin treatment. This exclusion would minimize the risk of overshoot platelet aggregation in patients with ACS with possible increased risk of thrombotic events after coronary surgery. Nevertheless, conventional methods for analysis of platelet function are time-consuming, operator dependent, and lack standardization. Methods that are deemed to be closely related to platelet function, such as blood aggregometry or platelet count ratio, are considered appropriate for measurement of clopidogrel's effect [62,63]. Consequently, in **Study IV**, we used a simple point of care method based on platelet count ratio. This method seems favorable for point of care measurements, since it is simple, inexpensive, quick to

perform (2-10 min), and uses non-centrifuged whole blood samples [50]. However, so far no point of care method has been validated to detect clopidogrel resistance on a routine basis.

CLOPIDOGREL, CABG, AND BLEEDING

Can bleeding be reduced to improve outcome in patients subjected to CABG while on clopidogrel treatment? This question is of interest since data from pooled observational studies indicate that in ACS patients without persistent ST-segment elevation, there is a strong, consistent, temporal, and dose-related association between bleeding and death [64]. Several studies have convincingly demonstrated that clopidogrel treatment within 4 days of CABG significantly increases blood loss, requires more reoperations for bleeding, and has greater transfusion requirements for red blood cells (6 to 11 times), plasma (2 to 4 times), and platelets (2 to 45 times) [18-20,65-67]. Furthermore, the usual combination of aspirin and clopidogrel has synergistic antiplatelet effects, because each agent affects platelet aggregation by a different mechanism. As expected, clopidogrel, together with aspirin, has been shown to be superior to aspirin alone for patients hospitalized with non-ST-elevation ACS [4]. Thus, Yende and Wunderink [20] found that the reoperation rate in patients undergoing CABG increased from 2.3% to 10.4% when the patients were treated with aspirin only and with the combination aspirin/clopidogrel, respectively. In comparison, their reoperation rate was 0% for patients who received neither aspirin nor clopidogrel. It is thus not surprising that the ACC/AHA 2004 Guideline Update for CABG Surgery [68], state that *"If clinical circumstances permit, clopidogrel should be withheld for 5 days before performance of CABG surgery."* (Class I Recommendation, Level of Evidence: B). This recommendation will most certainly be followed in patients who are to undergo elective CABG.

As a rule centers use an early interventional strategy for ACS similar to that described in Fast Revascularization During Instability in Coronary Disease Trial II (FRISC II) [16]. At least 5% of patients presenting for CABG may require urgent or acute surgery after clopidogrel administration [66]. The recent evaluation of ACS patients undergoing CABG surgery (n = 2,858) in the Can Rapid Risk Stratification of Unstable Angina Patients Suppress Adverse Outcomes With Early Application of the ACC/AHA Guidelines study (CRUSADE) revealed that 30% with unstable angina pectoris received clopidogrel before CABG surgery. In contrast to ACC/AHA guideline recommendation [69], 87% of these patients had surgery within ≤ 5 days of treatment. Obviously, cardiac surgeons and cardiac anesthesiologists note less bleeding in patients considered in need of preoperative clopidogrel.

This is probably explained by their increased experience in the management of patients on clopidogrel, including meticulous management of hemostasis during surgery, especially before closure of the sternum, the use of aprotinin, and, when needed, the use of platelet transfusion postoperatively. Altogether, in patients with ACS that require CABG, surgeons will *not* easily delay surgery, since it may lead to acute ischemic events. On the other hand, the drawback of urgent surgery is the risk of excessive bleeding induced by clopidogrel.

This concern may question the routine administration of clopidogrel before anticipated but undecided PCI. However, Fox et al. [14] reported that in patients with non-ST-elevation ACS treated with the combination of clopidogrel and aspirin, benefits and risks of early and long-term clopidogrel therapy (freedom from cardiovascular death, MI, stroke, or life-threatening bleeding) are similar, independent of revascularization (CABG or PCI). For patients undergoing CABG and continuing clopidogrel within five days before surgery, a non-significant trend of approximately 1 additional patient per 100 suffered a life-threatening bleeding and an additional 2.0 patients per 100 suffered a major bleed [14]. The contribution of aspirin to bleeding was uncertain because the timing of aspirin discontinuation was not recorded [14]. It is likewise not known if or how many of the CABG patients that were given aprotinin intraoperatively. In general the benefits of starting clopidogrel on admission appeared to outweigh the risks, even among those who proceeded to CABG during the initial hospitalization. Clearly, if excessive bleeding could be avoided the decision to commence surgery would be much easier. On this issue, the ACC/AHA 2007 Guideline Update for CABG Surgery [53] further states: *"Thus, many hospitals that use an early invasive approach for UA/NSTEMI delay starting clopidogrel until diagnostic angiography clarifies whether early CABG is indicated. However, when clopidogrel is given before catheterization, and urgent surgical intervention is indicated, some experience suggests "early" bypass surgery may be undertaken by experienced surgeons at acceptable incremental risk. More data are needed to formulate definitive recommendations on this issue."* This thesis suggests that a significant reduction of clopidogrel-induced bleeding and transfusion requirements after urgent CABG can be achieved with intraoperative full-dose aprotinin treatment. Furthermore, it appears feasible to restrict intraoperative aprotinin treatment to clopidogrel responders as suggested in **Study IV**.

Finally, alternative strategies may also be considered to decrease perioperative bleeding in patients on clopidogrel undergoing CABG. One possibility would be, as suggested by Jeppsson´s group in Gothenburg, to administer fibrinogen, since preoperative measurement of

fibrinogen concentration provides information about bleeding volume and transfusion requirements after CABG [70]. Another possibility would be to try to limit the effect of clopidogrel by giving proton-pump inhibitors to patients on clopidogrel that have to undergo urgent CABG.

BLOOD TRANSFUSIONS

Blood transfusions during cardiac surgery are associated with increased in-hospital morbidity (infectious complications [71-73], respiratory and renal failure, neurologic events, length of ICU and hospital stay) and mortality [74]. Moreover, the long term effects of transfusions of PRBC have been linked to increased long term mortality after CABG [65,67]. If urgent surgery is preferred, excessive bleeding induced by clopidogrel remains an issue. Undoubtedly, if excessive bleeding could be avoided the decision to commence surgery would be much easier.

It could be argued that prophylactic transfusion of platelets in clopidogrel responders undergoing coronary surgery may adequately reverse clopidogrel induced platelet disaggregation to facilitate postoperative hemostasis. This would make the alternative use of aprotinin redundant [75]. Unfortunately, transfusion of platelets is afflicted with drawbacks as it might severely influence the immunological system with possibly both acute and long-term morbidity and mortality, including leukocyte and Rh-alloimmunization (due to accompanying white and red blood cells), transfusion related acute lung injury, graft versus host disease, hemolysis, and anaphylaxis. Further risks consist of transferred viral and bacterial infections or sepsis, the latter due to the storage of platelets in room temperature [76]. The general drawbacks of transfusions cannot be ignored either [65,67,71-74]. Noticeably, Koch et al. [74]. concluded that perioperative transfusion of PRBC was the most powerful independent predictor of postoperative morbid events after isolated CABG. Furthermore, perioperative transfusion of PRBC was associated with a significantly reduced long-term survival among more than 10 000 patients who underwent isolated CABG. Thus, transfusion of PRBC was associated with risk-adjusted reductions in survival for both the early and late phases after CABG [67].

One should also consider that transfusion therapies are connected with high social costs and, in many situations, blood products remain a scarce commodity which should be predominantly used for the appropriate patient groups.

APROTININ

Patient selection in aprotinin studies

Aprotinin has officially been indicated for "prophylactic use to reduce perioperative blood loss and the need for blood transfusion in patients undergoing CPB in the course of CABG" but as of 2006 with the additional restriction "who are at an increased risk for blood loss and blood transfusion" (Trasylol Label approved by FDA on 12/15/2006, NDA no. 020304).

Most observational studies regarding the overall safety of aprotinin in patients undergoing cardiac surgery have, however, included several different cardiac procedures such as CABG, valve surgery, combined procedure, re-operations, and surgery on the ascending aorta [37-39,41-44]. In this thesis, aprotinin was given according to the policy at our department i.e. when clopdiogrel had been discontinued <5 days before CABG. In **Study III**, the selection was based on patients undergoing isolated primary CABG surgery after matching for age, gender and presence of ACS.

Aprotinin use in cardiac surgery has recently been studied in the large randomized Canadian BART study [45], which evaluated aprotinin versus lysine analogues in patients categorized as "high risk cardiac surgery patients". The inclusion criteria were re-operation for CABG, re-operation for aortic valve replacement, re-operation for mitral valve replacement or repair, initial mitral valve replacement, aortic and/or mitral valve replacement/repair with a CABG, multiple valve replacement/repair, and ascending aortic artery procedures. Notably, isolated patients with primary CABG:s were excluded, which implies that patients with ACS scheduled for primary CABG could not enter the trial. The authors concluded that "Despite the possibility of a modest reduction in the risk of massive bleeding, the strong and consistent negative mortality trend associated with aprotinin, as compared with the lysine analogues, precludes its use in high-risk cardiac surgery." Certainly, aprotinin had a more potent haemostatic effect as indicated by the fact that fewer patients in the aprotinin group of the BART study received platelets and at least one unit of packed red blood cells. This more potent effect of aprotinin could also explain why the observed frequency of cardiac death was higher in aprotinin-treated patients. However, 52% of eligible patients were excluded from the final analysis and their outcome has not been compared with those included in the trial. If an exclusion rate of this magnitude occurs in a multi-centre study

it may as least be possible that trialists have directly or/and indirectly selected the patients least likely to bleed for the study. Indeed, when patients receiving aprotinin were compared with those given TXA, the mortality was significantly higher *only* in patients <65 years old, without co-morbid illnesses, and with a pre-operative hemoglobin value of >140 g/L (Table 3, Appendix C [45]). Similarly, when aprotinin was compared with aminocaproic acid, the mortality was significantly higher *only* in patients without preoperative aspirin treatment, and with a pre-operative hemoglobin value of >140 g/L. Moreover, the definition of "high risk cardiac surgery" may not at all be equal to a high risk of suffering a haemostatic deficiency perioperatively. These limitations may have affected the results in the BART trial. Furthermore, only few (≤ 6%) of the patients in the BART study were subject to treatment with anti-platelet drugs other than aspirin. According to recent guidelines from The Society of Thoracic Surgeons and The Society of Cardiovascular Anesthesiologists [77], patients with the above criteria (<65 years old, hemoglobin value of >140 g/L, without co-morbid illnesses, without preoperative aspirin treatment) can not automatically be defined as patients with a high risk of bleeding and must thus be considered inappropriate for aprotinin treatment. In our opinion, the results indicate that aprotinin may possibly be prothrombotic in patients without deranged haemostatic conditions. Indeed, any drug with a possible prothrombotic effect should be carefully restricted to patients with a, preferably, measurable defect in hemostasis. Thus, the administration should not be based on the subjective judgment of the individual surgeon and/or risks of patient groups (with a large variation) as was the case in the BART trial.

In the light of the BART trial the FDA has suspended aprotinin use in US and in Europe government agencies have either stopped aprotinin completely for clinical use or restricted it to individual licensed administration.

Aprotinin and hypersensitivity

As aprotinin is a foreign protein, hypersensitivity reactions are possible, with an increased risk in patients re-exposed to aprotinin-containing products, in particular within a 6 month interval [78]. For first-time exposure, the incidence of anaphylaxis is estimated to be less than 0.1 %.

Aprotinin and risk of thromboses

Although uncommon, severe and fatal adverse events associated with extensive venous

and arterial thromboses have been reported, both with the full-dose regimen and with variations on the recommended dose. However, a meta-analysis of 35 randomized trials, involving 3879 patients [36], showed that aprotinin use was associated with significantly reduced perioperative transfusion and stroke rates, as well as a trend towards a lower incidence of postoperative atrial fibrillation. The analysis revealed no increased mortality, or increased risk of myocardial infarction, or renal failure in patients undergoing CABG. The following meta-analysis by Brown et al. [34] of 138 randomized trials comparing aprotinin with TXA and ε-caproic acid did not show any differences regarding mortality, stroke, myocardial infarction, or renal failure, but high-dose aprotinin significantly increased the risk of renal dysfunction from 8.4% to 12.9%. Renal dysfunction was defined as an increase of more than 0.5 mg/dl (37.5 μmol/L) in serum Cr. However, aprotinin significantly reduced the incidence of re-exploration (RR 0.49). High dose aprotinin reduced total blood loss by a mean of 184 ml (95% -256 to -112) compared with TXA. In the only multicenter trial [23] that used postoperative coronary angiographic assessment and the recommended full-dose in CABG patients, the incidence of saphenous vein graft thrombosis was not significantly higher in the treated group than in the placebo group when adjusted for risk factors associated with vein graft occlusion (aprotinin versus placebo risk ratio 1.05, 90% confidence interval, 0.6 to 1.8). When considering the above studies, one should be aware of the possible pitfalls of post-hoc analyses when small randomized clinical trials are pooled to meta-analyses unless each of them had the same primary endpoints.

Aprotinin and clopidogrel

In an animal model, the administration of aprotinin has been shown to shorten the prolonged bleeding time induced by clopidogrel treatment [30]. Clinical studies have confirmed that aprotinin reduces perioperative bleeding and transfusion rates when given indiscriminately to patients undergoing coronary surgery while on clopidogrel, i.e. with the last dose less than five days before surgery [42,47,48]. **Study IV** suggests an alternative option. If the degree of platelet aggregation in these patients would be tested preoperatively, the use of aprotinin could be restricted to clopidogrel responders. This would exclude administration of aprotinin to patients with none or a very limited effect of clopidogrel (<10% inhibition), where an already enhanced coagulation increases the risk of thromboembolic cardiac events [9-11,51,52].

Clopidogrel selectively acts to inhibit platelets by reducing activation and subsequent aggregation in response to ADP, whereas aprotinin positively affects platelet aggregation and adhesion in CPB patients through multiple mechanisms mediated by its effects on kallikrein, thrombin, and plasmin. These effects include the inhibition of undesirable platelet activation and aggregation by thrombin at PAR-1 [79,80] while allowing epinephrine and collagen to stimulate appropriate clot formation at wound sites [81]. These multiple mechanisms probably contribute to the clinical effects achieved by aprotinin. However, the exact biochemical mechanism behind the inter-action between aprotinin and clopidogrel is not completely understood. Nevertheless, the data suggest that ADP-induced platelet aggregation is not influenced by aspirin [82]. The results of **Study IV** suggest an influence on the level of ADP-receptors. Clopidogrel's blockage of ADP-receptors is thought to be irreversible and to last during the whole lifespan of the platelet, whereas functional platelets will aggregate maximally after addition of ADP. Since the platelet counts in the EDTA-tubes before and after aprotinin did not differ significantly, the drug itself is unlikely to activate the uninhibited ADP-receptors. This means that aprotinin does not increase platelet aggregation of normal functioning platelets. Consequently, aprotinin must interact with clopidogrel-blocked ADP-receptors, be it temporarily or permanently, and make the clopdiogrel inhibited platelet available to ADP stimulation. This may explain why aprotinin reduces bleeding in clopidogrel treated patients undergoing coronary surgery [42,47,48].

Aprotinin and duration of CABG

Our data of **Study I** indicate that the duration of the operation could be shortened by a mean of 51 minutes (p=0.05) with the use of aprotinin in clopidogrel treated patients. This effect is most likely due to shortened hemostatic measures at the end of the operation, since there were no differences in duration of CPB and cross-clamping time. This finding could not be reproduced in the following larger controlled clinical trial (**Study II)**, where the duration of surgery was only shortened by a mean of 8 minutes in the aprotinin group (p=0.55). One might have expected a greater difference also in Study II. However, one possible explanation may be that the blinding of treatment caused the surgeons to treat both groups in the same manner, including haemostasis.

Aprotinin and postoperative cardiac enzyme levels

Study II documented lower Troponin-T values postoperatively in the aprotinin group,

which is in accordance with the study by Taggart et al [83]. These findings may be explained by the antithrombotic and antinflammatory mechanisms of action of aprotinin [32] as the full-dose aprotinin regimen will cause anti-inflammatory and kallikrein inhibitory effects as well as plasmin inhibition, whereas the half-dose achieves only plasmin inhibition and hence this dose has primarily only antifibrinolytic activity [78].

Aprotinin and renal dysfunction

The possibility that treatment with aprotinin during cardiac surgery might impair renal function has attracted attention in a large observational study by Mangano et al.[38]. In this context the definition of postoperative renal function is of vital importance but still controversial. The relevant studies have used different definitions of renal dysfunction. Mangano et al. [38] defined it as "a postoperative Cr level of ≥ 177 μmol/L with an increase over the preoperative baseline levels of ≥ 62 μmol/L". On the other hand Kincaid et al. [84] defined it as "creatinine greater than 2.0 mg/dL" (150 μmol/L) "within 72 hours of surgery", whereas Karkouti et al.´s [42] definition was "a >50% increase in Cr during the first postoperative week to >100 μmol/L in women and >110 μmol/L in men, or a new requirement for dialysis support". In **Study III** we chose to present several different measurements to evaluate postoperative renal function, with ΔCrCl% as the primary method. Calculated CrCl is considered to be a convenient, and reproducible surrogate measure for estimation of glomerular filtration rate [85] which is advantageous to measured CrCl and Cr [49,86,87]. Notably, the accuracy and precision of measured CrCl has generally been low [85,88]. Furthermore, urine sampling is inconvenient and short collection times may magnify inaccuracies [89]. The comprehensive study by Wijeysundera et al [85] evaluated different measurements of renal function regarding clinical outcomes after cardiac surgery. They found that calculated CrCl is a valid substitute measure of perioperative renal function, which correlated well with patient-relevant clinical outcomes (mortality, dialysis, and prolonged hospitalization). Consequently, in **Study III** we based the calculation of ΔCrCl% on the difference between preoperative Cr and the highest Cr value registered on any postoperative day.

A drawback of the relevant non-randomized trials [37,38,42] is that the individual surgeon usually have decided who should receive aprotinin treatment and who not. So far, only one placebo-controlled randomized trial with more than 100 patients undergoing CABG has been published regarding the effect of aprotinin on postoperative renal function as the

primary outcome. That study demonstrated no significant difference between aprotinin-treated patients and placebo controls with respect to Cr, electrolytes, blood urea, nitrogen, or abnormal CrCl rates, except on postoperative day 7, when there was a transient increase in Cr levels in the aprotinin group [90]. This finding is consistent with **Study III** which showed a slight decrease in ΔCrCl% (-12% and -11%) after aprotinin and TXA treatment, respectively, in patients undergoing CABG.

One should appreciate that patients undergoing cardiac surgery are prone to excessive bleeding, which most often results in a high transfusion burden. This may also affect renal function. Kincaid et al. [84] showed that transfusions of PRBC, platelets, and low intraoperative hematocrit will increase the risk of perioperative renal failure. Notably, the study by Furnary et al. [40] including more than 11 000 patients undergoing cardiac surgery noticed that aprotinin was associated with an odds ratio of 1.5 for acute renal failure ($p=0.008$), when the number of transfused PRBC was not included. However, with the inclusion of transfused PRBC the odds ratio for acute renal failure was 1.23 per Unit of transfused PRBC ($p<0.0001$) and not with aprotinin ($p=0.23$). Furnary et al. concluded that acute renal failure in patients receiving aprotinin was directly related to increased number of transfusions and that aprotinin does not independently increase the risk for acute renal failure in cardiac surgery. Unfortunately, the number of transfusions was not reported in the studies by Mangano et al. and Karkouti et al. [38,42]. Their results could thus be explained as due to a greater propensity to prescribe aprotinin to patients at high risk for bleeding, who consequently have to carry a higher transfusion burden. It is worthy of note that a more recent publication from the Mangano group [91] concerning perioperative risk factors for renal failure after cardiac surgery did not mention aprotinin as a risk factor, although this study used the exact definition of renal dysfunction/failure and the same patient data set as were used in the original Mangano publication on aprotinin [38]. This latter study has been criticized extensively by Royston et al. who presented several compromising arguments [92]. A retrospective analysis of an observational study that uses propensity adjustment assumes that the model includes *all* covariates and confounders. However, 691 eligible patients (20%) had been excluded from an earlier study by the same authors [93], in which the mortality of excluded patients (8%) was three times higher than non-excluded. Mangano et al. have even admitted that patients receiving aprotinin had a higher risk of bleeding and transfusions as well as a higher risk of adverse outcome. Furthermore, risk factor adjustment did not include variables such as age, duration of CPB, use of aspirin, country, and center. These variables

have earlier been shown by the same authors to influence the prevalence of acute renal failure, myocardial infarction, heart failure, neurological outcome, and mortality [93,94]. By the same token, much of Royston's criticism can be applied to the two most recent observational studies indicating a worse outcome after aprotinin exposure [43,44].

Experimental studies have shown that aprotinin with its high affinity for the kidneys is deposited in the proximal tubular cells, and is not significantly secreted until 5-7 days after its administration [95,96]. These findings may explain why a reversible increase in Cr has been noted during the first postoperative week in patients receiving aprotinin treatment during cardiac surgery [90]. Since in **Study III** we measured Cr on multiple occasions during the first postoperative week it may be assumed that a possible difference between the two cohorts in this respect would have been detected. It is also possible that the slight decrease in postoperative fractional CrCl observed after aprotinin is restricted by its coincidental ability to lower the transfusion rate more efficaciously that TXA. Similarly, the observed decrease in postoperative fractional CrCl in the TXA group may be explained by its higher transfusion rate.

Were the matching criteria appropriate for Study III?

We used matching for age, presence of ACS, and gender in **Study III** to make the groups comparable regarding baseline variables, thus increasing the statistical precision with narrower confidence intervals. We recognize that matching for gender eliminates the possibility of studying the potential effect of gender on the association between aprotinin and renal dysfunction. However, this was neither a primary nor a secondary aim of the study. Furthermore, propensity score based matching would also have been feasible for this type of study. During the planning of Study III, and in collaboration with our biostatistician, we decided to use traditional matching for age, gender and presence of ACS. This resulted in well balanced groups.

The two cohorts in **Study III** were comparable except for a higher prevalence of earlier AMI, a higher rate of three-vessel disease, and a slightly reduced ejection fraction in the aprotinin group. The resulting higher number of anastomoses ought to have led to a higher risk of perioperative bleeding and a higher transfusion rate in the aprotinin group. In spite of this drawback the aprotinin group had a reduced overall transfusion requirement, although they were on clopidogrel <5 days preoperatively. The latter may explain the significantly higher platelet transfusion rate in the aprotinin group compared with the TXA group if we

assume a lower rate of patients receiving clopidogrel in the TXA group. Hypothetically, even if the fraction of patients in the TXA group on clopidogrel varies, it cannot be higher than that of the aprotinin group. Thus, an increased fraction of patients on clopidogrel could only have increased the transfusion requirements of the TXA group and not vice versa.

LIMITATIONS

Although **Study I** is a retrospective non-randomized study, the study data were prospectively gathered in our institution's database, which includes all patients operated at our institution. Still, the inevitable drawbacks of a nonrandomized study like this motivated us to follow up with a prospective randomized clinical trial (**Study II**). As we did not measure platelet function preoperatively, the groups may not have been comparable in that respect. However, resistance to clopidogrel is reported to be in the range of 5% to 15% [60,97], and even if some of the patients in the aprotinin group were resistant, it can not explain the large differences in blood loss and transfusion requirements. TXA was almost never used intraoperatively, but it was sometimes used postoperatively if excessive bleeding occurred and no clots were noted in the drains. This drug possesses similar antifibrinolytic properties as aprotinin but without the reported platelet benefits [98]. However, because TXA was used more often in the control group, it can not explain our findings. Furthermore, to eliminate any remaining doubt about its possible influence in **Study I**, we excluded patients who received TXA from the comparison. In spite of the small numbers, we still found significant differences between the aprotinin group (n = 15) and the control group (n = 8) regarding total bleeding (p <0.01) and total number of transfusions (p = 0.05). Hence, the effects must be attributed to aprotinin. Thus, the only remaining alternative explanation for the difference is some form of unknown patient selection, a drawback that is inherent in our study's nature, namely, a retrospective review of data concerning what probably is a first-time observation. Likewise, we have been reluctant to comment on the incidence of stroke in the control group, since the 95% confidence limit for stroke in 2 out of 15 patients is wide (3.7%-38.9%). One may speculate that patients with ACS are prone to thrombotic and embolic events in the whole arterial bed and not only in the coronaries.

To date, the potential role of TXA and aminocaproic acid in decreasing bleeding and transfusion rates in patients on clopidogrel undergoing surgery remain unexplored. The use of TXA is a possible confounder in the trial design of **Study II** although it was a double blinded randomized trial. So, in effect, one could argue that the aprotinin group is looking at two

antifibrinolytic agents, one of which has additional platelet preserving properties.

First, in **Study II** TXA (range 0 – 6 g IV) was given at the discretion of the anesthetist, if there was excessive drainage without clots after reversal of heparin with protamine sulfate and transfusion of platelets. Moreover, 55% of the patients treated with saline received 1.8±1.8 g TA, as compared with 25% of aprotinin-treated patients (p=0.008) receiving 0.5±0.9 g (p=0.001), respectively.

Second, since TXA possesses similar antifibrinolytic properties as aprotinin without the reported platelet benefit [98], the effects of aprotinin in this study must logically be attributed to platelet effects rather than antifibrinolytic properties. This view is supported by our observation that in **Study II** TXA was administered to fewer patients in the aprotinin group and at lower doses than in the control group.

Another limitation of **Study II** is the lack of power to evaluate infrequent clinical outcomes. We did for example not find any difference in stroke rate or mortality between the groups. To study such end points, far more patients would have been needed. By the same token, a type II error may explain the non-significant tendency toward more reoperations because of bleeding in the control.

There are also limitations to **Study III**. When trying to evaluate such a cohort study, the crucial question always is: are the analyzed groups really comparable or has some form of selection taken place? Therefore, it should be realized that the health care system in Sweden, financed by the notoriously high Swedish taxes, provides equal medical treatment, unrelated to socioeconomic factors, to any patient in need of acute medical care. Neither do patients in need of surgery select individual surgeons or hospitals. Before being merged in 2004, the two cardiothoracic departments compared here had a continuous interchange of surgeons, anesthetists, nurses, and perfusionists. Medical practice at the two departments was thus very similar. We have done our utmost to collect and present data on baseline patient and hospital characteristics. In our view it would be hard to find two more comparable hospitals. Indeed, before the merger of the two hospitals in 2004 an independent Norwegian medical consulting Agency (Helseplan) scrutinized the two institutions on behalf of Stockholm County. Helseplan did not find any differences regarding morbidity, mortality, patient outcome, or any other patient or surgically related variables. However, only one department started to use aprotinin in patients undergoing cardiac surgery when there was a high risk of perioperative

bleeding, namely, clopidogrel treatment [47,48], reoperations, and endocarditis. The other department did not.

Admittedly, in **Study III** different pump systems were used at the two centers. Roller pumps were used in the aprotinin group and centrifugal pumps in the TXA group. However, a recent evidence-based meta-analysis of randomized clinical trials comparing roller and centrifugal pumps concluded that there is no evidence in favor of a centrifugal pump over a roller pump in elective CABG with respect to blood loss or clinical outcomes [99]. Even more important, if the claim that centrifugal pumps cause less blood trauma were true, this would have unduly favored the TXA group and not the aprotinin group.

We did not perform a power calculation prior to conducting **Study III** because we used all patients available to us (209 patients receiving aprotinin during the study period) and this determined the sample size. According to the literature post hoc power calculations are generally and positively discouraged by biostatisticians [100]. Instead as suggested by our statistician and by this reference we present 95% CI regarding outcomes of interest. Finally, apart from the primary outcome measure of the study, the fractional change in calculated CrCl (ΔCrCl%), Study III is clearly underpowered to detect possible differences between groups regarding rare events e.g. early mortality, stroke, and reoperation rates.

The main limitation of **Study IV** was its small size. However, all patients were consecutive. The low number of patients in this study, the absence of a control group, and the risk of Type II errors did not allow for any conclusion regarding clinical outcome. Consequently, clinical data from **Study IV** are only presented under Results.

CLINICAL IMPLICATIONS

This thesis demonstrates that a significant reduction of clopidogrel-induced bleeding and transfusion requirements can be achieved with full-dose aprotinin treatment.

If urgent surgery is preferred, excessive bleeding induced by clopidogrel remains an issue. ACS patients presenting for CABG may require acute surgery after clopidogrel administration [66]. Thus, the surgical team is facing the question whether the patient should have surgery delayed for 5 days at the risk of ischemic events, or should be operated upon earlier at the risk of increased bleeding and morbidity. Clearly, if excessive bleeding could be avoided the decision to commence surgery would be much easier.

Study I indicates that a significant reduction of clopidogrel-induced bleeding and transfusion requirements can be achieved with full-dose aprotinin treatment.

In **Study II**, a double-blind, placebo-controlled, randomized clinical trial, these results were further confirmed by the fact that patients in the aprotinin group bled approximately 37% less postoperatively and received fewer units of PRBC, platelets, and total number of blood products versus patients in the placebo group.

The main finding of **Study III** was that aprotinin was not associated with postoperative renal impairment when compared with TXA in patients undergoing isolated primary CABG with a high risk of bleeding. However, the alternative treatment with TXA resulted in a similar slight decrease in postoperative fractional CrCl, but again the overall transfusion rate was higher.

The findings of **Study IV** suggest that aprotinin interacts with clopidogrel-blocked ADP-receptors, making the clopidogrel inhibited platelet available to ADP stimulation.

Screening of platelet function in patients on clopidogrel before acute or urgent coronary surgery makes it possible to select patients with adequate inhibition of platelets for aprotinin treatment. Thus, clopidogrel non- or low-responders would not be given aprotinin, which could minimize the risk of overshoot platelet aggregation with the possible risk of thrombotic events.

CONCLUSIONS

This thesis concludes that:

- Full-dose aprotinin reduces bleeding, transfusion requirements of packed red blood cells, platelets, and total number of blood units in patients on clopidogrel undergoing urgent CABG.

- Perioperative aprotinin treatment during primary coronary surgery in patient on clopidogrel treatment is not associated with impaired renal function postoperatively in comparison with patients receiving tranexamic acid.

- The use of aprotinin reduces the overall transfusions rate to a greater extent than tranexamic acid.

- Aprotinin reduces the antiplatelet effect of clopidogrel. This effect is restricted to patients with a platelet inhibition of $\geq 10\%$.

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