



## Regular Article

## Plasma activity of individual coagulation factors, hemodilution and blood loss after cardiac surgery: A prospective observational study

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## ARTICLE INFO

## Article history:

Received 31 March 2010

Received in revised form 11 May 2010

Accepted 31 May 2010

Available online 26 June 2010

## Keywords:

coagulation

cardiac surgery

hemodilution

## ABSTRACT

**Background:** Hemodilution and consumption of coagulation factors during cardiopulmonary bypass has been suggested to contribute to bleeding complications after cardiac surgery. The aim was to describe the activity of individual coagulation factors after CABG in relation to hemodilution and postoperative bleeding.

**Materials and Methods:** Plasma concentrations of fibrinogen and plasma activity of FII, FV, FVII, FVIII, FIX, FX, FXI and FXIII adjusted for hemodilution were analysed in 57 CABG patients before, and 2 h and 24 h after surgery. Postoperative bleeding was registered and correlations to coagulation factor activity were calculated.

**Results:** Adjusted plasma concentration of fibrinogen ( $-14 \pm 6\%$ ), and plasma activity of FII ( $-9 \pm 6\%$ ), FV ( $-13 \pm 8\%$ ), FX ( $-13 \pm 7\%$ ) and FXIII ( $-9 \pm 14\%$ ) were reduced two hours after surgery compared to baseline (all  $p < 0.001$ ). FVII ( $+3 \pm 12\%$ ,  $p = 0.34$ ) and FXI ( $+1 \pm 19\%$ ,  $p = 0.50$ ) were unchanged, while FVIII ( $+23 \pm 44\%$ ,  $p = 0.006$ ) and FIX ( $+23 \pm 17\%$ ,  $p < 0.001$ ) increased. Twenty-four hours after surgery fibrinogen ( $+45 \pm 27\%$ ), FVIII ( $+93 \pm 66\%$ ) and FIX ( $+33 \pm 26\%$ ) were all increased (all  $p < 0.001$ ), while FVII ( $-37 \pm 14\%$ ,  $p < 0.001$ ), FXI ( $-4 \pm 18\%$ ,  $p = 0.02$ ) and FXIII ( $-6 \pm 15\%$ ,  $p = 0.004$ ) were decreased.

Median postoperative blood loss was 380 ml/12 h. There were significant inverse correlations between postoperative blood loss and fibrinogen concentration 2 h after surgery ( $r = -0.33$ ,  $p = 0.019$ ) and between postoperative blood loss and pre- and postoperative FXIII activity ( $r = -0.34$ ,  $p = 0.009$  and  $r = -0.41$ ,  $p = 0.003$ , respectively), but not between blood loss and any of the other factors.

**Conclusions:** There is a marked dissociation in plasma activity of individual coagulation factors after CABG. Plasma concentration of fibrinogen and factor XIII activity correlates inversely to postoperative blood loss after CABG.

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## Introduction

Significant bleeding after cardiac surgery may be caused by both impaired hemostasis and surgical factors [1]. Impaired hemostasis may occur due to enhanced fibrinolysis, platelet dysfunction or loss, and/or coagulopathy secondary to the exposure of blood to artificial surfaces, hemodilution and the surgical trauma [2–4].

Consumption of coagulation factors during cardiopulmonary bypass has been suggested to contribute to coagulopathy after cardiac

surgery [2,3]. It is, however, not evident whether the activity of all coagulation factors responds similarly to cardiopulmonary bypass and surgical trauma. Furthermore, more refined methods to treat coagulopathies, including plasma-derived and recombinant coagulation factors, have in recent years become available. Increased knowledge about how individual coagulation factor activity varies after surgery may improve treatment. The first aim of the present study was to describe the activity of individual coagulation factors after cardiac surgery with cardiopulmonary bypass in relation to the inevitable hemodilution during and after the operation.

Our group and others have reported that the preoperative and postoperative plasma concentration of fibrinogen is associated with postoperative bleeding volume after cardiac surgery [5,6]. The results indicate that plasma concentration of fibrinogen is a limiting factor for postoperative hemostasis, and that preoperative fibrinogen concentration may be used as a biomarker to identify groups of patients with increased bleeding risk. The second aim was to investigate whether

**Abbreviations:** ACT, Activated clotting time; BMI, Body mass index; CABG, Coronary artery bypass grafting; CPB, Cardiopulmonary bypass; ECAT, External quality Control of diagnostic Assays and Tests.

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activity of any other plasma coagulation factor correlates to bleeding volume after cardiac surgery.

## Material and Methods

### Patients

Initially 59 consecutive patients, (mean age  $65 \pm 7$  years, 77% males) undergoing first time elective CABG with cardiopulmonary bypass (CPB) at Sahlgrenska University Hospital, were enrolled in a prospective descriptive non-interventional study. Predefined exclusion criteria were acute CABG and known bleeding disorder. Two patients were excluded from analysis: one due to change of surgical approach (off-pump instead of on-pump CABG), and one because of on-going medication with clopidogrel at the time of surgery, not noticed at inclusion. Thus, 57 patients were finally included in the study. All patients gave informed written consent before inclusion. The protocol was approved by the local Research Ethics Committee. Patient characteristics are given in Table 1.

### Clinical management

Anesthesia in all patients was induced with 200–300 µg of fentanyl and 3–5 mg/kg of thiopentone, followed by 0.1 mg/kg pancuronium and maintained with sevoflurane. During CPB, anesthesia was maintained with propofol. The patients received heparin (350 units/kg bodyweight) in order to maintain an activated clotting time (ACT) of more than 480 seconds. After CPB, the heparin was reversed by the administration of protamine sulphate (1 mg protamine/100 units of heparin) to an ACT of less than 130 s.

The CPB circuit included a membrane oxygenator and roller pumps. Standard non-pulsatile CPB technique with moderate hypothermia (bladder temperature 34–35 °C) and hemodilution was used. The CPB circuit was primed with 1400 ml of Ringer-Acetate (Fresenius Kabi AB, Uppsala, Sweden) and 200 ml of Mannitol (150 mg/ml) (Fresenius Kabi AB). Cardioprotection was achieved with antegrade cold blood cardioplegia. Weaning off CPB was performed after rewarming to a bladder temperature of 36 °C.

Aspirin was not discontinued before surgery. Clopidogrel was discontinued at least three days before surgery. All patients received 2 g tranexamic acid intravenously at anesthesia induction and at the end of surgery. Aprotinin was not used in any of the study patients.

**Table 1**  
Patient characteristics. Mean and standard deviation or number (%).

Age (years)	$65 \pm 7$
Male gender	44 (77%)
BMI (kg/m <sup>2</sup> )	$27 \pm 3.4$
Euroscore	$2.9 \pm 3.1$
CPB time (min)	$72 \pm 27$ (40–187)
Aortic clamp time (min)	$44 \pm 17$ (23–112)
Number of grafts	$3.3 \pm 0.9$
Preop aspirin	57 (100%)
Preop clopidogrel	0*
Preop LMWH	0
Preop warfarin	0
Unstable angina	19 (33%)
Hemoglobin (g/L)	$148 \pm 14$
Platelet count ( $\times 10^9$ /L)	$279 \pm 64$
Serum-creatinine (µmol/L)	$84 \pm 24$
Hematocrite	$0.44 \pm 0.04$
ACT after protamine reversal (seconds)	$122 \pm 11$
Blood group non O	38 (67%)

**Key:** ACT = Activated clotting time, BMI = body mass index, CPB = cardiopulmonary bypass, L = litre, LMWH = low molecular weight heparin,\*Clopidogrel was discontinued at least 3 days before surgery.

### Study design and analyses

Plasma concentration of fibrinogen and plasma activity of coagulation factor II (FII), FV, FVII, FVIII, FIX, FX, FXI and FXIII were analyzed at three time points: the day before surgery and 2 and 24 hours after surgery. Hemoglobin concentration, hematocrit and platelet count were analyzed at the same three time points. Coagulation factor activity is reported both as absolute values and values adjusted for hemodilution according to the formula: Adjusted activity = absolute activity  $\times$  (preoperative hematocrit / actual hematocrit) [7]. Correlation calculations between coagulation factor activity and postoperative bleeding were performed on absolute activity. The following pre- and perioperative patient variables were registered: age, gender, body mass index (BMI), Euroscore, type of angina, preoperative medication, number of grafts, CPB time and aortic clamp time. Postoperatively, the total amount of chest tube drainage was registered during the first 12 postoperative hours.

The preoperative blood samples were collected from an antecubital peripheral vein and the postoperative samples from a non-heparinized radial arterial line. During sampling, the first 10 ml of blood was discarded. Blood was collected in sodium citrate tubes (0.13 M, 9 parts blood, 1 part sodiumcitrate), and centrifuged at 2000 g for 20 minutes. The supernatant was filled in separate tubes and freezed in dry ice for further analysis.

All samples were analysed at the accredited coagulation laboratory at Sahlgrenska University Hospital. The laboratory participates in the ECAT foundation external quality assessment programme ([www.ecat.nl](http://www.ecat.nl)). Fibrinogen (reference range 2.0–4.5 g/L) was measured by the modified method of Clauss. FII (reference range 70–130%), FV (reference range 60–140%), FVII (reference range 50–160%), FVIII (reference range 50–200%), FIX (reference range 45–190%), FX (reference range 70–130%) and FXI (reference range 60–140%) were determined using one stage clotting assay with specific factor deficient plasma samples. The thromboplastin used for analysis of FVIII, IX and XI was STA C.K. PREST® and for FII, V, VII and X - Neoplastine® CL plus (both STA® (Diagnostica Stago, Asnieres, France) and start of clot formation was detected by viscosity based chronometric measurement on instrument STA-R (Diagnostica Stago). Activity of FXIII (reference range 70–140%) was measured by photometric method (Berichrom FXIII/Dade Behring, Marburg, Germany) on the instrument Cobas Mira (Roche, Basel, Switzerland). Hemoglobin concentration, hematocrit and platelet count were analyzed with clinical standard methods.

### Statistics

Results are expressed as mean and standard deviation (SD) or number and percent (%). Statistical significance was defined as a p-value <0.05. Since bleeding is not normally distributed, all statistical analyses involving bleeding were performed with non-parametric tests. Intergroup comparisons were performed with Mann-Whitney test, Kruskal-Wallis test or Chi-square-test, when appropriate. Correlation testing was performed with Pearson's test (normally distributed data) or Spearman rank sum test. Correlation between coagulation factor activity and postoperative bleeding volume was performed on absolute activities, without correction for hemodilution. Coagulation factor activity after surgery (normally distributed) was compared to baseline with paired T-test. The computer software used was SPSS 16.0 for Windows (SPSS Inc, Chicago Ill, USA).

## Results

### Clinical course

All patients could be discharged from hospital without serious complications.

**Table 2**

Plasma concentration of fibrinogen and plasma coagulation factor activity (absolute values and adjusted for hemodilution) before surgery and 2 and 24 hours after surgery. Mean and standard deviation.

	Before surgery	2 h Postop	24 h postop
Fibrinogen (g/L)			
Absolute	3.7 ± 0.9	2.5 ± 0.7***	3.9 ± 0.7*
Adjusted		3.1 ± 0.7****	5.2 ± 1.0***
FII (%)			
Absolute	108 ± 12	78 ± 13***	79 ± 11***
Adjusted		99 ± 13***	106 ± 20
FV (%)			
Absolute	111 ± 19	76 ± 18***	81 ± 16***
Adjusted		96 ± 20***	106 ± 19*
FVII (%)			
Absolute	121 ± 31	98 ± 28***	57 ± 20***
Adjusted		124 ± 32	76 ± 27***
FVIII (%)			
Absolute	194 ± 66	175 ± 59*	263 ± 76***
Adjusted		221 ± 70**	347 ± 95***
FIX (%)			
Absolute	149 ± 24	143 ± 30	146 ± 26
Adjusted		180 ± 29***	194 ± 38***
FX (%)			
Absolute	104 ± 18	72 ± 17***	70 ± 14***
Adjusted		91 ± 18***	93 ± 20***
FXI (%)			
Absolute	117 ± 25	91 ± 19***	83 ± 18***
Adjusted		115 ± 18	110 ± 24*
FXIII (%)			
Absolute	135 ± 30	97 ± 26***	94 ± 19***
Adjusted		122 ± 31***	125 ± 26**

### Bleeding and Transfusions

Median postoperative bleeding was 380 ml (range 150–1560 ml). During hospital stay, 20/57 patients (35%) were transfused with packed red blood cells, 3/57 (5.3%) with plasma and 5/57 (8.8%) with platelets. The mean volume of transfused packed red blood cells in the whole study group was  $1.3 \pm 3.1$  units (median 0, range 0–20), of plasma  $0.2 \pm 1.2$  units (median 0, range 0–9, and of platelets  $0.2 \pm 0.7$  units (median 0, range 0–4).

### Hemodilution

Mean hematocrit fell from  $44.1 \pm 4.0\%$  preoperatively to  $34.7 \pm 3.9\%$  2 h after surgery and to  $33.7 \pm 3.6\%$ , 24 h after surgery (both  $p < 0.001$  vs. preoperative).

### Coagulation factor activity

#### Preoperative activity

All patients had coagulation factor activity above the lower normal range for all the individual coagulation factors with the exception of one patient with FX activity just below the lower normal limit (68%, lower normal limit 70%). In contrast, a significant number of patients had coagulation factor activity above the upper normal limit (fibrinogen: seven patients (12%), FII: one (2%), FV: three (5%), FVII: six (10%), FVIII: twenty (35%), FIX: four (7%), FX: two (4%), FXI: seven (12%) and FXIII: sixteen (28%).

#### Correlation to age and gender

Baseline activity of FII and FX, but not any of the other factors, correlated inversely with age ( $r = -0.43$ ,  $p = 0.001$  and  $r = -0.50$ ,  $p < 0.001$ , respectively). There were no significant differences in baseline levels between men and women for any of the coagulation factors.

#### Two hours after surgery

The unadjusted plasma activity of all coagulation factors except factor FIX were significantly reduced two hours after surgery compared

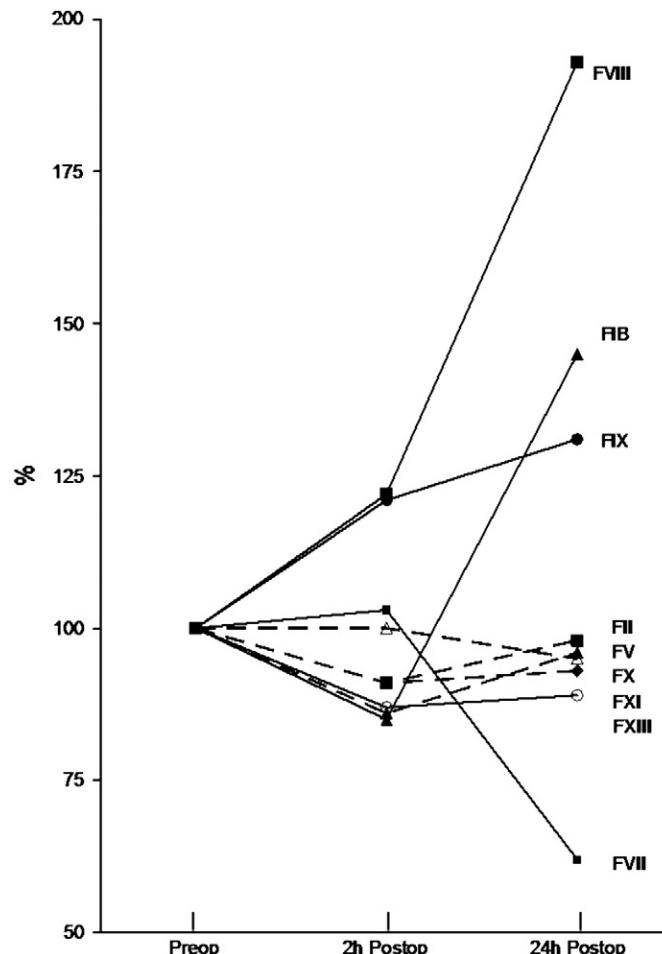
to baseline (fibrinogen concentration  $-32 \pm 11\%$ , FII activity  $-27 \pm 10\%$ , FV  $-32 \pm 11\%$ , FVII  $-19 \pm 13\%$ , FVIII  $-4 \pm 35\%$ , FX  $-31 \pm 10\%$ , FXI  $-20 \pm 17\%$  and FXIII  $-28 \pm 16\%$  (all  $p < 0.001$  except FVIII:  $p < 0.05$ ). FIX did not differ significantly ( $-4 \pm 16\%$ ,  $p = 0.08$ ), Table 2 and Fig. 1.

After adjustment for hemodilution, mean plasma concentration of fibrinogen ( $-14 \pm 6\%$ ), and mean plasma activity of FII ( $-9 \pm 6\%$ ), FV ( $-13 \pm 8\%$ ), FX ( $-13 \pm 7\%$ ) and FXIII ( $-9 \pm 14\%$ ) were significantly reduced (all  $p < 0.001$ ). Activity of FVII ( $+3 \pm 12\%$ ,  $p = 0.34$ ) and FXI ( $+1 \pm 19\%$ ,  $p = 0.50$ ) did not differ significantly compared to baseline, while FVIII ( $+23 \pm 44\%$ ,  $p = 0.006$ ) and FIX activity ( $+23 \pm 17\%$ ,  $p < 0.001$ ) had increased significantly, Table 2 and Fig. 1.

#### Twenty-four hours after surgery

The unadjusted plasma activity of all coagulation factors except fibrinogen, FVIII and FIX were reduced compared to baseline (FII  $-26 \pm 8\%$ , FV  $-27 \pm 9\%$ , FVII  $-53 \pm 10\%$ , FX  $-33 \pm 8\%$ , FXI  $-28 \pm 11\%$  and FXIII  $-29 \pm 16\%$  (all  $p < 0.001$ ) twenty-four hours after surgery. Fibrinogen ( $+9 \pm 18\%$ ,  $p = 0.016$  and FVIII ( $+45 \pm 46\%$ ,  $p < 0.001$ ) had increased while FIX did not differ significantly ( $-1 \pm 14\%$ ,  $p = 0.38$ ), Table 2 and Fig. 1.

When adjusted for hematocrit plasma concentrations of fibrinogen ( $+45 \pm 27\%$ ), and activity of FVIII ( $+93 \pm 66\%$ ) and FIX ( $+31 \pm 26\%$ ) were significantly higher (all  $p < 0.001$ ) compared with baseline, while FV ( $-4 \pm 12\%$ ,  $p = 0.014$ ), FVII ( $-38 \pm 14\%$ ,  $p < 0.001$ ), FX ( $-11 \pm 11\%$ ,  $p < 0.001$ ), FXI ( $-5 \pm 15\%$ ,  $p = 0.020$ ) and FXIII ( $-7 \pm 14\%$ ,  $p = 0.004$ ), were significantly lower compared with baseline. FII ( $-2 \pm 13\%$ ,  $p = 0.27$ ) did not differ compared with baseline level, Table 2 and Fig. 1.



**Fig. 1.** Plasma concentrations of fibrinogen and activity of FII, FV, FVII, FVIII, FIX, FX, FXI and FXIII before surgery and 2 and 24 h after surgery in % of the preoperative value.

**Table 3**

Bivariate correlation coefficient between plasma coagulation factors activity before and 2 h after surgery, and postoperative bleeding.

		r	p
Fibrinogen	Preop	-0.19	0.15
	2 h Postop	-0.33	0.019
FII	Preop	-0.11	0.42
	2 h Postop	-0.22	0.12
FV	Preop	0.04	0.79
	2 h Postop	-0.14	0.34
FVII	Preop	0.04	0.76
	2 h Postop	-0.06	0.66
FVIII	Preop	-0.06	0.65
	2 h Postop	-0.07	0.61
FIX	Preop	-0.09	0.52
	2 h Postop	-0.15	0.29
FX	Preop	0.04	0.78
	2 h Postop	-0.17	0.24
FXI	Preop	-0.12	0.39
	2 h Postop	-0.22	0.12
FXIII	Preop	-0.34	0.009
	2 h Postop	-0.41	0.003

#### Associations between postoperative blood loss and coagulation factor activity

The correlation between absolute individual coagulation factor activity at baseline and 2 h after surgery, and postoperative blood loss is shown in Table 3. There were significant inverse correlations between postoperative bleeding volume and postoperative fibrinogen ( $r = -0.33$ ,  $p = 0.019$ ), as well as between postoperative bleeding and pre- and postoperative FXIII activity ( $r = -0.34$ ,  $p = 0.009$  and  $r = -0.41$ ,  $p = 0.003$ , respectively), but not with any of the other coagulation factors. The correlation between preoperative FXIII and bleeding is further illustrated in Fig. 2 where patients were divided into three groups (lower quartile, mid-two quartiles and upper quartile) based on preoperative FXIII activity. There was a significant difference between the three groups with the largest postoperative bleeding in the lower quartile ( $p = 0.034$ ).

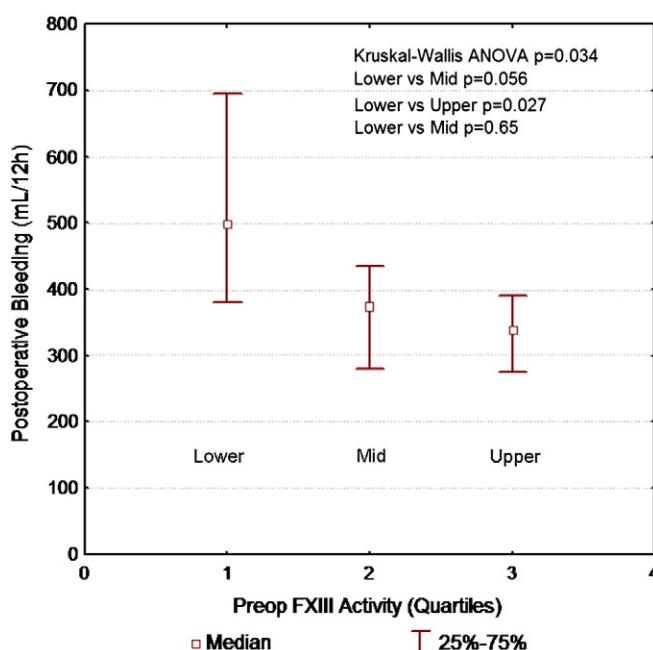


Fig. 2. Postoperative blood loss in patients with preoperative FXIII levels in the lower quartile, in the mid two quartiles and in the upper quartile. There was significant difference between the three groups with the largest postoperative bleeding in the lower quartile ( $p = 0.034$ ).

#### Association between bleeding and other pre- and perioperative variables

Postoperative bleeding volume did not correlate to patient age ( $r = -0.07$ ,  $p = 0.59$ ), body mass index ( $r = -0.09$ ,  $p = 0.50$ ), Euroscore ( $r = -0.11$ ,  $p = 0.40$ ), CPB time ( $r = 0.05$ ,  $p = 0.67$ ), preoperative hemoglobin levels ( $r = 0.18$ ,  $p = 0.19$ ), preoperative platelet count ( $r = -0.19$ ,  $p = 0.15$ ), or preoperative serum-creatinine ( $r = 0.11$ ,  $p = 0.41$ ). There was no difference in bleeding between women and men (360 (150-1010) vs. 390 (160-1560) ml/12 h (median and range),  $p = 0.29$ ), between patients with unstable angina and stable angina (375 (160-1560) vs. 385 (150-1110) ml/12 h,  $p = 0.84$ ), or between patients preoperatively treated with clopidogrel or not (380 (270-1560) vs. 380 (150-1520) ml/12 h,  $p = 0.59$ ).

#### Discussion

The main findings of the present study were the marked variation in plasma activity of individual coagulation factors after CABG, and the observation that plasma activity of fibrinogen and factor XIII correlated inversely to postoperative blood loss after CABG.

The aim of the present study was to investigate how plasma activity of individual coagulation factors responds to cardiac surgery with CPB, and its potential association to postoperative bleeding. Plasma activity, absolute and adjusted for hemodilution, was measured before and after surgery and correlated to postoperative blood loss. As far as we know, this study is the first where plasma activity of all factors involved in plasma coagulation have been investigated in a systematic fashion. Factor XII is deliberately omitted in spite of its role in contact activation because it does not affect bleeding tendency during cardiac surgery [8]. In the preoperative analyses virtually no patients had coagulation factor activity below the lower normal limit, but many patients had higher activity than the upper normal limit, in particular for fibrinogen, FVIII and FXIII. This finding confirms most likely the well known associations between age and increasing levels of fibrinogen, FVIII and FXIII [9]. The association between fibrinogen and FXIII levels and coronary disease has long been recognized [10,11] but causality is far from proven [12].

It is known that cardiac surgery with CPB and hemodilution can lead to a reduction in coagulation factor activity by up to 50% [13]. This decrease cannot be explained only by a dilutional effect since the reduction in hematocrit most often is in the range of 20–25%. Instead, there appears to be a consumption of coagulation factors during surgery, which also is illustrated by the marked increase in thrombin generation during CPB [14]. The present study confirms this pattern with a significantly reduced absolute activity (without adjustment for hemodilution) 2 h after CPB for all coagulation factors except FIX. However, when activity was adjusted for hemodilution, the pattern changed markedly with reductions of fibrinogen, FII, FV, FX and FXIII, unchanged activity for FVII and FXI and increased activity of FVIII and FIX. This demonstrates either a disparity in consumption of coagulation factors during cardiac surgery, or a difference in how fast new generation of the coagulation factors occurs. The present study cannot discriminate between these two mechanisms. In the 24 h time frame, the differences are clearly illustrated in Fig. 1. Five of the coagulation factors, FII, FV, FX, FXI, and FXIII behave similarly with moderate reductions in activity during the time period studied. Four of the coagulation factors contrast strikingly to the remaining factors. The plasma concentration of fibrinogen, FVIII and FIX was markedly increased 24 h after surgery, demonstrating an acute phase response while FVII decreased to approximately 60% of the preoperative levels, indicating a much slower recovery. This finding is not easy to explain regarding the fact that liver synthesis capacity seems to be well preserved after surgery and trauma [15]. The decrease of FVII may partly be due to its continued consumption in presence of abundant amount of tissue factor, and partly be due to its short half life (3–6 hours).

The mean preoperative activity of FIX was higher than in normal populations although only four patients had levels above the upper normal limit. However, it should be noted that the generally used normal ranges may not reflect the age and gender mix of patients undergoing CABG. It is thus possible that the increased activity in some of the coagulation factors reflect the age and gender of the studied population rather than the coronary artery disease and its activity. Accordingly, an age effect on FIX activity has previously been reported [9]. Furthermore, it is unlikely that the preoperative levels, in contrast to the postoperative levels, are influenced by an acute phase response. FIX is not generally considered as an acute phase response protein but unchanged levels after cardiac surgery has been reported before [16].

The problem to predict which patient is going to present with severe hemorrhage after cardiac surgery has been intriguing physicians and scientists for several decades. However, the coagulation process during and after cardiac surgery (with exposure of blood to foreign surfaces, intense mechanical stress and hemodilution) is complex, and the value of a single laboratory test to predict excessive bleeding in individual patients has not yet been proven. Numerous studies have tried to identify biomarkers associated with excessive blood loss. It is important to underline the huge discrepancy between the studies regarding e. g. study populations, definitions of blood loss, endpoints, sampling time points, local routine in surgical and anesthetic procedures and statistical analysis.

Out of all the nine factors analysed in the present study, only postoperative fibrinogen concentration and pre- and postoperative FXIII activity showed an association with postoperative blood loss. Fibrinogen, acting at the very end of the coagulation cascade, is an appealing biomarker to predict postoperative hemorrhage. The association between fibrinogen and postoperative blood loss has been investigated in several studies with diverging results. It is interesting that in the largest cohorts of patients undergoing cardiac operations requiring CPB, fibrinogen levels showed correlation with postoperative mediastinal drainage volume [6,17]. There has been a debate whether pre- or postoperative fibrinogen level predicts bleeding tendency best [18] and it is intuitively appealing to consider variables at the end of surgery as more predictive. On the other hand, the results of preoperative tests make therapeutic interventions possible earlier. In the present study, only fibrinogen levels two hours after skin closure correlated to postoperative bleeding volume, which is in contrast to our previous study on the subject [6]. However, the present study included fewer patients and it is possible that the lack of correlation reflects a statistical type II error. Furthermore, there was a strong correlation between pre- and postoperative fibrinogen level in our data ( $r=0.80$ ,  $p<0.001$ ), indicating that the time point when fibrinogen is measured might be less important.

Factor XIII is not only involved in hemostasis but also in angiogenesis and wound healing [11]. In plasma almost all FXIII is bound to fibrinogen in an inactivated form. The association between FXIII and bleeding after cardiac surgery has been investigated with varying results. In the smaller studies by Shainoff and Chandler, there were significant inverse relationships between FXIII activity and bleeding [19,20]. This could however not be reproduced in a larger study [5]. The results of the present study demonstrate that both preoperative and postoperative FXIII activity correlated to postoperative bleeding. It is intuitive to understand the postoperative relation, which also was stronger, but it is more difficult to explain why preoperative XIII levels correlate to postoperative bleeding. However, the observation is not new. Shainoff et al presented similar results already in 1994 [20]. One reason that also preoperative FXIII levels correlate to bleeding volume could be the close association between pre and postoperative levels ( $r=0.65$ ,  $p<0.001$  in the present material), i. e. that patients with high postoperative levels also have high preoperative levels. Another explanation could have been that there were patients included in the study with hereditary FXIII

deficiency. However, none of the patients had FXIII activity below or close to the lower normal limit.

The results indicate further that FXIII activity is at least as good as fibrinogen concentration to identify groups of patients with increased postoperative bleeding. However, neither FXIII nor fibrinogen is accurate enough to identify individual patients with increased bleeding risk. It is noticeable that bleeding volume correlated only with fibrinogen and FXIII, which both are implied in clot stability rather than in the initiation of the coagulation cascade. One may thus speculate that therapeutic measures, aimed to improve clot stability, may be effective to prevent or treat bleeding complications after cardiac surgery.

As mentioned above, none of the other coagulation factors correlated with postoperative blood loss. At first sight this might be surprising but the activity of the individual factors did, in the present study, not drop to the levels known to predispose bleeding. Fibrinogen and FXIII act as final steps in the coagulation cascade and seem to be crucial for the quality of the clot. It is known from clinical practice that so called "bypassing hemostatic agents" (e.g. activated prothrombin complex concentrate and recombinant activated VII) are effective in treating bleeding in patients with acquired hemophilia, not by substitution of a lacking factor, but by the ability to "jump over" the whole plasma coagulation to the final stages.

Therapeutic interventions with fibrinogen and FXIII to prevent and treat bleeding complications after surgery have already been tested. We have recently presented data suggesting that prophylactic infusion of plasma derived fibrinogen reduces bleeding after CABG [21] and Fenger-Eriksen et al have demonstrated that fibrinogen substitution reduces transfusion requirements in patients after radical cystectomy [22]. FXIII concentrate infusion before cancer surgery improves maximal clot firmness and reduces perioperative blood loss [23]. Interestingly, this was also associated with reduced fibrinogen loss. The safety of a new recombinant FXIII concentrate was successfully proven in a recent pilot study on patients undergoing cardiac surgery with CPB [24]. No effect on transfusion or chest tube drainage was seen but the study was underpowered regarding these endpoints. Sufficiently powered phase III studies with recombinant FXIII to prevent bleeding complications after cardiac surgery are in progress.

This study has several limitations. It is a single centre observational study, reflecting the real life patient management in our institution, which limits the generalizability of our results. It is important to underline that all patients were routinely treated with tranexamic acid which was not the case in most of the cited studies, where some even had use of antifibrinolytics as a distinct exclusion criteria. It is possible that the use of tranexamic acid may give bias towards the influence of clot stabilizing factors (fibrinogen and FXIII) on postoperative bleeding. On the other hand, the majority of patients, at least in Sweden and probably in the rest of the world, are currently treated with antifibrinolytics during cardiac surgery and thus, our results reflect the real world. We acknowledge that our results and conclusions are limited to the studied patient group, i. e. patients treated perioperatively with tranexamic acid. ACT or any other heparin sensitive assay was not measured beyond the immediate postoperative period. It is therefore possible that heparin rebound effects may have contributed to postoperative bleeding. Furthermore, it is acknowledged that postoperative blood loss is not an optimal endpoint. Blood loss is influenced by a number of factors besides hemostasis, such as surgical accuracy and technique, and placement and function of the mediastinal drains. However, other possible endpoints, such as transfusion requirements are also multifactorial, possibly to an even higher degree. In larger studies combined endpoints have been used, such as massive bleeding, defined as re-exploration for bleeding, death due to bleeding or transfusion of  $>10$  units of packed red blood cells [25], but this endpoint is unsuitable for smaller studies.

In conclusion, a marked dissociation in coagulation factors' response to cardiac surgery with cardiopulmonary bypass was demonstrated. Plasma levels of fibrinogen and FXIII correlated to postoperative bleeding. Further studies in larger patient populations are warranted to determine the clinical significance of our results.

## Conflict of interest

None.

## Acknowledgements

The study was supported by the Swedish Heart & Lung Foundation, AstraZeneca AB, Mölndal, Sweden, and the Sahlgrenska University Hospital (ALF/LUA grant). The study sponsors had no influence on the analysis and interpretation of data; in the writing of the report; or in the decision to submit the paper for publication.

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