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INFLUENCE OF COAGULATION SYSTEM CHANGES AND GENETIC POLYMORPHISMS ON POSTOPERATIVE BLEEDING AFTER ON PUMP CARDIAC SURGERY

Summary of the Doctoral Thesis

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LIST OF ABBREVIATIONS

ACE 16 intron I/D	Angiotensin converting enzyme gene insertion/ deletion polymorphism at 16-intron (rs4646994)
ACT	Activated coagulation time
Angle A	Angle Alpha
APTT	Activated partial thromboplastin time
BMI	Body mass index
BSA	Body surface area
CABG	Coronary artery bypass grafting
CTD	Chest tube drainage
CPB	Cardiopulmonary bypass
EF	Ejection fraction
EuroSCORE I	European System for Cardiac Operative Risk Evaluation I
Hb	Hemoglobin
hep.kTEG	Heparinase-modified kaolin activated thromboelastography thromboelastography
ICU	Intensive Care Unite
K	Clot formation time
kg	Kilograms
kTEG	Kaolin activated thromboelastography
LMWH	Low molecular weight heparin
MA	Maximum Amplitude
mL	Milliliters
NSAID	Non-steroidal anti-inflammatory drugs

PAI-1	Plasminogen activator inhibitor type-1
PAI- 1-675(4G5G)	Plasminogen activator inhibitor -1 gene 4Guanine/ 5Guanine polymorphism at 675 position (rs1799768)
PAI- 1 -844 A/G	Plasminogen activator inhibitor type-1 Adenosine/ Guanine polymorphism at 844 position (rs2227631)
PCR	Polymerase chain reaction
PI	Prothrombin index
PLT	Platelets
R	Reaction time
RAAS	Renin angiotensin aldosteron system
SD	Standard deviation
s	Seconds
t-PA	Tissue plasminogen activator
t-PA/PAI-1	Complex of tissue plasminogen activator and plasminogen activator inhibitor type-1
T0	Time point before surgery (preoperatively)
T1	Time point immediately after surgery on admission in ICU
T4	Time point four hours after surgery
T6	Time point four six after surgery
T24	Time point twenty-four hours after surgery
TEG	Thromboelastography

1. INTRODUCTION

Perioperative bleeding is a concern for all patients undergoing cardiac surgery [1]. The incidence of severe bleeding after cardiac surgery exceeds 10% [2]. Bleeding continues to be the most frequent complication requiring early mediastinal re-exploration after open heart surgery and is associated with worse outcomes [3]. Surgical re-exploration due to postoperative bleeding has an incidence in the range of 2% - 6% [4, 5], and in less than 50% of the cases a specific site of bleeding is identified during re-exploration.

At the Cardiac Surgery Center in Pauls Stradins Clinical University hospital we carry out more than 1000 operations with cardiopulmonary bypass (CPB) every year. Most of them are coronary artery bypass grafting (CABG) surgery, valve replacement and mixed (CABG + valve) operations. The local importance of the problem is emphasized by the fact that our incidence of re-exploration due to increased bleeding are similar (5.9%) with reported data in literature [4].

Cardiac surgery with concomitant CPB can profoundly alter hemostasis, predisposing patients to major hemorrhagic complications [1, 6]. Alterations in hemostasis may have a diversity of etiologies. These include the cardiac surgery per se as well as effects of the CPB on the coagulation and the inflammation cascades, and their cross-reactions with the fibrinolytic and the kinin-kallikrein systems [7, 8]. Moreover, hemodilution promotes trombocytopenia and drop of plasma coagulation factors by 30-50% [8, 9].

Pathophysiologically, the balance between bleeding, normal hemostasis and thrombosis is markedly influenced by the rate of thrombin formation, platelet aggregation and activation of the fibrinolytic system. The fibrinolytic system plays a pivotal role in the prevention of intravascular thrombosis but increasing evidence reports its importance in bleeding especially in patients undergoing cardiac surgery with the use of CPB [10, 11].

Fibrinolytic activity depends of balance between plasminogen activators - tissue plasminogen activator (t-PA) and plasminogen activator inhibitors - plasminogen activator inhibitor type -1 (PAI-1) and alpha-2 antiplasmin [12].

Recent evidence suggests that genetic variability modulates the activation each of these pathways. Genetic factors modulate the variability in blood loss after cardiac surgery, and increasing knowledge shows that combining genetic and clinical factors doubles our ability to predict bleeding [13, 14].

Numerous trials with different study design have been made to identify more precisely the biomarkers associated with bleeding. However, even today it is difficult to predict who is going to present with severe bleeding after cardiac surgery. Different genetic polymorphisms, standard coagulation tests - activated partial thromboplastin time (APTT), prothrombin index (PI), fibrinogen, platelet (PLT) count and point-of-care testing such as thromboelastography (TEG) and activated coagulation time (ACT) - are only few of markers which have been studied [6, 13, 15].

Trying to resolve the bleeding problem, we designed this study on a complex of factors including standard coagulation tests (APTT, PI, fibrinogen, PLT count), fibrinolysis markers (PAI-1, complex of t-PA/PAI-1), three genetic polymorphisms, influencing fibrinolytic activity, and TEG analysis.

Standard coagulation tests - APTT, PI, fibrinogen and PLT count. Considering the data in literature, there is a growing tendency to show that standard coagulation tests have failed to monitor hemostasis and to predict increased bleeding risk [15-17].

PAI-1 and complex of t-PA/PAI-1 - PAI - 1 is the main inhibitor of fibrinolytic system, synthesized in platelets as well as in endothelium and adipose tissues. PAI-1 protects the blood clot from premature lysis. The active form of PAI-1 is unstable with a half life of 30 minutes [18]. It binds rapidly to t-PA forming a stable t-PA/PAI-1 complex in the ration 1:1 that is cleared from

circulation in hepatic cells. The complex is considered to be an indicator of the concentration and function of active PAI-1 and t-PA in the blood [19, 20].

Genetic polymorphisms of PAI-1 un Angiotenzin Converting Enzyme (ACE) genes are demonstrated to affect seriously fibrinolytic activity [21]. The human PAI - 1 gene is located in chromosome 7 and contains nine exons and eight introns [22]. The PAI-1 promoter of the PAI-1 gene contains two common polymorphisms. -675 (4G/5G) and -844 A/G which could effect the fibrinolytic balance.

Functional insertion/deletion of PAI-1 gene - 675 4guanine/5guanine (4G/5G) polymorphism has been described in the promoter region in the 675 position of the PAI-1 gene. A single guanine base pair deletion (4G/4G) results in increased PAI-1 levels, which in turn decreases the effect of t-PA. From another, insertion of a guanine base pair (5G/5G) is associated with decreased PAI-1 levels in the circulation and most likely with greater fibrinolysis [23]. Numerous of studies have been reported the role of PAI-1gene -675 (4G/5G) polymorphism and bleeding after cardiac surgery [6, 24].

It has been suggested that PAI-1 gene - 844 adenosine/guanine (A/G) polymorphism also may be relevant as fibrinolytic activity is regarded. The -844 G allele could be associated with a higher bleeding tendency but A allele with increased risk of venous thrombosis [25].

Many links have been established between the renin angiotensin aldosteron system (RAAS) and fibrinolytic system [26, 27]. The RAAS affects fibrinolytic balance because angiotensin IV and aldosteron trigger PAI-1 production whereas its counterpart, bradykinin is the most important stimulus of secretion of t-PA. The ACE gene is located in chromosome 17. The gene comprises 26 exons and 25 introns. ACE insertion/deletion (I/D) polymorphism in the intron 16 may play a role in fibrinolytic activity, and consequently, in postoperative blood loss. The inserted allele is associated with a half of levels of ACE and PAI-1 and potentially an elevated fibrinolytic activity. Deletion of the allele is associated with elevated levels of both ACE and PAI-1 [26].

TEG - measures *in vitro* viscoelastic properties of the developing clot in whole blood. Specific patterns characterize the presence of clotting factor deficiencies, platelet dysfunction and thrombocytopenia, hypofibrinogenemia and fibrinolysis. There is still an ongoing discussion as to whether hemodilution causes hypo- or hyper-coagulability [28, 29] reflected by TEG and standard coagulation tests. Parameters of TEG have been demonstrated as more precise variables for increased bleeding and for hemostatic therapy management [15]. N. Porite with co-authors also reports that TEG guided transfusion algorithm reduces hemotransfusions in cardiac surgery [30].

1.1. Aim of the study

To identify and evaluate predictive potential markers of bleeding in patients undergoing elective cardiac surgery employing CPB.

1.2. Objectives

1. To evaluate changes in the standard coagulation tests (APTT, PI, fibrinogen, PLT count) during CPB and their correlation with 24-hour postoperative bleeding volume.
2. To analyze quantitatively the plasma concentrations of markers of fibrinolysis: PAI-1 and complex of t-PA/PAI-1 and their association with 24-hour postoperative bleeding volume.
3. To identify genetic polymorphisms in PAI-1 and ACE genes and determinate their associations with individual fibrinolytic activities and bleeding.
4. To estimating changes in the coagulation state after CPB, as determined by TEG and the standard coagulation tests.

1.3. Working hypothesis

1. As the standard coagulation tests are regarded, the plasma concentration of fibrinogen possibly could be one of the most precise predictor of greater postoperative bleeding.
2. Decreased plasma concentrations of PAI-1 preoperatively and t-PA/PAI-1 postoperatively may lower inhibitory potential, and consequently, cause greater bleeding tendency.
3. Genetic polymorphism can influence postoperative bleeding volume due to different individual fibrinolytic activity.
4. Parameters of TEG more precisely reflect changes of coagulation state in comparison with standard coagulation tests after CPB.

1.4. Scientific and practical diagnostic novelty

Scientific novelty mostly is based on the understanding of the individual kinetic of the markers of fibrinolytic system at different time points, as well as to evaluate the genetic predisposition to bleeding due to changed fibrinolytic activity in our population after cardiac surgery with CPB.

Current knowledge of the effect of genetic variability on fibrinolysis and bleeding is sparse but our knowledge about gene polymorphisms and their influence on individual plasma concentrations of PAI-1 and t-PA/PAI-1 may help us in the preoperative patient risk stratification before cardiac surgery employing CPB.

Evaluation of coagulation state after CPB by means of TEG parameters and standard coagulation tests could help to estimate the effect of hemodilution on hemostasis with a potential to improve hemostatic management.

1.5. Personal contribution

The author was involved in all stages of the study. The author participated in the design and administration of the study, informed the patients and obtained their written consent, selected genetic polymorphism and fibrinolytic markers under investigation, collected the clinical and laboratory data for analysis. Moreover, the author reviewed the literature, collected the data, performed the statistical analysis and interpreted the results.

1.6. Ethical concerns

The study protocol and the informed consent form were approved by the Ethics Committee of Development Society (approval Nr. 151209-4L) of Pauls Stradins Clinical University hospital. All patients gave their informed consent for participation in the study. A separate consent form was approved by Latvian Biomedical Research and Study Center and the patients gave in writing their informed consent that their genome could be included in the Latvian genome database.

1.7. Structure and size of the work

The Doctoral Thesis is written in english in classical structure. It consist of introduction, literature review, materials and methods, results, discussion, conclusions, practical recommendations and references. The Doctoral Thesis consist of 144 pages including 11 figures, 5 tables in literature review and 20 figures, 18 tables in the part of materials and methods and results. List of literature consist of 221 references.

2. MATERIALS AND METHODS

2.1. The study design

Between March 1st 2010 and July 30th 2011, 90 adult patients scheduled for cardiac surgery by the use of CPB, were enrolled into a prospective observational study. The study was carried out in the Center of Cardiac Surgery et the Department of Anesthesiology and Cardiac surgery of Pauls Stradins Clinical University hospital, Riga, Latvia. As well as in 21 and 16 Cardiac care wards where patients where selected, enrolled and informed for participation in the study. During the study cooperation was provided with Clinical Immunology Center of Pauls Stradins Clinical university hospital and Latvian Biomedical Research and Study Center.

Inclusion criteria:

- More than 18 years of age;
- First-time CABG and/or valve replacement under CPB;
- Predicted operative mortality calculated using the EuroSCORE I < 10%;
- Anticoagulants, antiplatelet and NSAID drugs were withdrawn at least five days prior to surgery. The last dose of LMWH was administered at the latest 12 hours before surgery.

Exclusion criteria:

- Emergency or urgent heart surgery;
- Redo operation;
- Preoperative hemostatic disorders with a history of hemorrhagic events or coagulopathy (PI < 50% or INR greater than 1.5, fibrinogen plasma concentration < 1.5 g/L, PLT count < 100 x 109/L);
- Severe renal failure;
- Hepatic dysfunctions or failure;
- Autoimmune disorders.

2.2. Methods

2.2.1. Anesthesia

The same anesthetic procedure was used in all patients. Anesthesia was induced with fentanyl (A/S Kalceks, Latvia) 0.2-0.3 mg and etomidate (Sagent Agila, India) 0.1-0.3 mg/kg. Cisatracurium (GlaxoSmithKline Manufacturing S.p.A, Italy) 0.2 mg/kg was used for muscle relaxation. All patients received tranexamic acid (Rottapharm, SL, Spain) 2-4 g during surgery.

Anesthesia was maintained with sevoflurane (Piramal Healthcare Ltd, United Kingdom) administered at MAC 0.8-1.2. During CPB, anesthesia was maintained with fentanyl 0.03-0.06 μg/kg/min, propofol 3-5 mg/kg/h (B. Braun Melsungen AG, Germany) and cisatracurium 0.1 mg/kg/h. Before the start of CPB, heparin (Panpharma S.A./Rotexmedica Gmbh, Germany) was administered in a dose of 300 to 400 units/kg initially followed by 5.000 to 10.000 units to achieve and maintain activated coagulatiuon time (ACT) above 480 seconds (s) during CPB.

Standard pulsatile CPB with an extracorporeal circuit consisting of a polypropylene membrane oxygenator (Admiral®, Eurosets TM, Italy) with moderate hypothermia (bladder temperature 34-35 ^{0}C) in combination with hemodilution was used. Extracorporeal circuit was filled up with the constant volume of 1400 ml solution of deltajonin (Pharma GmbH, Germany) for all the patients. Myocardial protection was achieved by using St. thomas 4:1 cardioplegia (Pharma GmbH, Germany) and volume used for cardioplegia dependent on anatomical features of the heart. Weaning off CPB was performed after rewarming the patient to a bladder temperature of at least 36 ^{0}C. After separation from CPB, protamine (Meda Pharma, Wien, Austria) in a dose of 1 mg per 100 units of heparin was administered initially, followed by additional doses until ACT had returned to baseline, or less than 130 s.

2.2.2. Data collection and analysis

During the study following parameters were analyzed:

- Demographic data: age, sex, body mass index (BMI), body surface area (BSA), ejection fraction (EF), EuroSCORE I, diagnosis, patient co-morbidities and preoperative medications (antiaggregants, anticoagulants);
- Surgical parameters: type of surgery, CPB, aortic clamp and reperfusion times, body temperature on CPB, ACT, extracorporeal priming volume of deltajonin and volume of St.thomas solution used for cardioplegia;
- Determination of genetic polymorphisms, assessment of standard coagulation tests and fibrinolysis markers in addition, hematocrit, hemoglobin (Hb) and PLT count were analyzed in different time points: T0 - day before operation, T1 - immediately after surgery on admission in Intensive Care Unit (ICU), T6 - 6 hours after surgery, T24 - twenty-four hours after surgery;
- Kaolin activated TEG (kTEG) with and without heparinase was performed immediately after surgery et T1;
- Postoperative bleeding volume in milliliters (mL) was recorded in T0, T4 - 4 hours after surgery and T24.

Standard coagulation tests. Analyzed T0, T1, T6 and T24 time periods.

APTT - was analyzed in citrated human plasma (Pathrombin*SL reagent, Siemens Healthcare Diagnostics, U.S.A.). Pathrombin*SL reagent enable rapid screening for disorders of the intrinsic coagulation system and sensitively defects of FVIII and FIX. In addition, it can be used for monitoring heparin therapy Normal range is 26-36 s.

PI - was analyzed with a prothrombin complex assay (Lyophilized Dade® and Innovin® reagent, Siemens Healthcare Diagnostics, U.S.A.). PT normal range is 70 - 120%.

Fibrinogen - was determined as described by Clauss [31]. In short, citrated plasma was brought to coagulation by administration of an excessive amount of thrombin 50 units/mL (Multifibren U reagent, Siemens Healthcare Diagnostics, U.S.A.). The reference value is 1.8-3.6 g/L.

Hb concentration and PLT count were analyzed by means of a Beckman Coulter LH 750 Hematology Analyzer. The Coulter LH 750 uses impedance technology to measure PLT count (normal range 150 - 450 x 10^9/L). A hemoglobin cyanide method was used to measure Hb concentration. Normal range Hb 120 - 160 g/L.

Assessment of fibrinolysis markers. PAI-1 was analyzed at T0 and t-PA/PAI-1 complex at T24. To confirm fibrinolytic activity D-dimer concentrations was determinated in three time periods after surgery: T1, T6 and T24.

PAI-1 and t-PA/PAI-1 complex were quantitatively assessed by means of an enzyme-linked immunosorbent assay (ZYMUTEST®, HYPHEN BioMed, France) with ELISA method. PAI-1 normal range is 1-25 ng/mL, t-PA/PAI-1 < 5 ng/mL.

D-dimer - for quantitative determination the immunoturbidimetric test was used (D-dimer PLUS®, Dade Behring, Marburg, Germany) Normal range is < 300 ng/mL.

Gene polymorphism determination - blood samples were taken preoperatively from a central vein at T0 into EDTA vacutainers. Gene determination consist of 5 steps: genomic deoxyribonucleic acid extraction, polymerize chain reaction (PCR), electrophoresis of agarose gel, purification of PCR products and sequencing. For determination of ACE I/D polymorphism insertion and deletion alleles were identified using PCR amplification of respective fragments from intron 16 in the ACE gene and fragment size determination by agarose gel electrophoresis. For determination of PAI-1 gene polymorphisms the Sanger sequencing was used.

Thromboelastography - TEG®5000 (TEG® Haemoscope Corporation, Niles, U.S.A.) was performed once at T1 on admission in ICU. According to the manufacturers instructions [32] blood samples from the radial artery catheter were collected by aspirating 20 ml of blood to perform heparinase-modified kaolin activated thromboelastography (hep.kTEG) and non-heparinase modified kaolin- activated thromboelastography (kTEG). Hep.kTEG has heparinase containing cups containing 2 units of lyophilized heparinase-1, kTEG contains kaolin (hydrated aluminum silicate).

The following TEG parameters were recorded: reaction time – R (normal range 4-8 minutes), clot formation time - K (normal range 1-4 min), the alpha angle – A (normal range 47^0-78^0) and maximum amplitude – MA (normal range 55-73 mm).

Postoperative bleeding volume was fixed in three time points after surgery: T1, T4 and T24 as milliliters (mL) from chest tube drainage (CTD) system.

2.2.3. Methods of statistical analysis

The statistical analysis was performed using Statistical Package for the Social Sciences (SPSS® version 20.0) and Microsoft Excel programs. In the present study continuous variables were described as the mean ± standard deviation (SD) and categorical variables as percentages (%). Statistical significance was defined as a $p < 0.05$. The parametric Student t-test, ANOVA tests were used for variables with regular distribution and Mann-Witney U, Kruskal-Wallis H and Wilcoxon paired tests were used as non-parametric tests. Correlation with postoperative bleeding volume was described as Pearson correlation coefficient.

3. RESULTS

3.1. Clinical results

Totally 90 consecutive adult cardiac surgical patients (47 men and 43 women) 65 ± 11 years (mean ± SD) of age were considered for inclusion in the study. Seven patients (7.8%) required re-operation due to excessive surgical bleeding, who were discarded from further data analysis. 83 patients were subjected to further analysis (42 men and 41 women), age 65 ± 11 years, EF 56 ± 8 %, BMI 28 ± 5 kg/m^2, EuroSCORE I 4.8 ± 1.8 %. Preoperative parameters for 83 patients are demonstrated in Table 3.1.

Table 3.1.

Preoperative parameters for 83 patients subjected to study

Preoperative parameters	Mean ± SD	Range (min.-max.)
Hemoglobin, g/dL	136 ± 15	90 - 166
APTT, s	34.4 ± 7	26 - 75
PLT, x 10^9/L	216 ± 58	120 - 450
Prothrombin indekss, %	89 ± 14	52 - 129
Fibrinogen, g/L	4.6 ± 1.3	1.8 - 10

Abbreviations: APTT, activated partial thromboplastin time; PLT, platelets. Data are given mean ± standard deviation (SD) and min.-max. range.

Preoperative medications. Preoperatively 59 (71%) patients were treated with one or more antiaggregants or anticoagulants. Fifty-six patients received aspirin within 7 ± 2 days before surgery, 16 patients - clopidogrel within 8 ± 2 days and 59 patients - LMWH with the last injection 12 hours before surgery. Four patients were treated with warfarin until 5 days before surgery. There were not statistically significant differences in 24-hour blood loss between patients treated preoperatively with various antiaggregants and anticoagulants.

Type of surgery. All the patients included in the study underwent elective cardiac surgery. The distribution of patients according to type of surgery was as follows: 34 patients (41%) underwent CABG, 31 patients (37%) had valve replacement surgery and 18 patients (22%) mixed (CABG + valve) surgery. Surgical parameters for 83 patients are recorded in Table 3.2.

Table 3.2.

Surgical parameters for 83 patients subjected to study

Surgical parameters	Mean ± SD	Range (min.-max.)
CPB duration (min)	105 ± 40	52 - 252
Aorta occlusion time (min)	66 ± 27	17 - 175
Reperfusion time (min)	34 ± 15	12 - 83
Heparin dose, mL	7.6 ± 1.4	5 - 13
Protamine dose, mg	302 ± 66	200 - 500
Baseline ACT , s.	141 ± 23	95- 202
Temp. on CPB, ^{0}C	35.3 ± 0.4	34 - 36.2
Deltajonin, mL	1503 ± 516	600 - 3200
Blood loss, mL T1	60 ± 32	15 - 150
Blood loss, mL T4	208 ± 120	55 - 510
Blood loss, mL T24	569 ± 270	80 - 1250

Abbreviations: CPB, cardiopulmonary bypass; ACT, activated coagulation time; s, seconds; temp, temperature; T1, on admission in ICU; T4, four hours after surgery; T24, twenty-four hours after surgery. Data are given mean ± standard deviation (SD) and min.-max. range.

Diagnosis and co-morbidities

From 83 patients requiring open heart surgery, (32.5%) had history of myocardial infarction, (20.5%) had hypercholesterolemia and (15.7%) had chronic angina pectoris. Most often observed co-morbidities were primary hypertension (52%), diabetes mellitus type 2 (25.3%) and chronic obstructive pulmonary disease (COPD; 13.3%).

3.2. Coagulation tests in association with bleeding

APTT, PI, fibrinogen and PLT count were analyzed from standard coagulation tests in three time periods after surgery (T1, T6, T24). Association between standard coagulation tests and postoperative bleeding volume was evaluated.

Additionally, two fibrinolysis markers were selected for analysis: PAI-1 preoperatively (T0) and complex of t-PA/PAI-1 postoperatively 24 hours after surgery (T24) and their associations with postoperative bleeding volume.

3.2.1 Standard coagulation tests: APTT, PI, PLT count and fibrinogen

Activated Partial Thromboplastin Time (APTT)

All patients had preoperative APTT values above the lower limit of 26 s. Higher or equal APTT values as compared to the normal value of 36 s preoperatively were registered in 27 patients and 21 of them received LMWH.

At T6 the highest values were detected, which statistically differed as compare to APTL values at T0. The mean values were increased by about 18% at T6. APTT mean values at different time points are shown in Table 3.3.

Table 3.3.

Mean APTT values at different time points

Time point	Mean ± SD	Range (min.-max.)
APTT, s T0	34.4 ± 6.9*≠	26 - 75
APTT, s T1	34 ± 4.8	25 - 48
APTT, s T6	42 ± 13*	29 - 104
APTT, s T24	38 ± 7≠	28 - 74

Abbreviations: APTT, activated partial thromboplastin time; s, seconds; T0, day before surgery; T1, on admission in ICU; T6, six hours after surgery; T24, twenty-four hours after surgery. Data are given mean ± standard deviation (SD) and min.-max. range, *$p < 0.05$ between APTT at T0 and T6; ≠$p < 0.05$ between APTT at T0 and T24

Only preoperative APTT showed medium positive correlation with 4-hour postoperative blood loss ($r = 0.3$, $p = 0.01$).

Prothrombin Index (PI)

PI values postoperatively did not differed significantly as compared with PI at T0 and showed the smallest changes after CPB surgery. Mean values of PI at different time points are shown at Table 3.4.

Table 3.4.

Mean PI values at different time points

Time point	Mean ± SD	Range (min.-max.)
PI, % T0	89 ± 14	52 - 129
PI, % T1	90 ± 12	62 - 125
PI, % T6	87 ± 12	57 - 116
PI, % T24	88 ± 12	61 - 125

Abbreviations: PI, prothrombin index; T0, day before surgery; T1, on admission in ICU; T6, six hours after surgery; T24, twenty-four hours after surgery. Data are given mean ± standard deviation (SD) and min.-max. range.

Platelet count (PLT)

PLT demonstrated the most pronounced changes after CPB surgery. During the first 24 hours, there is an obvious tendency for PLT count to decrease. PLT values postoperatively statistically decreased in all three time periods as compare with PLT count at T0. The lowest mean PLT count 140 ± 47 $\times 10^9$/L was observed 24 hours after surgery when it decreased by 35.2% of baseline. Mean PLT values at different time points are shown at Table 3.5.

Table 3.5.

Mean PLT values at different time points

Time point	Mean ± SD	Range (min.-max.)
PLT, $\times 10^9$/L T0	216 ± 58*	120 - 450
PLT, $\times 10^9$/L T1	144 ± 47*	75 - 346
PLT, $\times 10^9$/L T6	146 ± 49*	56 - 345
PLT, $\times 10^9$/L T24	140 ± 47*	48 - 324

Abbreviations: PLT, platelets; T0, day before surgery; T1, on admission in ICU; T6, six hours after surgery; T24, twenty-four hours after surgery. Data are given mean ± standard deviation (SD) and min.-max. range. *$p < 0.05$, when compare with PLT count at T0

Analyzing postoperative blood loss we noticed that patients with PLT count < 150 x 10^9/L at T6 and T24 time points showed statistically greater bleeding volume after surgery when compare with those who had PLT count ≥ 150 x 10^9/L, respectively, 623 mL *vs*. 429 mL ($p = 0.03$) at T6 and 637 mL *vs*. 460 mL ($p = 0.01$) at T24. As expected, we found a correlation between PLT count and postoperative blood loss. The highest correlation was observed between T24 PLT count and T24 blood loss ($r = - 0.3$, $p = 0.01$).

Fibrinogen

Fibrinogen decreased by 22% from baseline at T1 and by 15% at T6, which statistically differed from fibrinogen values at T0. Fibrinogen level

started to increase after 6 hours and continued to raise 24 hours after surgery. Mean plasma levels of fibrinogen at different time points are shown in Table 3.6.

Table 3.6.

Mean levels of fibrinogen at different time points

Time point	Mean ± SD	Range (min.-max.)
Fibrinogen, g/L T0	4.1 ± 1.3* ≠	1.8 - 10
Fibrinogen, g/L T1	3.2 ± 1.05*	1.5 - 7.3
Fibrinogen, g/L T6	3.5 ± 0.9 ≠	1.8 - 6.6
Fibrinogen, g/L T24	4 ± 0.9	2.2 - 6.4

Abbreviations: T0, day before surgery; T1, on admission in ICU; T6, six hours after surgery; T24, twenty-four hours after surgery. Data are given mean ± standard deviation (SD) and min.-max. range, *$p < 0.05$ between fibrinogen at T0 and T1; ≠$p < 0.05$ between fibrinogen at T0 and T6

As expected, fibrinogen showed the highest correlation with postoperative bleeding. Preoperative level of fibrinogen correlated with postoperative blood loss at T1 ($r = - 0.3$, $p = 0.01$), at T4 ($r = - 0.4$, $p < 0.0001$) and at T24 ($r = - 0.5$, $p < 0.0001$), as shown in Figure 3.1.

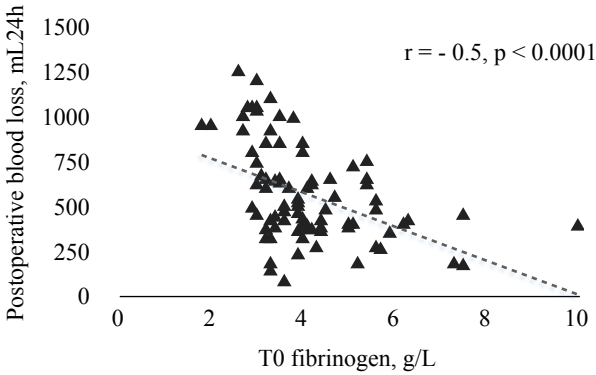

Figure 3.1. Preoperative (T0) fibrinogen plasma level correlation with postoperative 24-hour blood loss

3.2.2. Fibrinolysis parameters: PAI-1 and t-PA/PAI-1 complex

Plasminogen activator inhibitor type-1 (PAI-1)

Mean preoperative PAI-1 plasma concentration was 24 ± 12 ng/mL ranging from 3 to 50 ng/mL. PAI-1 values that are higher or equal to as normal of 25 ng/mL were noticed in 30 patients with a mean concentration of 38 ± 7.5 ng/mL. Out of the total of 83 patients, 53 had lower values than 25 ng/mL. The mean PAI-1 plasma concentration was 16 ± 6 ng/mL.

Patients with lower preoperative levels of PAI-1 than 25 ng/mL showed significantly greater postoperative bleeding volume: at T1 ($p = 0.008$) at T4 ($p = 0.003$) and at T24 ($p = 0.002$), as shown in figure 3.2.

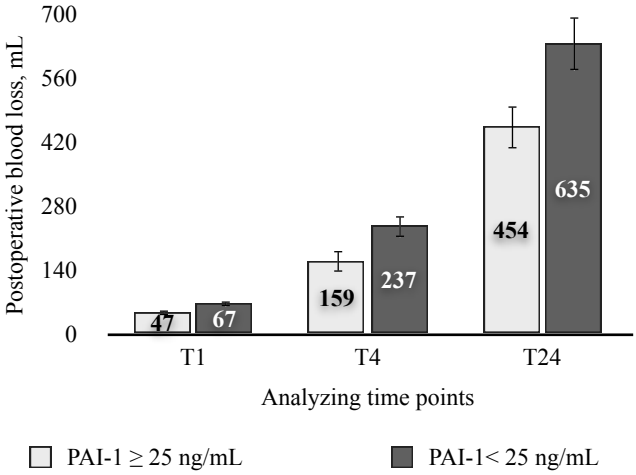

Figure 3.2. Preoperative plasminogen activator inhibitor type-1 (PAI-1) plasma concentrations and postoperative blood loss at three time points (T1, T4 and T24) between patients with preoperative PAI-1 levels ≥ or < 25 ng/mL

Preoperative PAI-1 showed correlation with 24-hour blood loss ($r = -0.3$, $p = 0.01$).

Complex of tissue plasminogen activator/plasminogen activator inhibitor-1 (t-PA/PAI-1)

Mean postoperative t-PA/PAI-1 plasma concentration measured 24 hours after surgery was 3.6 ± 2.1 ng/mL with a range from 1 to 9.5 ng/mL. Eighteen patients had a complex concentration ≥ 5 ng/mL with a mean plasma complex concentration of 7.2 ± 1.3 ng/mL. Of the 83 patients, 65 had lower values than 5 ng/mL with a mean t-PA/PAI-1 plasma concentration of 2.6 ± 0.9 ng/mL.

Patients with lower postoperative levels of t-PA/PAI-1 than 5 ng/mL showed significantly greater postoperative bleeding: at T1 ($p = 0.02$), at T4 ($p = 0.02$) and at T24 ($p = 0.01$). Figure 3.3.

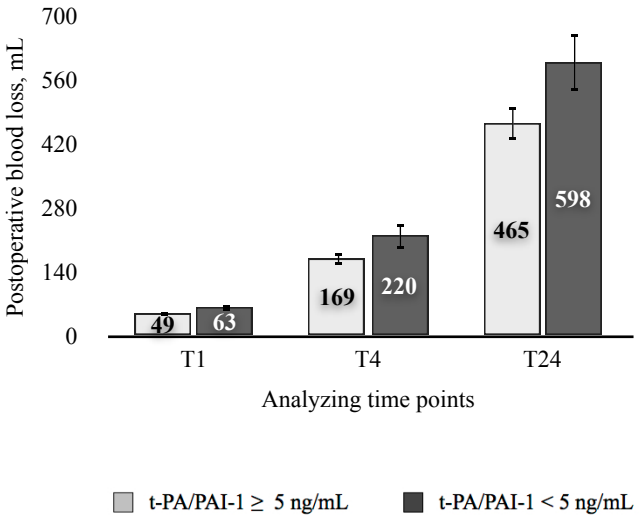

Figure 3.3. Postoperative plasma concentrations of tissue plasminogen activator/ plasminogen activator inhibitor type-1 complex (t-PA/PAI-1) and postoperative blood loss at three time points (T1, T4 and T24) between patients with postoperative t-PA/PAI-1 levels ≥ or < 5 ng/mL

Complex of t-PA/PA-1 did not show any significant correlation with postoperative bleeding volume determinated at three times points.

3.3. Genetic polymorphisms and fibrinolytic bleeding

In the presented study three polymorphisms were investigated which could affect fibrinolytic activity. PAI-1 gene -675 (4G/5G) and -844 A/G polymorphisms and ACE gene intron 16 I/D polymorphism. Fibrinolytic activity was observed by analyzing PAI-1 and t-PA/PAI-1 plasma concentrations, as well as D-dimer levels measured after surgery.

3.3.1.PAI-1 gene -675 (4G/5G) polymorphism

The distribution of PAI-1 polymorphism was as follows: 21 patients were (25%) in the 4G/4G genotype group, 42 (51%) in the 4G/5G group, and 20 (24%) of the 83 patients studied in 5G/5G group. All alleles were in the Hardy-Weinberg equilibrium.

Preoperative PAI-1 levels differed significantly between carriers of genotypes 5G/5G and 4G/5G (17 ± 10.8 vs. 27 ± 13, p = 0.004) and of genotypes 5G/5G and 4G/4G (17 ± 10.8 vs. 24 ± 9.6, p = 0.04), respectively. Figure 3.4.

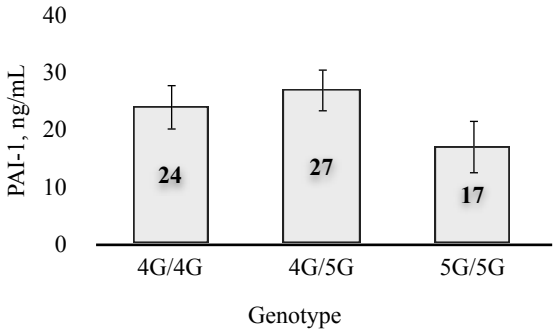

Figure3.4. Preoperative plasminogen activator inhibitor type-1 (PAI-1) levels according to PAI-1 -675 (4G/5G) genotype

With respect to t-PA/PAI-1 complex measured 24 hours after the surgery, the mean plasma concentrations were: 5G/5G 3.6 ± 2.4 ng/mL, 4G/5G 3.9 ± 2.1 ng/mL and 4G/4G 3.1 ± 1.8 ng/mL. The levels of t-PA/PAI-1 complex did not differ statistically between the three genotype groups.

Genotype group 5G/5G displayed the highest postoperative D-dimer levels at all the three time points and the greatest 24 hour postoperative blood loss. Table 3.7.

Table 3.7.

D-dimer levels et different time points and postoperative blood loss according to plasminogen activators inhibitor type-1 (PAI-1) -675 (4G/5G) genotypes

	4G/4G n = 21	4G/5G n = 42	5G/5G n = 20	p value
D-dimer, ng/mL T1	239 ± 225	253 ± 181	334 ± 224	0.2
D-dimer, ng/mL T6	232 ± 185*	256 ± 178	371 ± 226*	0.03
D-dimer, ng/mL T24	209 ± 160*	226 ± 147	326 ± 206*	0.04
Blood loss, mL T24	432±168*	568±192	609±321*	0.02

Abbreviations: T1, on admission in ICU; T6, six hours after surgery; T24, twenty-four hours after surgery. Data are given mean ± standard deviation. *$p < 0.05$

3.3.2. PAI-1 gene -844 A/G polymorphism

We found the following distribution of PAI-1 polymorphism among the 83 patients studied: 22 patients (26.5%) had genotype G/G, 38 patients (46%) had genotype A/G group, and 23 (27.5%) belonged in the A/A group. All alleles were in the Hardy-Weinberg equilibrium.

Preoperative PAI-1 plasma concentrations differed according to the genotype of the patient. Thus, in genotype G/G group, the PAI-1 plasma level

was 18 ± 12 ng/mL, and correspondingly, 24 ± 13 ng/mL and 28 ± 12 ng/mL, in genotype groups A/G and A/A, respectively. Moreover, preoperative PAI-1 levels differed significantly between carriers of PAI -844 G/G and A/A genotypes (p = 0.004). Figure 3.5.

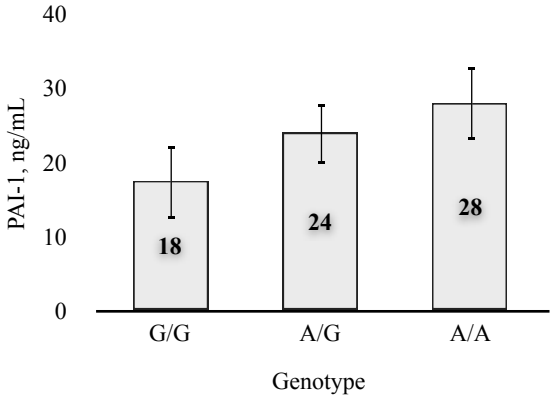

Figure 3.5. Preoperative plasminogen activator inhibitor type-1 (PAI-1) levels according to PAI-1 -844 A/G genotype

G/G carriers showed the lowest values of t-PA/PAI-1 complex 24 hours after surgery but without statistical difference 3.4 ± 2.4 ng/mL, 3.6 ± 2.1 ng/mL and 3.8 ± 1.8 ng/mL in genotype groups G/G, A/G and A/A, respectively.

Additionally, we found that G/G carriers were tendended to have higher postoperative D-dimer levels at T24 in comparison with those of genotypes A/A and A/G.

Regarding to 24 hour blood loss statistically significant differences were found between the G/G and A/A genotypes (p = 0.03) and the A/A and A/G genotypes (p = 0.03).Table 3.8.

Table 3.8.

D-dimer levels et different time points and postoperative blood loss according to plasminogen activators inhibitor type-1 (PAI-1) -844 A/G genotypes

	G/G n = 22	A/G n = 38	A/A n = 23	p value
D-dimer, ng/mL T1	287 ± 255	312 ± 213	251 ± 170	0.5
D-dimer, ng/mL T6	255 ± 203	312 ± 200	289 ± 210	0.5
D-dimer, ng/mL T24	267 ± 168*	184 ± 129*	234 ± 187	0.04
Blood loss, mL T24	601 ± 221*	604 ± 308	436 ± 267*	0.03

Abbreviations: T1, on admission in ICU; T6, six hours after surgery; T24, twenty-four hours after surgery. Data are given mean ± standard deviation. *$p < 0.05$

3.3.3. ACE gene intron 16 I/D polymorphism

The distribution of ACE gene Intron 16 I/D polymorphism was as follows: 22 patients (26.5%) presented with genotype I/I, 42 (51%) with genotype I/D and 19 (23.5%) of the 83 patients studied had genotype D/D. All alleles were in the Hardy-Weinberg equilibrium.

Preoperative PAI-1 plasma concentrations differed according to the patient's genotype. Carriers of genotype I/I presented with preoperative PAI-1 of 18.4 ± 10.7 ng/mL, which was the lowest registered. Preoperative PAI-1 levels differed significantly between carriers of ACE gene Intron 16 I/I and D/D genotypes ($p = 0.02$), as depicted in figure 3.6.

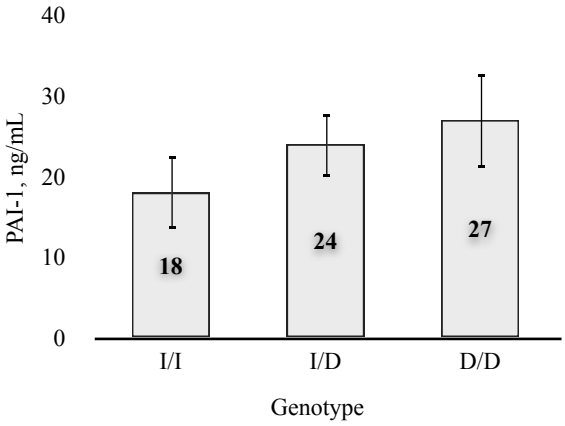

Figure 3.6. Preoperative plasminogen activator inhibitor type-1 (PAI-1) levels according to angiotensin converting enzyme (ACE) Intron 16 I/D genotype

ACE gene Intron 16 I/I carriers also presented with the lowest plasma levels of t-PA/PAI-1 complex. Statistically significant difference was reached between the I/I and the I/D carriers ($p = 0.02$), as shown in figure 3.7.

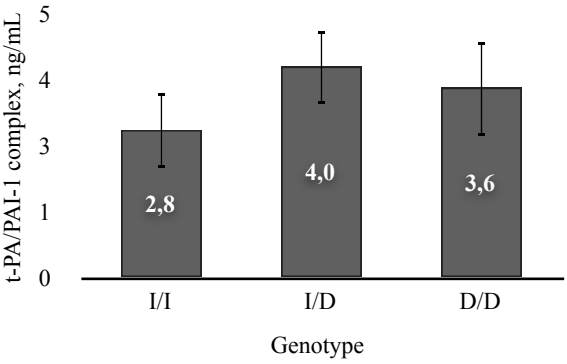

Figure 3.7. Postoperative plasma levels of tissue plasminogen activator/plasminogen activator inhibitor type-1 (t-PA/PAI-1) complex according to angiotensin converting enzyme (ACE) Intron 16 I/D genotype

ACE gene Intron 16 I/I genotype group presented higher D-dimer levels at all three time points after surgery showing tendency of enhanced fibrinolysis postoperatively. There were found similar 24-hour postoperative blood loss in all three ACE gene Intron 16 I/D genotype groups with a small tendency towards higher blood loss in the group of patients with genotype I/I. Data are presented in Table 3.9.

Table 3.9.

D-dimer levels et different time points and postoperative blood loss according to Angiotenzin Converting Enzyme (ACE) 16 intron I/D genotypes

	I/I n = 22	I/D n = 42	D/D n = 19	p value
D-dimer, ng/mL T1	367 ± 203*	294 ± 238	234 ± 161*	0.03
D-dimer, ng/mL T6	331 ± 218	291 ± 204	244 ± 182	0.4
D-dimer, ng/mL T24	274 ± 167	247 ± 165	208 ± 173	0.2
Blood loss, mL T24	589 ± 262	546 ± 276	544 ± 331	0.65

Abbreviations: T1, on admission in ICU; T6, six hours after surgery; T24, twenty-four hours after surgery. Data are given mean ± standard deviation. *$p < 0.05$

3.4. Changes of coagulation state after CPB detected by thromboelastography and standard coagulation tests

In the present study, we investigated the changes of coagulation state influenced by hemodilution during CPB as assessed by TEG after cardiac surgery employing CPB in 83 patients. The patients included in the study were allocated to two groups depending on the deltajonin volume used for priming of the CPB circuit. For all patients initial priming volume in the extracorporeal circuit was constant at 1400 ml. In addition, a volume of deltajonin was used as

required to provide adequate priming of the circuit based on the individual BSA of the patient. Considering the fact that the mean volume of deltajonin per patient was 809 ± 256 ml/m^2 (range 375 - 1500 ml/m^2), the patients were divided into two groups:

Group I (n = 40) was the most diluted group. The extracorporeal circuit priming volume of deltajonin was more than the calculated mean. The deltajonin volume was 1015 ± 200 mL/m^2 (range 800 - 1500 mL/m^2). The BSA of these patients were 1.9 ± 0.2 m^2.

Group II (n = 43) was the less diluted group. The extracorporeal circuit priming volume of deltajonin was less than the calculated mean. The deltajonin volume was 620 ± 116 mL/m^2 (range 375 - 778 mL/m^2). The BSA of these patients were 1.8 ± 0.2 m^2.

All kTEG parameters were out of the normal range in the more diluted patients of Group I and statistically differed from Group II, respectively, R 12.3 ± 6.4 min *vs.* 9.7 ± 4.9 min (p = 0.04), K 5.1 ± 2.8 min *vs.* 3.8 ± 2.5 min (p = 0.02), angle A 40 ± 120^0 *vs*. 50 ± 13^0 (p = 0.001) and MA 56 ± 10 mm *vs.* 60 ± 10 mm (p = 0.04). Concomitantly, we noticed significant differences between Group I and II as hep.kTEG parameters were regarded with differences in K (p = 0.02), A (p = 0.03) and MA (p = 0.04).

In contrast, except for the plasma concentrations of fibrinogen in Group I and II (2.9 ± 0.8 vs. 3.5 ± 1.2, p = 0.01) we found no significant intergroup differences in the standard coagulation variables after CPB.

For comparison of qualitative and quantitative TEG analysis between more and less diluted patients depending on the priming volume of deltajonin used in the extracorporeal circuit as calculated on the basis of the patient's BSA (m^2) figures 3.8. and 3.9. are presented.

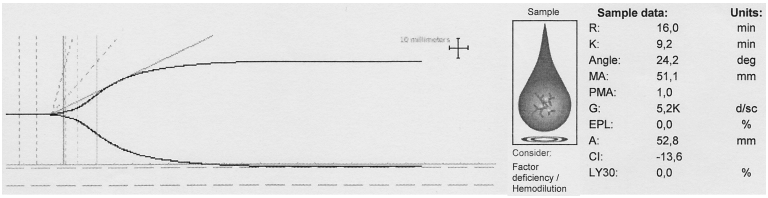

Figure 3.8. Thromboelastography data of qualitative and quantitative analysis. Patient from group I, extracorporeal circuit priming volume of deltajonin 1157ml/m^2, postoperative 24-hour blood loss 890 mL

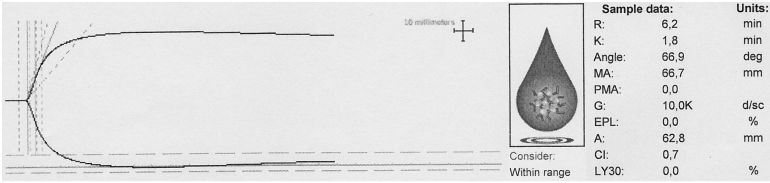

Figure 3.9. Thromboelastography data of qualitative and quantitative analysis. Patient from group II, extracorporeal circuit priming volume of deltajonin 500 ml/m^2, postoperative 24-hour blood loss 320 mL

As expected, there were differences in blood loss analyzed at three time points postoperatively. Group I had significantly higher blood loss at 4-hour and 24-hour after surgery in comparison with Group II ($p = 0.04$ and $p = 0.01$) respectively, as evident from Table 3.10.

Table 3.10.

Postoperative blood loss at three time points between two groups

	Group I n = 40	Group II n = 43	p value
Blood loss, mL T1	67 ± 32	55 ± 31	0.2
Blood loss, mL T4	237 ± 119*	182 ± 116*	0.04
Blood loss, mL T24	647 ± 254*	496 ± 267*	0.01

Abbreviations: T1, on admission in ICU; T4, four hours after surgery; T24, twenty-four hours after surgery. Data are given mean ± standard deviation. *$p < 0.05$

4. DISCUSSION

Bleeding complications in cardiac surgery with CPB have multifactorial causes [1, 4]. The present study focuses on the association between the standard coagulation tests (APTT, PI, PLT count and fibrinogen) and fibrinolysis markers (PAI-1 and t-PA/PAI-1) with 24-hour postoperative blood loss. Moreover, genetic polymorphisms which could influence fibrinolytic balance and therefore bleeding tendency was analyzed and TEG was performed to determinate changes of coagulation state in patients undergoing heart surgery employing CPB.

4.1. Coagulation tests association with bleeding after CPB

Standard coagulation tests. According to our results APTT and PLT count demonstrated the most significant changes after CPB, but preoperative values of fibrinogen had the highest predictive value for postoperative bleeding.

Activated Partial Thromboplastin Time. Twenty-seven patients had higher or equal than normal APTT values at T0 and 21 patients of them received the last dose of LMWH 12 hours before surgery although for those patients were not found greater postoperative blood loss. Similar results to our finding was published by *Medalion B. et al.* [33], who analyzed whether the use of LMWH before CABG could be associated with an increase in bleeding in parallel with increased transfusion of blood products after the operation. There was no difference in CTD among the patients who received enoxaparin 8-10 hours before surgery or placebo group at 24 hour and in the amount of blood products transfused. APTT values showed significant changes after CPB, particularly et T6, when APTT increased by 69% and et T24 by 61% as compare with baseline. This could be explained by the “heparin rebound phenomena” [34]. Higher APTT values after surgery also can be explained by

hemodilution and reduction of the coagulation factors by 50% [8]. Despite the fact that more than a half of the patients had higher than normal APTT values after cardiac surgery at T6 and at T24, we did not find any postoperative APTT association with 24-hour postoperative blood loss. There are several studies investigating the relationship between APTT and postoperative blood loss reporting similar results to ours [35, 36]. We found an correlation between preoperative APTT and postoperative bleeding volume 4 hours after surgery, which is consistent with finding in a large study including 894 cardiac surgery patients where APTT had a low predictive value as postoperative bleeding is regarded [16].

Analyzing ***prothrombin index*** data, it showed the smallest changes after CPB in our results. Preoperatively, only 7 patients had PI lower than normal, and those did not show greater bleeding tendency after the surgery. After CPB, nobody had lower levels of PI than normal. We speculate, that one of the explanation could be the administration of fresh frozen plasma or crioprecipitate in operating theater. We were not able to find any correlation between PI and 24-hour postoperative blood loss. Results from the literature are controversial. Some authors [15, 35, 37] have been found significant relationship between PI measured after termination of CPB with postoperative CTD and have been demonstrated that PI differed significantly between bleeders and non-bleeders. From another, *Ti et.al.* [38] and *Blome et al*. [39] failed to find any difference in PI measurements and postoperative bleeding, supporting our results.

In the present study ***platelet count*** showed a convincing tendency to decrease after CPB. At T24 more than a half of the patients presented with a reduced PLT count by 32.5%. We speculate that the main reasons could be hemodilution and consumption of PLT during CPB, when PLT count is known to decrease by about 30-50% [40]. Although previous studies have shown conflicting results, examining the relationship between PLT count and bleeding, most of the reports support the tendency, that PLT count predicts and influences

postoperative blood loss after cardiac surgery [39, 41]. Our results show similar finding, suggesting that the PLT count after CPB may affect the CTD volume 24 hours after surgery. In this study, we found a correlation between PLT count and 24-hour postoperative blood loss. Contradictory results have been published by *Gravlee et al.* [16]. Thus, a study of 894 patients aimed at screening the relationships between standard blood clotting tests and postoperative bleeding in cardiac surgical patients found no correlation between PLT count and increased postoperative bleeding.

The role of ***fibrinogen*** plasma concentration as a predictor of bleeding after cardiac surgery has been investigated in many studies. However, the results of these investigations have been inconsistent [6, 17, 39, 42]. Thus, few authors demonstrated a significant association between the fibrinogen level and postoperative blood loss [6, 17], supporting our results. In a recent study [39] the relationship between FXIII, fibrinogen, blood coagulation screening tests and postoperative bleeding in 98 patients undergoing cardiac surgery with CPB was investigated. These investigators found strong associations between preoperative and postoperative fibrinogen levels and postoperative bleeding. Consistent with the findings presented in this thesis, *Karlsson et al.* [17] found a strong correlation between postoperative bleeding volume and preoperative fibrinogen concentration in the study of 170 patients. Nowadays a multi-center trial [43] is going on investigating the role of prophylactic fibrinogen infusion to reduce postoperative bleeding and transfusion requirements after CABG in patients with endogenous fibrinogen levels in the lower normal range.

PAI-1 and t-PA/PAI-1 complex. The present study revealed that lower levels of PAI-1 preoperatively and of t-PA/PAI-1 complex postoperatively are associated with increased blood loss in the first 24 hours after the operation.

Since PAI-1 is a more stable indicator of fibrinolysis, as compared to t-PA, whose concentration peaks during CPB [20], we determined PAI-1 before the operation and t-PA/PAI-1 complex after the surgery. Our findings indicated that those presenting lower preoperative PAI-1 plasma level had a larger blood

loss at the data collection time points during the first 24 hours after surgery. Moreover, we found correlation between preoperative PAI-1 concentrations and 24-hour blood loss. Other investigators have noticed similar results. In an investigation of *Rivera et al.* [6] PAI-1 plasma concentrations were measured before and after cardiac surgery. Bleeders (> 1L/24h) had significantly lower PAI-1 plasma levels before and immediately after surgery (25.5 ng/mL and 35.5 ng/mL) in comparison with non-bleeders (< 1L/24h) and the latter also presented with significantly higher PAI-1 plasma levels (43.5 ng/mL vs. 76.6 ng/mL). In a recent, PAI-1 activity was studied in relation to the risk for perioperative bleeding complications in 62 patients scheduled for transurethral resection of the prostate [44]. In those with low plasma level and activity of PAI-1 75% had bleeding complications requiring re-operation against 28% in patients with normal PAI-1 levels and activity. Contrary to our results the authors could not present any correlation between intra- and postoperative bleeding volume and PAI-1 plasma levels.

As to the best of our knowledge, the literature is scanty on reports focusing on the importance of t-PA/PAI-1 complex and its relationship with enhanced bleeding after CPB. One of the few studies is published by *Rivera et al.* [6] who determined t-PA/PAI-1 complex concentrations in 26 patients immediately after cardiac surgery and showed that those with a lower level of complex have significantly higher 24-hour blood loss after surgery. Our results is consistent showing that patients with lower complex levels have greater bleeding tendency after heart surgery with CPB.

Recently, investigators reported favorable effects of administration of a modified PAI-1 protein with a very long half-life > 700 hours on bleeding in PAI-1 deficient mice [45]. Most recently, a clinical application of very long half life PAI-1 has been patented. One of the indication is bleeding related to hyperfibrinolysis, moreover, prevention of bleeding and topical application to control localized bleeding [46].

4.2. Genetic polymorphism and fibrinolytic bleeding

The plasma concentrations of PAI-1 and of t-PA/PAI-1 complex that are supposed to be the main regulators of fibrinolysis in humans, are both characterized by wide variations that may explain the large inter-individual differences in fibrinolytic activity [47]. Several recent studies have described the influence of genetic factors [24, 48, 49].

PAI-1 gene -675 (4G/5G) polymorphism. Much attention has has been paid to this polymorphism and fibrinolytic activity [24, 48, 50]. According to our data, PAI-1 gene 5G/5G polymorphism may be associated with lower preoperative plasma concentration of PAI-1, with higher levels of D-dimer postoperatively and larger bleedings after on-pump cardiac surgery. We observed by 29% lower levels of PAI-1 for 5G/5G carriers as compare with 4G/4G homozygous. These results are consistent with several recent investigations [6, 24, 49]. Unfortunately, we could not find any statistically significant difference in the complex plasma concentrations comparing three PAI-1 genotypes. Even more, the 4G/4G carriers demonstrated the lowest plasma concentrations of the t-PA/PAI-1complex 24 hours after surgery, and 4G/5G carriers the highest. We speculate that complex level could be influenced by CPB as it is well known that the PAI-1 and t-PAI/PAI-1 concentrations start to increase 2-3 hours after CPB as part of the “fibrinolytic shut down” [20]. Patients with 5G/5G genotype had the greatest bleeding volume after surgery. Additionally, D-dimer reached higher postoperative levels in the 5G/5G group as compared with the 4G/5G and the 4G/4G genotype groups confirming enhanced fibrinolysis.

So far, no studies have been published concerning ***PAI-1 gene -844 A/G polymorphism*** in patients undergoing cardiac surgery with CPB and its influence to bleeding volume. *Abboud N. et al.* [51] investigated the association between PAI-1 -844 A/G and changes in PAI-1 and t-PA levels in 305 patients

with myocardial infarction in comparison with 328 healthy controls. The elevation of PAI-1 levels was more pronounced in 844 A carriers. *Verschuur et al.* [52] have supported our results showing that patients with PAI-1 -844 G/G genotype might have higher fibrinolytic activity due to lower levels of PAI-1 and complex. We wished to show that G/G carriers may be predisposed to higher bleeding risk after heart surgery with CPB due to enhanced fibrinolysis. Such a tendency was found in presented study because G/G homozygous showed lower PAI-1 preoperative levels by 36%, lower t-PA/PAI-1 concentrations 24 hours after surgery and higher postoperative bleeding volume as compare with A/A carriers. Additionally, G/G group had higher D-dimer levels at T24.

ACE gene Intron 16 I/D polymorphism has been paid little attention to, and the few reports are conflicting [13, 49, 53]. In the present study, we observed statistical significant difference with regard to preoperative PAI-1 and postoperative t-PA/PAI-1 between I/I and D/D carriers. The I/I genotype group had approximately a 33% lower PAI-1 plasma concentration before surgery as compared with the D/D carriers and a 22% lower levels of complex in comparison with I/D carriers. Some authors [13, 49] have published higher postoperative bleeding after CPB in parallel with lower plasma PAI-1 levels in I/I carriers. However, we detected higher D-dimer levels after surgery in the I/I homozygous, 24-hour postoperative bleeding volume was not different in the three genotype groups. Surprisingly results have published *Pola et al.* [53] in patients during total hip replacement surgery showing that the D allele is a risk factor for increased blood loss, although I/I patients had higher D-dimers suggesting that a more efficient activation of coagulation occurred in this group.

4.3. Changes of coagulation state after CPB detected by thromboelastography and standard coagulation tests

In this comparison of TEG and standard coagulation tests, we demonstrated that kTEG and hep.kTEG might be reliable parameters for evaluation of the coagulation state after CPB affecting by hemodilution. There is still an ongoing discussion as to whether hemodilution causes hypo- [28] or hyper-coagulability [29]. Our results demonstrated that TEG are progressively affected during CPB by hemodilution showing hypocoagulation, although standard coagulation tests did not reflect hypocoagulability due to hemodilution, excepting fibrinogen. Moreover, more diluted patients showed higher bleeding volume after surgery. Such a tendency have been published by few authors in a large comparative study of 613 adult patients undergoing CABG surgery. There was not correlation between standard coagulation tests and hemodilution [54]. *Ternstrom et al.* [9] support or finding showing that there is a a marked dissociation in the plasma activity of individual coagulation factors after CPB after investigating the individual plasma coagulation factor activity after CABG in relation to hemodilution and postoperative bleeding. In our study, the infusion of higher saline volumes were strongly associated with an abnormal kTEG and hep.kTEG parameters that might reflect reduction of plasma activity and concentration of various coagulation proteins, fibrinogen plasma level and impaired platelet function due to dilution coagulopathy. In the study [55] were *in vitro* the influence of hemodilution with saline or hemaccel on TEG parameters was studied authors concluded that hemodilution per se increases the initiation of coagulation and speed of clot formation of whole blood *in vitro*, but saline hemodilution has a more marked effect on the final clot strength. We noticed in kTEG significantly prolonged R, K time and decreased A-angle and MA time in the group with more diluted patients and the same tendency was found in hep.kTEG. We speculate, that most likely, patients

with greater hemodilution might have prolonged speed of initial clot formation and decreased initial clot cross linking reactions with activated platelets and clots that have less strength and firmness. It could be caused by a greater decrease in circulating procoagulant activity than anticoagulant activity.

We believe that several factors influence blood loss during and after cardiac surgery employing CPB. Therefore, an important issue is to find the most appropriate bleeding marker, and many studies are made in this field. There is no single bleeding marker, which by itself has the necessary sensitivity and specificity to predict excessive bleeding. Nevertheless, there are still questions to answer and additional studies are warranted to find more precise bleeding markers or their combination as well as coagulation changes affecting by CPB.

5. CONCLUSIONS

1. APTT and PLT count constitute the most significant changes from standard coagulation tests after CPB. Decreasing in PLT count can suggest of increased bleeding tendency after cardiac surgery.

2. Preoperative plasma fibrinogen concentration documented the highest predictive value for a greater 24-hour postoperative blood loss, which could be as a possible predictor of increased bleeding risk in the postoperative period.

3. Our investigation indicates that low plasma levels of PAI-1 preoperatively and of t- PA/PAI- 1 complex postoperatively, in parallel with increased plasma concentration of D-dimer can be useful predictors of fibrinolysis, and thus, of increased postoperative blood loss.

4. Tendency to enhanced fibrinolysis affecting postoperative blood volume showed PAI-1 gene -675 5G/5G, PAI-1 gene -844 G/G and ACE Intron 16 I/I genotypes.

5. Our results indicate that kTEG and hep.kTEG can reflect hypocoagulability after CPB that could not be detected by standard coagulation tests.

6. Postoperative 24-hour blood loss can be affected by the amount of saline solution used in the extracorporeal circuit.

6. PRACTICAL RECOMMENDATIONS

1.We recommend to use standard coagulation tests before and after cardiac surgery with CPB for all patients and to focus more attention on preoperative plasma fibrinogen measurements and on changes in PLT count after surgery.

- Preoperative fibrinogen level < 3.6 g/L should be observed as a marker for increased bleeding risk after cardiac surgery with CPB.
- If PLT count is lower then 150 x 109/L transfusion of platelets should be considered for bleeding patient in ICU after cardiac surgery.

2.The established two fibrinolytic system markers PAI-1 and t-PA/PAI-1 should be introduced for practical diagnostic use for bleeding risk stratification before cardiac surgery employing by CPB. Therefore, by including screening of fibrinolytic markers pre – and postoperatively, we might be able to identify patients with low fibrinolytic inhibitory potential, who might benefit the most from antifibrinolytic therapy prior to cardiac surgery.

- We recommend for patients with preoperative PAI-1 levels < 25 ng/mL administration of tranexamic acid 20-25 mg/kg followed by 1mg/kg/h infusion instead of 10 mg/kg followed by 1 mg/kg/h infusion during surgery.
- We propose for bleeding patient with postoperative t-PA/PAI-1 complex plasma concentration < 5 ng/mL to consider additional administration of tranexamic acid in a dose 10-20 mg/kg in ICU after cardiac surgery.

3.Current knowledge of the effect of gene variability to fibrinolytic activity and bleeding is still limited. Gene analysis could be an additional criterion for diagnosing increased bleeding risk in most complicated clinical cases.

4.Taking into account the results of TEG performed after cardiac surgery with CPB, it is possible to evaluate the compromised coagulation state more precisely with TEG as with standard coagulation tests.

- We recommend to perform TEG for bleeding patients after cardiac surgery to evaluate influence of hemodilution to coagulation state.

- We propose to perform TEG for bleeding patient to provide more precise initial hemostatic therapy guided by TEG parameters: R > 11 min, 2-4 units of FFP or 10-15 ml/kg, alpha angle < 450 , CRIO 1-2 bags/10 kg, maximum amplitude < 54 mm, Desmopressin 3 μg/kg following by platelet transfusion if necessary.

7. LIST OF PUBLICATIONS

1. Ozolina A., Strike E., Jaunalksne I., Krumina A., Bjertnaes L.J., Vanags I. PAI-1 AND t-PA/PAI-1 COMPLEX POTENTIAL MARKERS OF FIBRINOLYTIC POSTOPERATIVE BLEEDING AFTER CARDIAC SURGERY EMPLOYING CARDIOPULMONARY BYPASS.

BMC Anesthesiology, 2012; 12:27. Pub Med (PMID: 23110524)

2. Ozolina A., Strike E., Jaunalksne I, Serova J., Romanova T., Nikitina-Zake L., Sabelnikovs O., Vanags I. INFLUENCE OF PAI-1 GENE PROMOTER-675 (4G/5G) POLYMORPHISM TO FIBRINOLYTIC ACTIVITY AFTER CARDIAC SURGERY EMPLOYING CARDIOPULMONARY BYPASS.

Medicina (Kaunas) 2012 48; (10): 515-520. Pub Med (PMID: 23324247)

3. Ozolina A., Strike E., Sondore A., Vanags I. COAGULATION TESTS AND THEIR ASSOCIATION WITH BLOOD LOSS AFTER CARDIAC SURGERY WITH CARDIOPULMONARY BYPASS.

Acta medica Lituanica, 2012; (19/3):166-171. (EBSCO 84526136)

4. Ozolina A., Strike E., Bekkers M., Arklina B., Lacis R., Sondore A.,Vanags I. Тромбэластография и стандартная коагулограмма при гемодилюции и послеоперационном кровотечении у пациентов после операций на сердце с применением искусственного кровообращения.

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5. Ozolina A., Strike E., Vanags I. PLASMA FIBRINOGEN LEVEL AND POSTOPERATIVE BLEEDING AFTER ON-PUMP CARDIAC SURGERY.

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6. Ozolina A., Strike E., Vanags I. FIBRINOLYTIC ACTIVITY AND GENETIC POLYMORPHISMS IN RELATION TO THE RISK FOR POSTOPERATIVE BLEEDING AFTER ON-PUMP CARDIAC SURGERY. *Rīga Stradiņš University, Research articles in medicine&pharmacy, Collection of Scientific Papers*, 2010; 78 - 84

7. Ozolina A., Strike E., Harlamovs V., Porite N. EXCESSIVE BLEEDING AFTER CARDIAC SURGERY: REASONS AND MANAGEMENT. *Acta Chirurgica Latviensis*, 2009; (9): 86-91

8. REPORTS ON THE STUDY THEME

Oral presentations

1. Ozolina A.,Strike E., Jaunalksne I., Nikitina Zake L., Harlamovs V.,Vanags I. GENETIC FACTORS CONTRIBUTE TO ENHANCED FIBRINOLYTIC ACTIVITY IN PATIENTS UNDERGOING CARDIAC SURGERY. The European Anaesthesiology Congress, Barcelona, Spain, 1-4 June, 2013. Participation in the Best Abstract Price Competition. Obtained an award from European Society of Anaesthesiology.

2. Ozolina A., Strike E., Daukste M., Lace A., Vanags I. INFLUENCE OF HEMODILUTION TO COAGULATION STATE IN PATIENTS AFTER ON-PUMP CARDIAC SURGERY. Rīga Stradiņš University Scientific Conference. Riga, Latvia, 21th-22th March, 2013; 121

3. Ozolina A., Strike E., Sondore A., Vanags I. DOES TEG REFLECT HYPOCOAGULATION AFTER ON-PUMP CARDIAC SURGERY? 6th International Baltic Congress of Anesthesiology and intensive care, Vilnius, Lithuania, 18-20 October, 2012, Acta medica Lituanica, 2012; (19/3): 299

4. Ozolina A., Strike E., Jaunalksne I., Vanags I. PAI-1 AND t-PA/PAI-1 COMPLEX ASSOCIATION WITH POSTOPERATIVE BLEEDING IN

CARDIOPULMONARY BYPASS PATIENTS. 26th Annual Meeting of the European Association of Cardiothoracic Anesthesiologists, Austria, Vienna, 1.-4.June, 2011; S16-S17

5. Ozolina A., Strike E., Vanags I. FIBRINOLYTIC ACTIVITY AND GENETIC POLYMORPHISMS IN RELATION TO THE RISK FOR POSTOPERATIVE BLEEDING AFTER ON-PUMP CARDIAC SURGERY. Rīga Stradiņš University Scientific Conference. Riga, Latvia, 18th-19th March, 2010; 94

6. Ozolina A., Strike E., Vanags I. FIBRINOLYTIC ACTIVITY AND GENETIC POLYMORPHISMS IN RELATION TO THE RISK FOR POSTOPERATIVE BLEEDING AFTER ON-PUMP CARDIAC SURGERY. 5th International Baltic Congress of Anesthesiology and Intensive care, Estonia, Tartu, 21.-23.October, 2010; 92-93

Poster presentations

1. Ozolina A., Strike E., Freiberga I., Daukste M., Sondore A., Vanags I. INFLUENCE OF HEMODILUTION TO COAGULATION STATE DETECTED BY TEG IN PATIENTS AFTER ON-PUMP CARDIAC SURGERY. The European Anaesthesiology Congress, Barcelona, Spain, 1-4 June, 2013.

2. Ozolina A., Strike E., Jaunalksne I., Vanags I. PAI-1 AND t-PA/PAI-1 COMPLEX POTENTIAL FIBRINOLYTIC MARKERS FOR POSTOPERATIVE BLEEDING IN CARDIOPULMONARY BYPASS PATIENTS. 15th WFSA World Congress of Anesthesiologists, Buenos Aires, Argentina, March 25-30, 2012; ii217-ii218

3. Ozolina A.,Strike E., Nikitina-Zake L., Jaunalksne I., Serova J., Romanova T., Vanags I. INFLUENCE OF PAI-1 PROMOTER POLYMORPHISM TO FIBRINOLYTIC ACTIVITY OF PATIENTS AFTER ON-PUMP CARDIAC SURGERY. 15th WFSA World Congress of Anesthesiologists, Buenos Aires, Argentina, March 25-30, 2012; ii218

4. Ozolina A.,Strike E., Nikitina-Zake L., Jaunalksne I., Serova J., Romanova T., Vanags I. INFLUENCE OF PAI-1 PROMOTER POLYMORPHISM TO FIBRINOLYTIC ACTIVITY OF PATIENTS AFTER ON-PUMP CARDIAC SURGERY. Rīga Stradiņš University Scientific Conference. Riga, Latvia, 29th -30th March, 2012; 153

5. Ozolina A. ,Strike E., Nikitina-Zake L., Jaunalksne I., Serova J., Romanova T., Vanags I. PAI-1 POLYMORPHISM INFLUENCES FIBRINOLYSIS AFTER ON-PUMP CARDIAC SURGERY. 6th International Baltic Congress of Anesthesiology and Intensive care, Vilnius, Lithuania, 18-20 October, 2012, Acta medica Lituanica, 2012; (19/3): 343

6. Ozolina A. Strike E., Vanags I. PLASMA FIBRINOGEN LEVEL AND POSTOPERATIVE BLEEDING AFTER ON-PUMP CARDIAC SURGERY. Rīga Stradiņš University Scientific Conference. Riga, Latvia,14th-15th April, 2011; 123

7. Ozoliņa A., Strike E., Vanags I. PLASMA FIBRINOGEN LEVEL AND POSTOPERATIVE BLEEDING AFTER ON-PUMP CARDIAC SURGERY. 26th Annual Meeting of the European Association of Cardiothoracic Anesthesiologists, Austria, Vienna, 1.-4.June, 2011; S45-S46

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