The role of fibrinogen in cardiac surgery

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The role of fibrinogen in cardiac surgery

De rol van fibrinogeen in de hartchirurgie

(met een samenvatting in het Nederlands)

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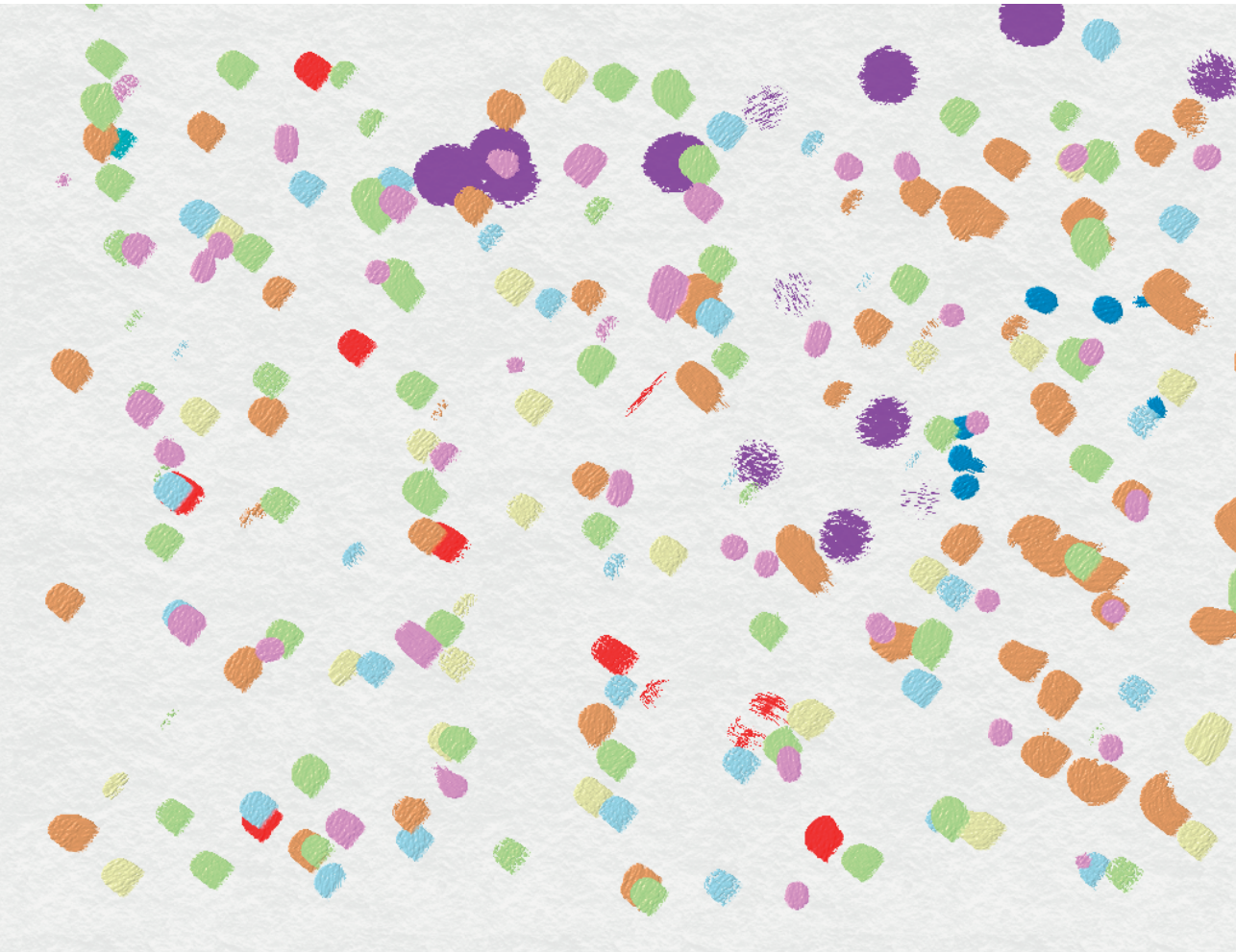
Chapter 1

General introduction



Excessive bleeding is one of the most common complications in cardiac surgery and is caused most frequently by insufficient surgical hemostasis or impairments of the coagulation system or a combination of both. Although a majority of patients undergoing elective cardiac surgery will not receive transfusion, a substantial number of patients consume disproportionately high amounts of blood products.[1] Transfusion of blood products is associated with negative outcomes such as early and late mortality, increased risk for infections, prolonged hospital stay and decrease of long term quality of life.[2-5] Each unit of red cells transfused is associated with incrementally increased risk for an adverse outcome.[6-8] Considering these findings, a more judicious use of the blood products is required.

There are several methods for reducing transfusion in cardiac surgery. One important method is the pharmacological intervention with tranexamic acid, desmopressin, prothrombin complex concentrate, recombinant factor VIIa and fibrinogen concentrate. At the start of this project, there was already an increase in the use of the procoagulant fibrinogen concentrate in patients with severe coagulopathic bleeding in various fields of surgery.[9-13] This interest was supported by the publication of several in-vitro and in-vivo studies which have shown that fibrinogen concentrate reversed dilution coagulopathy caused by colloid fluid infusions.[14-18] However, these studies were not performed in the domain of cardiac surgery.

Plasma fibrinogen (coagulation factor I) plays an important role in hemostasis by acting as an endogenous substrate for fibrin formation, promoting clot formation and platelet aggregation by binding platelet glycoprotein IIb/IIIa receptors.[19,20]

Commercially available fibrinogen concentrate is produced from pooled human plasma using a specific cryoprecipitation procedure (Cohn/Oncley method) and is labelled for reversing coagulopathic bleeding found in congenital hypo-, dys- and afibrinogenemia and in acquired hypofibrinogenemia.

Acquired hypofibrinogenemia as a result of dilution and/or consumption is the most common cause of low fibrinogen levels during surgery inducing coagulation disorders which eventually may lead to severe bleedings.[21] Suppletion of low plasma fibrinogen levels with the procoagulant medication fibrinogen concentrate is an interesting therapeutic option. Few studies with small number of patients addressed the use of fibrinogen concentrate in cardiac surgery both reporting a

reduction in postoperative blood loss.[22-24] Consequently, questions regarding the effects of fibrinogen concentrate on blood loss and transfusion in the domain of cardiac surgery remained unanswered.

The procoagulant fibrinogen concentrate was used for the first time in the Isala Zwolle in December 2006 in cardiac surgery procedures. This procoagulant was infused in patient who had excessive coagulopathic bleeding despite treatment with blood products and conventional antifibrinolytic and procoagulant medication to improve hemostasis. At the start of this thesis project, there was no solid evidence for the use of fibrinogen concentrate to reduce blood loss and transfusion in cardiac surgery. The effects of fibrinogen concentrate on clinical adverse events was also unknown.

The lack of evidence and the questions that were raised on the effects of fibrinogen in cardiac surgery, together with the aspiration to optimize hemostasis in this domain, have led to the formulation of this thesis project.

MAIN QUESTION OF THIS THESIS

What is the role of fibrinogen on blood loss and transfusion in cardiac surgery?

OBJECTIVES OF THIS THESIS

This thesis has the following objectives:

1. To explore, in an initial analysis, the effects of fibrinogen concentrate on blood loss, transfusion and clinical adverse events in a large cohort study with high-risk cardiac surgery patients.
2. To evaluate the effects of introducing a cardiac surgery-specific transfusion protocol on transfusion of blood products and clinical adverse events.
3. To design a prediction model that can predict excessive blood loss during and after surgery.

4. To determine which levels of plasma fibrinogen measured at the end cardiopulmonary bypass increase and which levels reduce blood loss and transfusion in cardiac surgery.
5. To design and conduct a placebo-controlled, double-blind and randomized clinical trial to determine the effects of targeted fibrinogen concentrate infusion on blood loss, transfusion and the occurrence of clinical adverse events.

OVERVIEW OF THIS THESIS

In *chapter 2* the effect of fibrinogen concentrate in patients who underwent complex cardiac surgery from 2007-2010 was investigated. A large cohort study was used to quantify whether the infusion of fibrinogen concentrate during complex cardiac surgery reduced postoperative blood loss and transfusion. Whether the use of fibrinogen concentrate was associated with the occurrence of clinical events was also investigated.

In *chapter 3* the effects of a transfusion protocol specifically designed for cardiac surgery procedures was investigated. To reduce unnecessary transfusion of blood products, a cardiac surgery-specific transfusion protocol was introduced in Isala Zwolle in January 1, 2009. The purpose of this tailor-made transfusion protocol was to systematically direct the use of blood products and pro-hemostatic medication to prevent unnecessary transfusions and reduce the number of transfused patients. The index group was transfused according to a tailor-made transfusion protocol (operation in 2009/2010) and the control group was transfused according the CBO Dutch National Transfusion Guideline (operation in 2007/2008).

In *chapter 4* a prediction model with EuroSCORE variables was constructed to predict excessive bleeding at two different time points: during cardiac surgery (intraoperative period) and during ICU stay (early postoperative period). In addition, it was quantified whether preoperative laboratory variables had added predictive ability for intraoperative blood loss, and if adding intraoperative clinical and laboratory variables (including plasma end-CPB fibrinogen concentration) improved prediction of blood loss in the early postoperative period. In an additional analysis,

the numerical EuroSCORE (low, medium and high risk) was related to excessive intraoperative and (early) postoperative blood loss.

In *chapter 5* the association between the end of cardiopulmonary bypass (end-CPB) plasma fibrinogen levels and post-operative blood loss (≥ 1000 mL) and post-operative transfusion of allogeneic blood products (≥ 1 unit) in cardiac surgery procedures was investigated. In cardiac surgery, the plasma fibrinogen concentration measured specifically at the end of cardiopulmonary bypass is of special interest, as this is a suitable moment for targeted infusion of fibrinogen concentrate or other procoagulant medication as part of the perioperative hemostasis management after separation from CPB. The association between end-CPB plasma fibrinogen levels and the occurrence of clinical adverse events was also explored.

Chapter 6 presents the results of the placebo-controlled, double-blind and randomized clinical trial designed to investigate the effect of fibrinogen concentrate infusion targeted to post-infusion plasma levels of 2.5 g/L in high-risk cardiac surgery patients with diagnosed coagulopathic bleeding that was determined by the "5-minute bleeding volume test" by suctioning of blood from the thoracic cavity (pleura and pericardium) for a period of 5 minutes. The primary endpoint was blood loss between intervention with study medication (i.e. infusion of fibrinogen concentrate or placebo after removal of cardiopulmonary bypass) and closure of the chest. Secondary endpoints were blood loss measured upon admission in the ICU as chest tube drainage volume collected in the first 24 hours and transfusion requirements in the first 24 hours. Data on the occurrence of adverse events within 30 days of follow-up was also collected.

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Chapter 2

Fibrinogen concentrate therapy in complex cardiac surgery

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ABSTRACT

Objectives: Fibrinogen concentrate is increasingly used to treat coagulopathic bleeding in cardiac surgery, although its effectiveness and safety have not been demonstrated. We conducted a cohort study to quantify the effects of fibrinogen concentrate on postoperative blood loss and transfusion and the occurrence of clinical adverse events in complex cardiac surgery patients.

Design: Cohort analysis using prospectively collected data.

Setting: A teaching hospital.

Participants: 1075 patients who underwent complex cardiac surgery in the years 2007 to 2010.

Intervention: Non-randomized intervention with fibrinogen concentrate during complex cardiac surgery.

Measurements and Main Results: Of the 1075 patients, 264 (25%) received fibrinogen concentrate during surgery (median dose 2g.). In the adjusted analysis, the effect of fibrinogen concentrate on blood loss and transfusion in the ICU showed a ratio of geometric means of 1.02 (0.91-1.14) and an odds ratio of 1.14 (0.83-1.56), respectively. For the risk of 30-day mortality, myocardial infarction, CVA/TIA, renal insufficiency/failure, total infections and prolonged mechanical ventilation the adjusted odds ratios were 0.96 (0.48-1.92), 1.10 (0.53-2.27), 1.16 (0.50-2.72), 0.62 (0.29-1.32), 1.18 (0.72-1.95) and 1.44 (0.83-2.49), respectively.

Conclusions: Fibrinogen concentrate infusion during surgery did not reduce postoperative blood loss and transfusion and no increased risk for clinical adverse events was measured. The lower doses and the relatively late intervention with fibrinogen concentrate might have attenuated the hemostatic effect of fibrinogen concentrate. This study reports the initial clinical use of fibrinogen concentrate in

complex cardiac surgery. A randomized clinical trial has been initiated to investigate the hemostatic role of fibrinogen concentrate in cardiac surgery.

Keywords fibrinogen concentrate, cardiac surgery, coagulopathy, transfusion

Cardiac surgery is often complicated by excessive peri- and postoperative bleeding which is commonly treated with transfusion of allogenic blood products. However, this is known to be associated with adverse events, significant cost, early and late mortality.[1-9] Recently, there has been increasing interest in the use of fibrinogen concentrate in patients with severe coagulopathic bleeding in various types of surgery.[10-16] Only a few small studies addressed the use of fibrinogen concentrate in cardiac surgery reporting a reduction in postoperative blood loss and transfusion requirements.[17-19] Consequently, it is yet unknown whether fibrinogen concentrate infusion reduces blood loss in cardiac surgery and to what extent it is able to reduce transfusion of blood products. Furthermore, it is unknown whether infusion of fibrinogen concentrate results in unintended clinical adverse events.

In this paper we evaluated the effect of fibrinogen concentrate in patients who underwent complex cardiac surgery in the years 2007, 2008, 2009 and 2010. Using a cohort analysis, we quantified whether the use of fibrinogen concentrate during complex cardiac surgery reduced postoperative blood loss and transfusion. We also investigated whether the use of fibrinogen concentrate was associated with the occurrence of clinical adverse events.

METHODS

This study was designed as a single center (Isala Zwolle, The Netherlands) cohort analysis using systematically and prospectively collected data. At the Isala Zwolle, fibrinogen concentrate (Haemocomplettan® P, CSL Behring, Marburg, Germany) became available at the end of year 2006. The analyses presented in this paper were based on all patients who underwent complex cardiac surgery between January 1st, 2007 and December 31st, 2010. Complex cardiac surgery was defined as coronary artery bypass grafting (CABG) with valve(s) procedures or aortic surgery (root, ascending, arch or descending aorta). Patients who received fibrinogen concentrate in the ICU and patients who had re-sternotomy after initial surgery were excluded from the analysis in order to exclude a surgical source of postoperative

bleeding. This study has been approved by the institutional medical ethical committee (reference 11.0668, June 16, 2011).

Anesthesia and surgical treatment was performed according to the standard procedures of the Isala Zwolle and have been described previously.[20] All patients in this cohort received initial hemostasis management involving the use of conventional procoagulant and antifibrinolytic therapy. Tranexamic acid was given to all patients at the initiation of cardiopulmonary bypass (CPB) with a dose of 2 grams and after CPB with a dose of 1 gram. If indicated, desmopressin was infused with a dose of 0.3µg/kg towards the end of CPB. During surgery, the transfusion of red blood cell concentrate (RBC), fresh frozen plasma (FFP) and platelet concentrate was according to the following transfusion protocol: When hematocrit during CPB was below 0.23, 1 unit of RBC was transfused. One unit of platelet concentrate was administered when thrombocyte count was below $100x10^9$/L. When plasma loss measured with the cell saver and the blood loss was above 1L or 2L, 2 units or 4 units of FFP were administered respectively. In case the initial hemostatic management was not effective and a surgical source of bleeding was excluded, fibrinogen concentrate infusion was considered during surgery. Recombinant factor VIIa was considered if bleeding persisted after intervention with fibrinogen concentrate. Cryoprecipitate was not used during the whole study period.

The primary outcome measure was postoperative blood loss in the ICU, measured as chest drain production (expressed in ml) from admission to the ICU until removal of the chest drains. The secondary outcome measure was transfusion of RBC, FFP and platelets in the ICU, analyzed as dichotomous variables. We also investigated the occurrence of major clinical events. Thirty-day mortality was defined as death from any cause within 30 days after the surgical procedure. Myocardial infarction was defined as myocardial specific creatine kinase (CKMB) value ≥180 U/L (7.5 times upper reference limit) plus a peak CKMB/creatine kinase ratio >10%, or pathological new Q waves on a postoperative electrocardiogram (ECG).[21] CVA was defined as a new motor or sensory deficit with its origin in the central nervous system, or an unexplained coma status lasting longer than 24 hours. TIA was defined as a brief episode of neurological dysfunction resulting from focal temporary cerebral ischemia lasting less than 24 hours and not associated with ce-

rebral infarction. Renal insufficiency was considered to be renal injury and/or acute renal failure, defined according to the Second International Consensus Conference of the Acute Dialysis Quality Initiative Group (ADQI).[22] Total infection is defined as any infection (skin infection, mediastinitis, sternal infection, pulmonary infection or any other infection) occurring in the postoperative period during hospital stay. Prolonged ventilation was defined as mechanical ventilation in the ICU for more than 24 hours.

For statistical analysis, we estimated the crude effect of fibrinogen concentrate intervention on the primary and secondary outcome variables. As the primary outcome variable was non-normally distributed, we performed a logarithmic transformation and performed a linear regression analysis using $\log_{10}$ (Blood loss ICU) as the dependent variable and fibrinogen intervention as the single independent variable. The resulting regression coefficients b were back-transformed as 10^b, resulting in an estimate of the ratio of geometric means (RGM), with its associated 95% confidence interval. The RGM can be interpreted as follows: a RGM of 1 (reference value) would indicate that the blood loss in the patients receiving fibrinogen equals the amount of blood loss in the non-fibrinogen concentrate group. Values below 1 indicate that patients with fibrinogen concentrate have less blood loss at the ICU (hence: the intervention is effective) and values above 1 indicate that patients who receive fibrinogen concentrate have more blood loss at the ICU. The dichotomous outcome variables were analyzed using logistic regression analysis; results are presented as odds ratios (OR) with the corresponding confidence intervals (CI). In parallel with the RGM, values below 1 indicate that the intervention with fibrinogen concentrate is effective. Values above 1 indicate that the outcome occurs more frequently in patients who did receive fibrinogen concentrate.

Due to the non-randomized design of this study, the association between infusion of fibrinogen concentrate and each of the outcomes was likely biased by potential confounders. Variables considered as potential confounders were baseline patient characteristics (age, gender, body surface area (BSA), Euroscore), procedure related characteristics (type of surgery [elective, urgent or emergent], CPB time and minimal core temperature) and characteristics related to the course of the procedure (total blood loss during surgery, transfusion of FFP and platelets

during surgery, use of desmopressin and recombinant FVIIa during surgery, and whether the patients were operated before or after a new transfusion protocol was put into place in the department). These potential confounders were included in the analysis using propensity score analysis, which was conducted as follows: first, three propensity scores for being treated with fibrinogen concentrate were calculated based on the patient characteristics, procedure related characteristics and characteristics related to the actual course of the procedure before fibrinogen concentrate was considered, respectively. To calculate these scores three regression models were fit using the abovementioned variables. Secondly, the probabilities resulting from these models were included in a multivariable regression analysis, with fibrinogen concentrate as the primary independent variable of interest and the three propensity scores as additional covariables. This second step was performed for each of the outcome measures.

Although very limited, there were missing data for one or more confounders with rates ranging from 0% to 3.3%. Missing values were singly imputed using a regression model approach with addition of a random error component before conducting the analyses. A p-value <0.05 was considered statistically significant. Analyses were performed using the statistical software SPSS 16.0 (SPSS Inc., Chicago, Illinois, USA).

RESULTS

Over a period of 4 years (2007 to 2010), 1173 patients underwent complex cardiac surgery. Of these patients, 95 (8.1%) had rethoracotomy after initial surgery. Three other patients were excluded because of use of recombinant factor VIIa without initial fibrinogen concentrate use. Eventually, 1075 patients met the inclusion criteria, of which 264 (25%) received fibrinogen concentrate during surgery (fibrinogen group) and 811 (75%) patients did not receive fibrinogen concentrate (no fibrinogen group). Patient characteristics were comparable between the two groups except for the Euroscore which was higher in the fibrinogen group (median value 8 vs. 7, $p<0.01$, Table 1). Regarding procedural characteristics, the fibrinogen

group compared to the control group had more frequently urgent or emergency surgery (8% and 10% vs. 3% and 2% respectively, p<0.01), had a longer cardio-pulmonary bypass time (221 min vs. 171 min, p<0.01) and aortic occlusion time (133 min vs. 116 min, p<0.01), had lower core temperature (28 vs. 30 °C, p<0.01), had more blood loss during surgery (2608ml vs. 1383ml, p<0.01) and had a higher percentage of patients needing transfusion of blood products during surgery (78% vs. 47%, p<0.01). More patients in the fibrinogen group received desmopressin (67% vs. 45%, p<0.01).

Table 1. Patient and procedure characteristics.

Patient and procedure characteristics	Fibrinogen, N=264 (25%)	No Fibrinogen, N=811 (75%)	p-value	Missing
Age (y), median (IQR 25th; 75th)	71 (64;77)	72 (65;78)	0.17	0
Gender, male (%)	164 (62%)	525 (65%)	0.44	0
BSA, mean (SD)	1.95 (0.22)	1.96 (0.20)	0.34	0
Euroscore, median (IQR 25th; 75th)	8 (6;11)	7 (6;9)	<0.01	0
Pre-operative use of anticoagulation medication				
ASA	99 (38%)	256 (32%)	0.08	0
Clopidogrel	11 (4%)	52 (6%)	0.18	0
LMWH	38 (14%)	101 (13%)	0.41	0
Warfarin	26 (10%)	101 (13%)	0.26	0
LVEF				
<30%	33 (13%)	104 (13%)	0.79	0
30-50%	86 (33%)	281 (35%)		0
>50%	145 (54%)	426 (52%)		0
Indication				
Elective	216 (82%)	770 (95%)	<0.01	0
Urgent	20 (8%)	24 (3%)		0
Emergency	28 (10%)	17 (2%)		0
CABG & valve(s) (%)	148 (56%)	698 (86%)	<0.01	0
Aortic surgery (%)	116 (44%)	113 (14%)		0
CPB time in min, median (IQR 25th; 75th)	221 (164;310)	171 (143;213)	<0.01	0
Aortic occlusion time in min, median (IQR 25th; 75th)	133 (105;170)	116 (96;139)	<0.01	0
Total blood loss surgery ml, median (IQR 25th; 75th)	2608 (1554;4867)	1383 (967;1927)	<0.01	2 (0.2%)
Minimal core temperature °C, median (IQR 25th; 75th)	28 (28;30)	30 (28;32)	<0.01	1 (0.1%)
Cell Saver use	261 (99%)	770 (95%)	<0.01	0

Table 1. Patient and procedure characteristics. (continued)

Patient and procedure characteristics	Fibrinogen, N=264 (25%)	No Fibrinogen, N=811 (75%)	p-value	Missing
Number of patients transfused during surgery with:				
RBC	183 (69%)	334 (41%)	<0.01	0
FFP	124 (47%)	124 (15%)	<0.01	0
Platelets	130 (49%)	86 (11%)	<0.01	0
Any blood product	206 (78%)	379 (47%)	<0.01	0
Fibrinogen concentrate in gram, median (IQR 25^{th}; 75^{th})	2 (2;3)	0		0
Number of patients infused with:				
Desmopressin	178 (67%)	363 (45%)	<0.01	0
Recombinant factor VIIa	23 (9%)	0		0

P<0.05 is considered a significant difference between groups. IQR: inter quartile range; SD: standard deviation; BSA: Body surface area; ASA: acetylsalicylic acid;
LMWH: low molecular weight heparin; LVEF: left ventricle ejection fraction; CPB: cardiopulmonary bypass. RBC: red blood cells; FFP: fresh frozen plasma.

There was more postoperative ICU blood loss in the fibrinogen group compared to the control group (670 ml vs. 560 ml, p<0.01, Table 2). The estimated ratio of geometric means for blood loss in the ICU was 1.13 (1.02 - 1.25), indicating that the fibrinogen group had 13% more blood loss in the ICU compared to the control group (Table 3). After adjustment for potential confounders, the fibrinogen group seemed to have 2% more postoperative blood loss in the ICU compared to the control group, with an estimated ratio of geometric means of 1.02 (0.91 - 1.14), although this was not significant. Regarding the need for transfusion in the ICU, more patients in the fibrinogen group received blood products in the ICU compared to the control group (55% vs. 43%, p<0.01) with an odds ratio (OR) of 1.57 (1.19-2.08) which reduced to 1.14 (0.83-1.56) in the adjusted analyses (Table 3). This means that in the unadjusted analysis, 57% more patients in the fibrinogen group (as compared to the no fibrinogen group) received transfusion of any blood product in the ICU. In the adjusted analysis this figure decreased to 14% more patients in the fibrinogen group transfused with any blood product in the ICU, this was not statistically significant.

Table 2. Outcome variables.

	Fibrinogen, N=264	No Fibrinogen, N=811	p-value
Postoperative blood loss and need for transfusion (ICU)			
Blood loss ICU ml, median, (IQR 25^{th};75^{th})	670 (420;1080)	560 (400;850)	<0.01
Number of patients transfused during ICU stay with:			
RBC	124 (47%)	313 (39%)	0.02
FFP	62 (24%)	98 (12%)	<0.01
Platelets	50 (19%)	81 (10%)	<0.01
Any blood product	144 (55%)	351 (43%)	<0.01
Adverse clinical events			
30-day mortality	18 (7%)	33 (4%)	0.07
Myocardial infarction	14 (5%)	30 (4%)	0.25
CVA/TIA	11 (4%)	20 (3%)	0.15
Renal insufficiency/ failure	13 (5%)	38 (5%)	0.87
Total infections	29 (11%)	74 (9%)	0.37
Prolonged mechanical ventilation	52 (20%)	45 (6%)	<0.01

P<0.05 is considered a significant difference between groups. ICU: intensive care unit; IQR: inter quartile range; RBC: red blood cells;
FFP: fresh frozen plasma. CVA: cerebrovascular accident; TIA: transient ischemic attack.

Prolonged mechanical ventilation (more than 24 hours) was higher in the fibrinogen group (20% vs. 6%, p<0.01) with an OR of 4.18 (2.72-6.40) which reduced to 1.44 (0.83-2.49) in the adjusted analysis. The occurrence of 30-day mortality, myocardial infarction, CVA/TIA, renal insufficiency or failure and infections was not different between the fibrinogen group and the control group in both the unadjusted and adjusted analysis.

Table 3. Unadjusted and adjusted analysis for the outcome measures.

	unadjusted		adjusted	
In the ICU	Model 1	Model 2	Model 3	Model 4
Blood loss ICU, RGM (CI)	1.13 (1.02-1.25)	1.10 (1.00-1.22)	1.01 (0.91-1.12)	1.02 (0.91-1.14)
Need for transfusion ICU, OR (CI)	1.57 (1.19-2.08)	1.37 (1.03-1.83)	1.23 (0.91-1.66)	1.14 (0.83-1.56)
Adverse clinical events, OR (CI)				
30-day mortality	1.73 (0.95-3.12)	1.25 (0.68-2.32)	0.81 (0.40-1.60)	0.96 (0.48-1.92)
Myocardial infarction	1.46 (0.76-2.79)	1.14 (0.58-2.22)	1.02 (0.50-2.09)	1.10 (0.53-2.27)
CVA/TIA	1.72 (0.81-3.64)	1.39 (0.65-3.00)	1.13 (0.49-2.58)	1.16 (0.50-2.72)
Renal insufficiency/ failure	1.05 (0.55-2.01)	0.88 (0.45-1.71)	0.60 (0.29-1.26)	0.62 (0.29-1.32)
Total infections	1.23 (0.78-1.94)	1.15 (0.73-1.84)	1.16 (0.71-1.89)	1.18 (0.72-1.95)
Prolonged mechanical ventilation	4.18 (2.72-6.40)	3.13 (1.99-4.93)	1.62 (0.96-2.72)	1.44 (0.83-2.49)

OR (Odds ratio), CI (confidence interval), RGM (ratio of geometric mean ($10^{(\text{ß-coefficient of regression line} \pm \text{CI})}$).
Model 1 = Unadjusted analysis.
Model 2 = Model 1 + Propensity score of patient characteristics: Age, gender, body surface area (BSA), Euroscore.
Model 3 = Model 2 + Propensity score of procedure characteristics: Indication for surgery, cardiac bypass time and minimal core temperature.
Model 4 = Model 3 + Propensity score of: Total blood loss surgery, transfusion of FFP, transfusion of platelets, infusion of desmopressin and rFVIIa, new transfusion protocol.

DISCUSSION

Fibrinogen concentrate is increasingly used in cardiac surgery procedures complicated by excessive bleeding. In our study, fibrinogen concentrate did not have a statistically significant effect on blood loss and transfusion of blood products in the ICU and no increased risk in clinical adverse events was found after adjusting for potential confounders.

It is possible that fibrinogen concentrate is truly not effective in patients undergoing complex cardiac surgery. However, our findings can probably also be (partly)

explained by some issues that we will now briefly discuss. First, due to the non-randomized nature of our study, the infusion of fibrinogen concentrate probably was inconsistent and created the potential problem of selective administration of fibrinogen. This is reflected by the differences in the baseline patient and procedure characteristics between the fibrinogen group and the no fibrinogen group. Patients who had a longer cardiopulmonary bypass time or who had more blood loss and transfusion during surgery were more likely to receive fibrinogen concentrate. To reduce or minimize this effect of confounding by indication, we used the propensity score method for the multivariate regression analysis. To verify the use of the propensity scores for adjustment, we investigated the distribution of the propensity scores, by stratifying the propensity score across quintiles. Good overlap was seen within each propensity model concerning patient and procedure characteristics and good overlap in the final model in which all variables and potential confounders were included. As a sensitivity analysis, we have repeated our analyses using other methods for covariable adjustment for the primary outcome of blood loss in the ICU along with variations in the propensity score methodology. These methods all yielded comparable results, confirming the stability of our original findings.

Second, the dose of fibrinogen concentrate seems to be important in reversing coagulopathic bleeding. Fibrinogen concentrate has a dose-dependent effect on platelet aggregation and overall clot strength independent of platelet count.[23] In the study of Rahe-Meyer et al., a median dose of 7.8 g fibrinogen concentrate was infused in a study population which is comparable to our cohort.[18] The median dose of fibrinogen concentrate infusion in our study was relatively low with 60% of the study population in the fibrinogen group receiving 1 or 2 gram of fibrinogen concentrate. These relatively low doses may have attenuated the effect of fibrinogen concentrate on postoperative blood loss.

Third, next to dosing, the timing of infusion might be crucial in coagulation management. In the study of Karlsson et al., fibrinogen was infused on a prophylactic basis in cardiac surgery patients, suggesting that early intervention during coagulation management might possibly result in an improved hemostatic condition.[17] Another example of early treatment is a study by Solomon et al. in 39 cardiac surgery patients. These patients received a mean dose of fibrinogen concentrate

of 6.5g. as a first line treatment in coagulopathic bleeding, which resulted in an increase of plasma fibrinogen levels and point-of-care thromboelastometric variables mirroring fibrinogen polymerization.[24]

In our study, fibrinogen concentrate was infused when a surgical source of bleeding was excluded, when (after removal of cardiopulmonary bypass) the ACT was less than 140 seconds, when thrombocyte count and hematocrit was corrected and when tranexamic acid and desmopressin therapy deemed unsuccessful in the treatment of coagulopathic bleeding. This strategy has led to a relatively late intervention with fibrinogen concentrate during coagulation management. Late intervention leads to ongoing, untreated coagulopathic bleeding with wasting of coagulation factors consequently leading to worsening of hemostasis. These factors probably contributed to a reduced hemostatic effect of fibrinogen concentrate therapy. For the occurrence of clinical adverse events in the adjusted analysis, no increase in 30-day mortality, myocardial infarction, CVA/TIA, renal insufficiency or failure, combined total infections or prolonged ventilation was measured in the fibrinogen group.

There are limitations to our study. The fibrinogen group had a higher risk for prolonged mechanical ventilation in the unadjusted analysis, which was explained by the longer cardiopulmonary bypass time, more blood loss and transfusion in this group, indicating a high risk group for a complicated outcome. Although the baseline characteristics between the fibrinogen group and control group are different, we used the propensity score method in order to adjust for these differences. Despite this statistical approach, confounding by unknown or immeasurable factors cannot be ruled out, especially in non-randomized studies as ours. Only a randomized study would counteract these pitfalls.

A recently published review by Levy et al. gives a complete overview of the current status of fibrinogen concentrate therapy in acquired bleeding states. The author mentions the growing number of studies concerning fibrinogen replacement in the treatment of massive bleeding across a broad range of clinical settings. Most of these studies are retrospective or performed in prospective trials with limited participant numbers. The author underlines the need for prospective, randomized controlled trial to determine the role of fibrinogen concentrate in various clinical

settings and help to further define optimal trigger concentrations and doses for fibrinogen supplementation.[25]

We conclude that fibrinogen concentrate infusion during complex cardiac surgery did not reduce postoperative blood loss and transfusion. Furthermore, no increase in clinical adverse events was measured. The lack of a clinical effect on blood loss and transfusion might be explained by the low doses and the relatively late intervention with fibrinogen concentrate during coagulation management. Although a randomized clinical trial on the effect of fibrinogen concentrate is lacking to support any recommendation, there is probably much to gain with a higher dose and an earlier fibrinogen concentrate infusion, preferably guided by laboratory plasma concentrations of fibrinogen or thromboelastometry. A randomized, double blinded and placebo controlled trial is needed to answer these very important questions concerning effectiveness, timing and dosing.

This study only reports the initial clinical use of fibrinogen concentrate in complex cardiac surgery and has methodological limitations due its non-randomized design. Therefore, with the experience gained in the past years and the results of this study, we designed a randomized clinical trial to determine the hemostatic role of fibrinogen concentrate in complex cardiac surgery, with a focus on adequate timing and dosing of therapy (ClinicalTrials.gov identifier NCT01124981).

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Chapter 3

Effectiveness of a cardiac surgery-specific transfusion protocol

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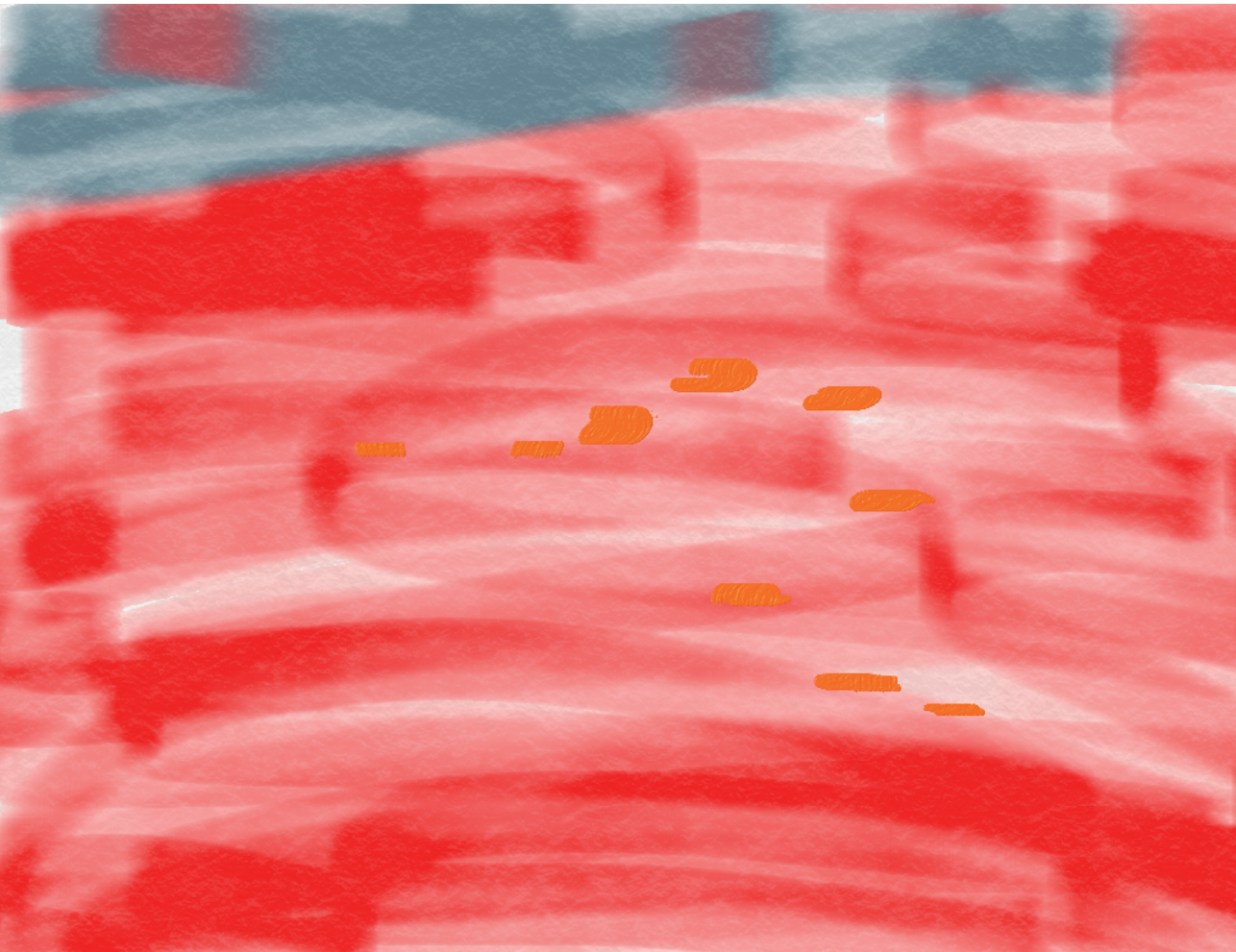


ABSTRACT

Background: Cardiac surgery is often complicated by excessive bleeding which is commonly treated with blood products. In the year 2009 a transfusion protocol was introduced specifically designed for cardiac surgery procedures. This study aims to evaluate the effect of this protocol on transfusion of blood products and the occurrence of clinical events.

Study design and methods: This was a non-randomized intervention study. The index group was transfused according to a tailor-made transfusion protocol (operation in 2009/2010) and the control group was transfused according the Dutch national transfusion guideline (operation in 2007/2008).
The primary outcome was mean units transfused and proportion of patients transfused. Secondary outcomes were; in-hospital mortality, myocardial infarction, cerebrovascular accident or transient ischemic attack, renal injury or failure, rethoracotomy and prolonged mechanical ventilation.

Results: The control group comprised 2685 patients and the index group 2534 patients. The tailor made transfusion protocol resulted in a decrease of patients transfused with RBC and FFP during surgery with odds ratio of 0.69 (95% confidence interval (CI); 0.55–0.86) and 0.63 (95% CI; 0.46–0.86) respectively. Less myocardial infarctions were observed in the index group with OR 0.67 (95% CI; 0.47–0.96).

Conclusion: The cardiac surgery-specific transfusion protocol resulted in less patients transfused with RBC and FFP and a lower incidence of myocardial infarction. This tailor made protocol has led to a more judicious use of blood products and is a basis for further refinement of coagulation management during cardiac surgery procedures.

Keywords Transfusion practices, blood management and hemostasis

Excessive bleeding is one of the most common complications in cardiac surgery. While the majority of patients undergoing elective cardiac surgery will not receive allogenic blood products, a substantial number of patients consume disproportionately high amounts of blood products.[1] Especially prolonged cardiopulmonary bypass times, re-operations, urgent surgeries and complex cardiac surgical procedures are associated with excessive blood loss and attendant transfusion of blood products.[2,3]

Not treating a severely anemic cardiac surgery patient increases the rate of adverse clinical events such as stroke, myocardial infarction and death.[4,5] While on the other hand, administration of blood products is also associated with negative outcomes such as early and late mortality, increased risk for infections, prolonged hospital stay and decrease of long term quality of life.[6-9] Each unit of red cells transfused is associated with incrementally increased risk for adverse outcome.[10-12] Several studies have compared a strict transfusion regimen to a more liberal transfusion regime showing that a strict regime can reduce consumption of blood products with similar or more favorable patient outcome.[13-15] Considering all these findings, a more judicious use of the blood products is required.

Accordingly, in order to reduce unnecessary transfusion of blood products in our hospital, a cardiac surgery-specific transfusion protocol was introduced in our hospital January 1st, 2009. The purpose of this tailor made transfusion protocol was to systematically direct the use of blood products and hemostatic medication to prevent excessive transfusion and reduce the number of transfused patients. The present non-randomized comparative study evaluated the effectiveness of this cardiac surgery-specific transfusion protocol in reducing the use of blood products and the occurrence of clinical events in cardiac surgery.

MATERIALS AND METHODS

Design and Patients

The study was designed as a non-randomized intervention study using prospectively collected data from a single institution; Isala Zwolle in The Netherlands. The study protocol was approved by the institutional medical ethics committee (Medical

Ethics Committee of Isala Academy, reference 11.0668, June 16, 2011). All patients who underwent cardiac surgery in the years 2007, 2008, 2009 and 2010 and who gave written informed consent were included in the study. For patients operated more than once within the study period, only the initial cardiac surgery procedure was analyzed.

The cardiac surgery-specific transfusion protocol was introduced on January 1st of 2009. The control group included all patients who underwent cardiac surgery in the years 2007 and 2008, in whom the transfusion protocol was not applied. The index group included the patients operated in 2009 and 2010, in whom the transfusion protocol was applied.

Anesthesia techniques

During the entire study period, anesthesia management and surgical treatment was performed according to the same standard procedure of the Isala Zwolle, as described previously.[16] In short, all patients underwent surgery through a median sternotomy. Myocardial protection during cardiopulmonary bypass (CPB) was achieved with antegrade blood or crystalloid cardioplegia. One surgeon used the combination of retrograde and antegrade crystalloid cardioplegia for aortic valve surgery. CPB was managed using nonpulsatile flow applied by a centrifugal pump and pH management with the α-stat principle.

From admission until hospital discharge, all clinical data of patients undergoing cardiac surgery were prospectively registered in an electronic patient data management system. In the operating room, the attending anaesthesiologists documented the patients' demographic data and intraoperative data on a dedicated form and also entered these variables in the electronic database. Clinical data during ICU stay were collected by the ICU medical staff. Clinical data after the ICU period were recorded by nurses from the research team. After the patient's discharge from the hospital, all data collection forms were verified with double check by another member of the research team to confirm accuracy of the data entry.

Control group

In the control group the national transfusion guideline introduced in the year 2004 was adhered to.[17] In this guideline, the '4-5-6 mmol/L' rule is used for trans-

fusion of red blood cells (RBC) in acute normovolemic anemia (Table 1). To ease interpretation, the unit g/dL was also added by multiplying mmol/L by 1.6.

Table 1: The '4-5-6 mmol/L' rule for RBC transfusion in acute normovolemic anemia

Consider transfusion when Hb < 6.4g/dL (4.0 mmol/L) in cases with:
- Acute blood loss in healthy patients (ASA-I) with age < 60 years, normovolemic and blood loss on 1 location
- Chronic asymptomatic anemia
Consider transfusion when Hb < 8.0g/dL (5.0 mmol/L) in cases with:
- Acute blood loss in healthy subjects (ASA-I) with age > 60 years, normovolemic and blood loss on 1 location
- Acute blood loss in healthy subjects with age < 60 years, normovolemic and bleeding on multiple loci (i.e. multi-trauma patient)
- Preoperative patient with age < 60 years, with expected blood loss of > 500 mL
- Fever
- Postoperative phase of an uncomplicated cardiac surgery procedure
- ASA-II and ASA-III patients
Consider transfusion when Hb < 9.6g/dL (6.0 mmol/L) in cases with:
- ASA-IV patients
- Patient not able to increase heart minute volume to compensate for hemodilution
- Patient with sepsis or who are intoxicated
- Patient with severe lung disease
- Patient with symptomatic cerebrovascular disease

ASA: American Society of Anesthesiologist physical status classification system

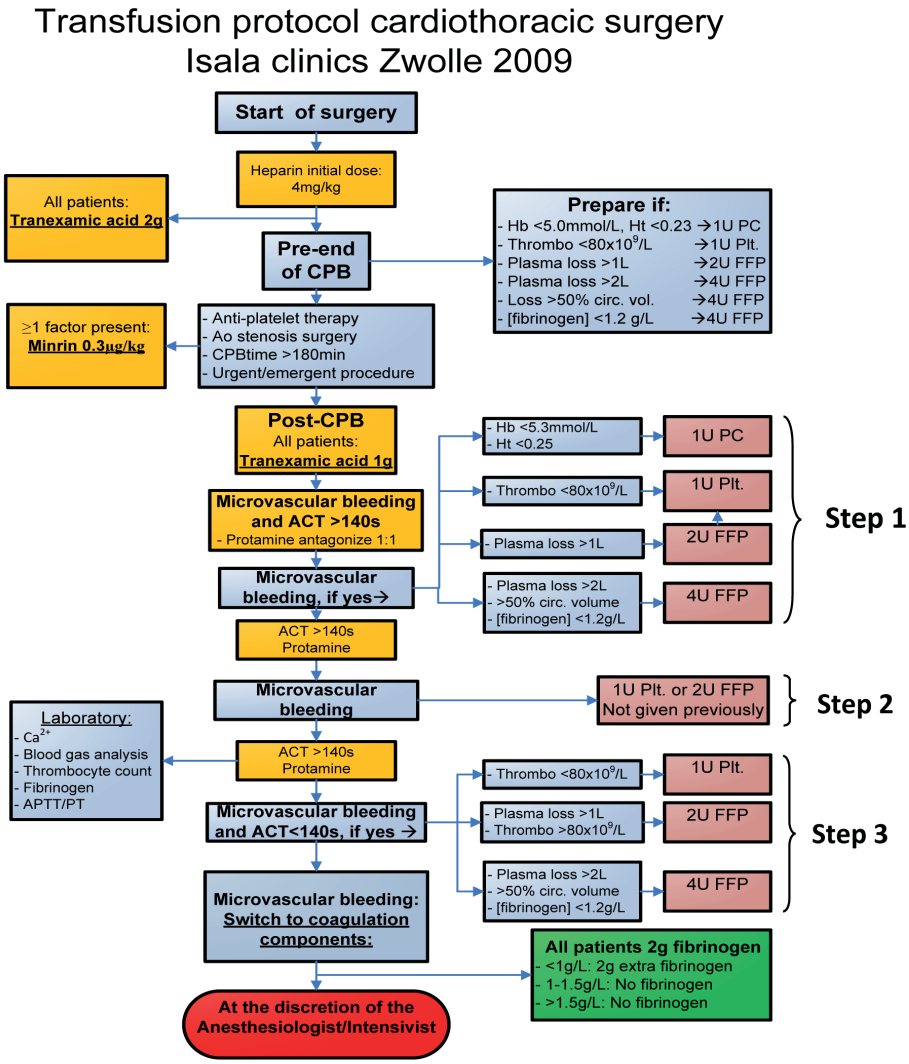
The ASA physical status classification system	
I	A normal healthy patient
II	A patient with mild systemic disease
III	A patient with severe systemic disease
IV	A patient with severe systemic disease that is a constant threat to life
V	A moribund patient who is not expected to survive without the operation
VI	A declared brain-dead patient whose organs are being removed for donor purposes

The '4-5-6 mmol/L' rule in short: transfusion of red blood cells (RBC) was considered when Hb < 6.4 g/dL (< 4.0 mmol/L) in healthy patients aged < 60 years who were normovolemic and had blood loss from one locus only. Transfusion of RBC's was considered when Hb < 8.0 g/dL (< 5.0 mmol/L) in healthy patients with aged > 60 years who were normovolemic and had blood loss from one locus, or age lower than 60 years with bleeding from multiple loci. Transfusion of RBC's was considered when Hb < 9.6 g/dL (< 6.0 mmol/L) in patients with severe heart or lung disease.

Concerning the transfusion of fresh frozen plasma (FFP), no clear triggers for transfusion in cardiac surgery procedures were mentioned in the guideline. Regarding platelet transfusion, a thrombocyte count of at least 100 x 10^9 was recommended.

Index group

On January 1st of 2009, the cardiac surgery-specific transfusion protocol specifically designed for cardiac surgery procedures was introduced (Figure 1). In this protocol, specific attention was given to the timing and dosing of RBC, FFP and platelets transfusions, also taking into account the use of cell saver techniques during surgery. Furthermore, the use of procoagulants and antifibrinolytics was standardized. In detail, one unit of RBC is administered when hemoglobin level is lower than 8.5 g/dL (5.3 mmol/L) or when hematocrit is lower than 0.25. When plasma loss measured with the cell saver is more than one liter, two units of FFP are transfused. Four units of FFP are transfused when plasma loss exceeds two liters or with more than 50% loss of circulating volume or when plasma fibrinogen concentration is lower than 1.2 g/L. The trigger for platelets transfusion was lowered from 100x10^9/L to 80x10^9/L; when thrombocyte count is less than 80x10^9/L, one unit of platelet concentrate (five donors) is transfused (Step 1 in Figure 1). Regarding the use of antifibrinolytics and pro-coagulants, two grams of tranexamic acid is given routinely at the beginning of CPB to all patients and is repeated with a second dose of one gram at the end of CPB. Desmopressin (0.3 µg/kg) is given intravenously at the end of CPB in patients with one of the following conditions; treated with anti-platelet agents, CPB time of > 180 minutes, aortic stenosis surgery or urgent/emergent surgery. When microvascular bleeding persists, one unit of platelet concentrate or two units of FFP is given (Step 2, Figure 1). If despite these measures microvascular bleeding continues to exist, Step 3 in the protocol is followed. Hereafter, if microvascular bleeding still persists, a switch to coagulation components is performed. Further treatment is at the discretion of the attending anesthesiologist. The APTT and PT values are measured for coagulation management during ICU stay.



Protocol violation is always allowed after consulting of attending senior Anesthesiologist/Intensivist

Figure 1. Cardiac surgery-specific transfusion protocol.
CPB = Cardiopulmonary bypass; Hb = Hemoglobin; Ht = Hematocrit; Ao = Aortic; U = Unit; PC = Packed red cells; FFP = Fresh frozen plasma; Plt = Platelet concentrate; ACT = Activated clotting time; APTT = Activated Partial Thromboplastin Time, PT = Prothrombin time.

Outcomes

The primary outcome was the mean units of blood products transfused and the proportion of patients transfused during surgery. Secondary outcome was the occurrence of clinical adverse events. The measured clinical adverse events were in-hospital mortality, myocardial infarction, cerebrovascular accident (CVA) or transient ischemic attack (TIA), renal injury or renal failure, rethoracotomy after initial surgery and prolonged mechanical ventilation. Myocardial infarction was defined as myocardial specific creatine kinase (CKMB) value ≥120 U/L (five times upper reference limit) plus a peak CKMB/creatine kinase ratio >10%, or pathological new Q waves on a postoperative electrocardiogram (ECG).[18] Renal injury and failure was defined by the RIFLE criteria.[19] The remaining definitions of clinical events are shown in table 2.

Table 2: Clinical adverse events and definitions

In-hospital mortality (30 days)	All-cause mortality within 30 days after the initial cardiac surgery procedure
Myocardial infarction	CKMB value ≥120 U/L (five times upper reference limit) plus a peak CKMB/CK ratio >10%, or pathological new Q waves on a postoperative electrocardiogram
CVA	New motor or sensory deficit of the central nervous system or an unexplained coma status lasting ≥ 24 hours
TIA	Neurological dysfunction from focal temporary cerebral ischemia lasting < 24 hours, no cerebral infarction.
Renal injury/failure	Renal injury and failure was defined by the RIFLE criteria.
Rethoracotomy (within 30 days)	Re-thoracotomy within 30 days after initial cardiac surgery.
Prolonged ventilation (≥24 hours)	Mechanical ventilation after initial surgery lasting longer than 24 hours in the ICU period.

Statistical analyses

First, the index and control group were compared on the potential confounders. For categorical variables the Chi-squared test was used and data were presented as odds ratio with 95% confidence interval. For continuous variables the independent sample t-test (for normally distributed variables) or Mann-Whitney U test (for non-normally distributed variables) was used and expressed as medians with 25th and 75th percentile.

Due to the non-randomized design of this study, the association between the determinant (tailor made transfusion protocol) and the primary outcome was likely to be biased by potential confounders. Based on the literature and clinical expertise, we considered the following pre- and perioperative characteristics as potential confounders; age, gender, comorbidity (chronic obstructive pulmonary disease (COPD), hypertension, unstable angina, diabetes type 1 and 2), left ventricular hypertrophy, pulmonary hypertension, statins use, beta-blocker use, previous cardiac surgery, euroscore, indication for surgery (elective or urgent), use of medication (acetylsalicylic acid (ASA), clopidogrel, low molecular weight heparin (LMWH), warfarin), left ventricle ejection fraction (LVEF), cell saver use and type of cardiac surgery procedure (valves only, CABG and valves, aortic surgery).[20-28]

For the secondary outcome related to clinical adverse events, cardiopulmonary bypass time (CPB time) was also considered a potential confounder and was additionally included in the adjusted analysis.

For the primary outcome variables, we used linear regression (outcome of mean transfusion during surgery) and logistic regression (outcome proportion of patients transfused) to correct for pre- and perioperative confounding variables.

For the secondary outcome measures logistic regression analysis was used to correct for confounders. Both the crude and adjusted analysis for the primary and secondary outcomes are expressed as odds ratios (OR) with 95% confidence intervals (CI).

Although very limited, some patients had missing values for one or more variables ranging from 0.1% (CPB time) to 1.1% (mechanical ventilation time). Missing data seldom occur completely at random. Deleting subjects with a missing value does not only lead to loss of statistical power but also commonly leads to biased results. Therefore, imputing missing values is generally preferred to complete case analysis.[29-32] Missing variables were imputed using a regression model with logistic regression for dichotomous variables and linear regression for continuous variables. The imputation model included all other variables including the outcome variables, as it has extensively been reported that imputation of missing covariate data should always be done with all available data including the outcome. [29-32] A p-value <0.05 was considered statistically significant. Analyses were performed using the statistical software IBM SPSS 20.0 (IBM SPSS Statistic, New York, USA).

RESULTS

In the control group 2685 patients underwent cardiac surgery, whereas in the index group 2534 patients underwent cardiac surgery. The index group included older patients with less hypertension but more diabetes, more use of ASA (but less use of clopidogrel and warfarin) compared to the control group. Also, in the index group there were more patients with a LVEF above 50%. Regarding procedure characteristics, there were more isolated CABG and less combined CABG with valve(s) procedures in the index group. Furthermore, the index group used the blood salvages technique (cell saver) more frequently (76% vs. 58%, P<0.01) compared to the control group, table 3.

Table 3: Baseline characteristics

	Controls	Index group	
	N=2685	*N=2534*	*P*
Age (years), median (IQR 25th; 75th)	69 (61;75)	70 (62;76)	<0.01
Gender, male (%)	1847 (69%)	1755 (69%)	0.71
Euroscore, median (IQR 25th; 75th)	5 (3;8)	5 (3;7)	0.12
Co-morbidity (%)			
COPD	346 (13%)	368 (15%)	0.09
Hypertension	1362 (51%)	1207 (48%)	0.03
Unstable angina pectoris	171 (6%)	160 (6%)	0.94
Diabetes mellitus	543 (20%)	597 (24%)	<0.01
Pulmonary hypertension	72 (3%)	55 (2%)	0.23
Left ventricular hypertrophy	684 (26%)	666 (26%)	0.51
Pre-operative use of medication (%)			
ASA	1802 (67%)	1790 (71%)	<0.01
Clopidogrel	607 (23%)	500 (20%)	0.01
LMWH	607 (23%)	546 (22%)	0.36
Warfarin	368 (14%)	249 (10%)	<0.01
Statins	1620 (60%)	1557 (61%)	0.41
Beta-Blocker	1959 (73%)	1884 (74%)	0.26
LVEF (%)			<0.01
<30%	193 (7%)	178 (7%)	
30-50%	901 (34%)	707 (28%)	
>50%	1591 (59%)	1649 (65%)	

Table 3: Baseline characteristics (continued)

	Controls	Index group	
	N=2685	*N=2534*	*P*
Previous cardiac surgery procedure	164 (6%)	124 (5%)	0.06
Indication (%)			0.47
Elective	2492 (93%)	2373 (94%)	
Urgent	112 (4%)	91 (4%)	
Emergency	81 (3%)	70 (3%)	
Type of surgery (%)			<0.01
CABG only	1452 (54%)	1481 (59%)	
Valve(s) only	488 (18%)	481 (19%)	
CABG and valve(s)	536 (20%)	416 (16%)	
Aortic surgery	141 (5%)	122 (5%)	
Rest	68 (3%)	34 (1%)	
CPB time in min., median (IQR 25^{th}; 75^{th})	110 (80;161)	108 (79;152)	0.06
Aortic occlusion time in min., median (IQR 25^{th}; 75^{th})	69 (50;104)	70 (52;99)	0.75
Cell Saver use (%)	1558 (58%)	1933 (76%)	<0.01

COPD= chronic obstructive pulmonary disease; ASA= acetylsalicylic acid; LMWH= low molecular weight heparin
LVEF= left ventricle ejection fraction; CABG= coronary artery bypass graft; CPB= cardiopulmonary bypass;
IQR= interquartile range

Primary outcomes

The mean transfusion during surgery between control group and index group was 0.88 units vs. 0.78 units per patient for RBC transfusion and 0.47 units vs. 0.37 units for FFP transfusion and 0.12 units vs. 0.13 units for platelet transfusion, respectively. In both the crude and adjusted analysis no difference between the control and index group was measured regarding the amount of transfusion with RBC, FFP, platelets and all transfusions combined.

For the proportion of patients transfused, in the adjusted analysis, the index group had less patients transfused with RBC and FFP during surgery with respectively 29% vs. 27% and 11% vs. 9%, with adjusted odds ratios and confidence intervals of 0.69 (0.55 – 0.86) and 0.63 (0.46 – 0.86), respectively. The proportion of patients transfused with any blood product was also reduced in the adjusted analysis with odds ratio and confidence interval of 0.74 (0.60 – 0.92), table 4.

Table 4: Frequencies and Odds Ratios of primary and secondary outcomes between the Controls and Index group

	Controls *N=2685*	Index group *N=2534*	Crude Odds Ratio	*95% CI*	Adjusted Odds Ratio	*95% CI*
Primary outcome						
Mean transfusion during surgery *						
RBC	0.88	0.78	0.91	0.82-1.01	1.05	0.87-1.28
FFP	0.47	0.37	0.91	0.82-1.01	1.15	0.94-1.42
Platelets	0.12	0.13	1.01	0.99-1.04	1.04	0.99-1.08
Any blood product	1.46	1.29	0.84	0.68-1.03	1.26	0.85-1.85
Proportion of patients transfused during surgery *						
RBC	29%	27%	0.88	0.78-0.99	0.69	0.55-0.86
FFP	11%	9%	0.81	0.68-0.97	0.63	0.46-0.86
Platelets	9%	10%	1.11	0.92-1.34	1.22	0.89-1.68
Any blood product	33%	31%	0.91	0.81-1.02	0.74	0.60-0.92
Secondary outcome						
Adverse clinical events (% of patients) †						
In-hospital mortality (30 days)	2.5%	2.3%	0.92	0.65-1.31	1.13	0.61-2.10
Myocardial infarction	6.6%	5.3%	0.79	0.62-0.99	0.67	0.47-0.96
Cerebrovascular event: CVA/TIA	1.4%	1.7%	1.20	0.78-1.87	1.73	0.91-3.31
Renal injury/failure	3.4%	3.0%	0.86	0.63-1.17	0.83	0.51-1.33
Rethoracotomy (within 30 days)	8.2%	9.5%	1.17	0.97-1.42	0.92	0.69-1.24
Prolonged ventilation (≥24 hours)	5.8%	5.7%	0.98	0.77-1.23	0.89	0.60-1.32

CI = Confidence interval; RBC= Red Blood Cells; FFP= Fresh Frozen Plasma; CVA= Cerebrovascular Event; TIA=Transient Ischemic Attack.
* = Adjusted for: Age, gender, year of surgery, co-morbidity (COPD, hypertension, unstable angina, diabetes mellitus), left ventricle hypertrophy, pulmonary hypertension, beta-blocker use, statins use, previous cardiac surgery, euroscore, indication for surgery (elective or urgent), use of medication (acetylsalicylic acid, clopidogrel, LMWH, warfarin), left ventricle ejection fraction, cell saver use and type of surgery (valves only, CABG and valves, aortic surgery).
† = Adjusted for variables of * and CPB time.

Secondary outcome

The index group had less myocardial infarction compared to the control group (5.3% vs. 6.6%) with adjusted odds ratio of 0.67 (0.47 – 0.96). The occurrence of in-hospital mortality, cerebral vascular events, renal injury or failure, rethoracotomy and prolonged mechanical ventilation was not different between the control and the index group, table 4.

DISCUSSION

In this study, the implementation of a cardiac surgery-specific transfusion protocol resulted in significantly less patients transfused with RBC and FFP during surgery and less myocardial infarction. With the implementation of a cardiac surgery-specific transfusion protocol in 2009, we aimed to reduce both unnecessary transfusions and the number of patients transfused. Although most elements of the protocol were already practiced, the infusion of hemostatic medication with specific focus on timing and dosing of intervention was relatively new for the clinical practice. We hypothesized that the introduction of a cardiac surgery-specific transfusion protocol would lead to a more judicious use of blood products, eventually leading to fewer transfusions and less patients transfused with blood products.

As expected, the differences in the amount of transfusion between the control and index group were small in this study. An explanation for the small differences could be the already restrictive use of blood products. When comparing the number of patients transfused in this study with data reported in studies from the United States, differences in transfusion practices are evident. For example, a study of Bennet-Guerrero et al. investigated the consumption of blood products in the year 2008 in 798 hospitals in the USA for primary isolated CABG surgery. This study showed that 56.1% received RBC's, 19.3% received FFP and 24.7% received platelets.[33] When analysing the subgroup primary isolated CABG surgeries in our cohort, these transfusion figures are considerably lower for RBC, FFP and platelets namely 18.2%, 3.4% and 2.5% respectively in the control group. Maddux et al.

studied the transfusion practice of 144 institutions in the USA for primary isolated CABG surgery. This study showed that mean RBC transfusion was between 0.81 units and 1.06 units per patient.[34] The mean number of units transfused was also lower in our cohort; the mean RBC transfusion for primary isolated CABG was 0.43 units per patient in the control group.

Interestingly, significantly fewer cases with myocardial infarction were measured in the index group while the incidence of other adverse events was comparable. Even after adjusting for potential confounders, the cardiac surgery-specific transfusion protocol was still associated with a lower probability of myocardial infarction. Liberal transfusion of blood products has been associated with more cardiac events.[6,9,13] Our result concerning myocardial infarction is in line with the results of Hebert et al.[35] They found that the restrictive strategy for RBC infusion - hemoglobin between 7.0 g/dL and 9.0 g/dL compared to liberal strategy of 10.0 g/dL to 12.0 g/dL - resulted in less myocardial infarction. On the contrary, the study of Bracey et al. showed that a restrictive transfusion threshold for RBC with Hb <8.0 g/dL compared to < 9.0 g/dL, did not affect the occurrence of myocardial infarction.[36]

Clearly, there are conflicting data on the relation between transfusion practice and myocardial infarction. We have no straightforward explanation for the decreased risk for myocardial infarction in the present study, especially in light of the relatively small reductions in transfusion achieved with the cardiac surgery-specific protocol. The results of the remaining clinical adverse events in-hospital mortality, CVA or TIA and renal injury or failure are in line with previous studies.[35-38] During the study period, most patients were operated electively (93%). In an additional analysis, the proportion of patients receiving preoperative inotropic support was 3% in the control group and 2% in the index group, p= 0.17. Patients receiving preoperative intra-aortic balloon pump (IABP) support was 5% in the control group and 4% in the index group, p= 0.07. In this group of patients receiving either inotropic or mechanical support (IABP), the relative risk for 30-day mortality was 5. As these variables were equally distributed between the two groups, they were not incorporated in the regression analysis. During the study period, there were no patients on extracorporeal membrane oxygenation (ECMO).

There are some limitations to our study. First, adherence to a new transfusion protocol remains a challenge, despite availability of transfusion guidelines.[39-41] It is likely that not all patients in this study were transfused according to the tailor-made transfusion protocol. To reduce the effects of confounding, we used multivariate regression analysis.

Secondly, as this study is non-randomized intervention study in a pre- and post-intervention design, there is a potential bias to the internal validity. For example, there might be an effect of time. Throughout the 4 years of analysis, there was a steady and constant increase in the use of the cell saver and procoagulant medication. By incorporating the use of the cell saver in the regression analysis, we largely corrected for this effect. However, residual or unmeasurable confounding is still possible. A randomized clinical trial is the preferred study design to compare two treatment options. However, in the setting of comparing two transfusion guidelines, it might be very difficult to blind the treatment. Furthermore, a new protocol which is based on evidence based medicine, compared to the "older" protocol, could create ethical issues due to lack of clinical equipoise.

Thirdly, there was a small amount (ranging from 0 to 1.1%.) of missing data present in patient variables. Although the amount of missing data was limited, imputation using a regression model was used to complete the data.

Finally, this study is a single center experience. The results of this study can be extrapolated to other cardiac surgery centers, but not automatically to other non-cardiac surgery specialties.

In conclusion, this study evaluated a cardiac surgery-specific transfusion protocol. This protocol resulted in significantly less patients transfused with red blood cells and fresh frozen plasma and a significant lower risk for myocardial infarction. This tailor made protocol, specifically designed for cardiac surgery procedures, has led to a more judicious use of blood products and is a basis for further refinement of coagulation management during these procedures.

ABBREVATIONS

CPB = Cardiopulmonary bypass; Hb = Hemoglobin; Ht = Hematocrit; Ao = Aortic; U = Unit; PC = Packed red cells; RBC= Red Blood Cells; FFP = Fresh frozen plasma; Plt = Platelet concentrate; ACT = Activated clotting time; APTT = Activated Partial Thromboplastin Time, PT = Prothrombin time.
CI = Confidence interval. IQR= interquartile range. CVA= Cerebrovascular Event; TIA=Transient Ischemic Attack; COPD = chronic obstructive pulmonary disease; ASA = acetylsalicylic acid; LMWH = low molecular weight heparin; LVEF = left ventricle ejection fraction; CABG = coronary artery bypass graft; CPB = cardiopulmonary bypass.

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Chapter 4

Predicting blood loss in cardiac surgery: A new application of the EuroSCORE

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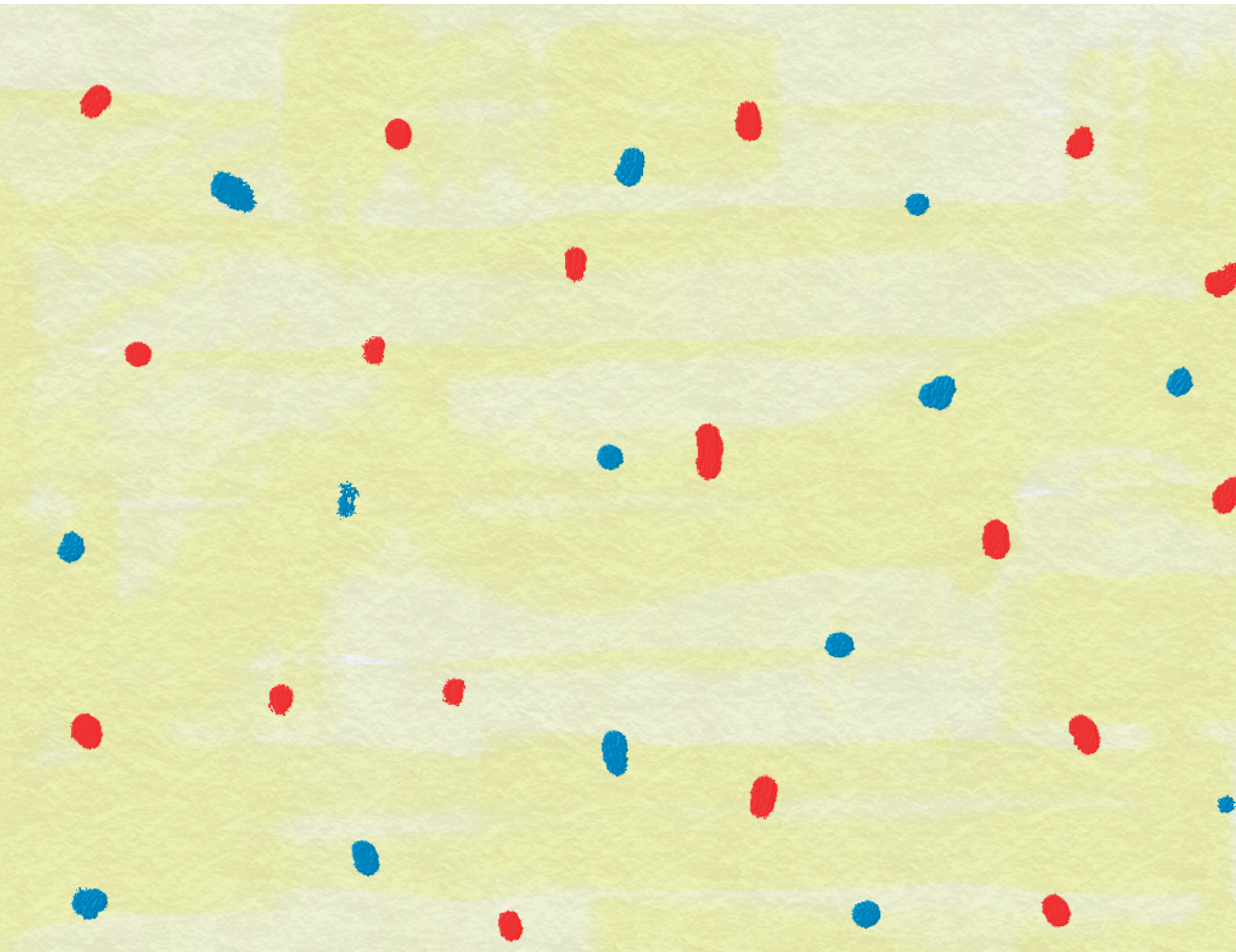
Submitted



ABSTRACT

Background: Excessive bleeding remains one of the most common complications in cardiac surgery. Early identification of these patients is necessary for adequate hemostasis management. We were interested whether the widely used EuroSCORE prediction model also predicts blood loss in cardiac surgery.

Objective(s): A model with EuroSCORE variables was constructed to predict excessive bleeding during and shortly after cardiac surgery. The added value of specific clinical and laboratory variables was also assessed.

Design: Cohort study. Reported with STROBE statement.

Setting: Large teaching hospital (Isala Zwolle, The Netherlands).

Patients: Patients who underwent cardiac surgery in the years 2007, 2008, 2009 and 2010 were included in the study.

Main outcome measures: In model 1 all EuroSCORE predictors were used to predict excessive intra-operative blood loss (≥ 2000 mL); this model was extended with preoperative laboratory variables. In model 2 all EuroSCORE predictors including intra-operative variables were used to predict excessive blood loss in the ICU (≥ 1000 mL); this model was extended with laboratory variables at the end of cardiopulmonary bypass (end-CPB).

Results: In total 5490 patients were included in the study. Model 1 had a ROC area under the curve (AUC) of 0.84 (95% confidence interval (CI) 0.83-0.86). Adding preoperative laboratory measurements did not increase the AUC. Model 2 had a ROC AUC of 0.74 (95% CI 0.72-0.76). Adding end-CPB laboratory measurements only slightly increased the AUC to 0.75 (95% CI 0.73-0.76). Calibration was good for both models and did not improve by adding laboratory variables.

Conclusions: The EuroSCORE predictors have good performance to predict the occurrence of excessive intra-operative blood loss in cardiac surgery. For excessive blood loss in the ICU the predicting ability of the EuroSCORE was lower. Addition of laboratory variables did not improve these models.

Trail registration: Isala Zwolle NL08.2013.3.01

Excessive bleeding remains one of the most common complications in cardiac surgery. While the majority of patients undergoing elective cardiac surgery will not receive allogeneic blood products, a substantial number of patients consume disproportionately high amounts.[1] Several prediction models were developed to predict transfusion of blood products in cardiac surgery to enable early identification of the patient with high risk for transfusion.[2-6]

A commonly used prediction model in cardiac surgery is the EuroSCORE (European System for Cardiac Operative Risk Evaluation; www.euroscore.org).[7,8] However, the EuroSCORE model calculates predicted operative in-hospital mortality for patients undergoing cardiac surgery and was not developed for predicting blood loss and transfusion.[9] We were interested whether the widely used EuroSCORE prediction model also predicts blood loss in cardiac surgery. The aim of this study was to determine whether the variables of the EuroSCORE model can also accurately predict excessive blood loss at two different time points: During cardiac surgery (intraoperative period) and during ICU stay (early postoperative period). In addition, we quantified whether preoperative laboratory variables have added predictive ability for intraoperative blood loss, and if adding intraoperative clinical and laboratory variables (including plasma end-CPB fibrinogen concentration) improves prediction of blood loss in the early postoperative period. In an additional analysis, the numerical EuroSCORE (low, medium and high risk) was related to the study outcomes.

METHODS

The study was designed as a cohort study using existing data from a large teaching hospital (Isala Zwolle, The Netherlands). All patients who underwent cardiac surgery in the years 2007, 2008, 2009 and 2010 were included in this study. Due to the retrospective nature of the study, informed consent was waived. This study has been approved by the institutional medical ethical committee (reference: NL08.2013.3.01). During the entire study period, anaesthesia management and surgical treatment was performed according to the standard procedure of the Isala

Zwolle, which was described previously in detail.[10] All patients underwent surgery through a median sternotomy. Myocardial protection during cardiopulmonary bypass (CPB) was achieved with antegrade blood or crystalloid cardioplegia. One surgeon used the combination of retrograde and antegrade crystalloid cardioplegia for aortic valve surgery. CPB was managed using non-pulsatile flow applied by a centrifugal pump and pH management with the α-stat principle. Heparin was given to achieve an activated clotting time >480 seconds during CPB. After weaning from CPB, protamine was administered with ratio 1:1. After surgery all patients were transferred to the ICU.

From admission until hospital discharge, all clinical data of patients undergoing cardiac surgery were prospectively registered in an electronic patient data management system. After the patient's discharge from the hospital, all data collection forms were verified with double check by another member of the research team to confirm accuracy of the data entry.

Statistical analysis

For the baseline data, mean and standard deviation (SD) was used for normally distributed continuous variables and median with interquartile range (IQR) 25^{th} and 75^{th} was used for non-normally distributed continuous variables. Normality was determined with de Kolmogorov-Smirnov statistical test. Frequencies with percentages were used for categorical variables.

In this study two prediction models were built: Model 1 predicts excessive intraoperative blood loss and model 2 predicts excessive early postoperative blood loss. The intraoperative period was defined as the period between start of surgery (incision) to the end of surgery (leaving the operating room).

The postoperative period was defined as the period between admission to the ICU until removal of the chest drains. The intraoperative blood loss measurement was performed according to local practice. Surgical gauzes were weighed and added up to blood loss measured with the suction devices, including the cell saver (Haemonetics Cell Saver 5, Braintree, Massachusetts, The definition of excessive blood loss for both models was as follows: For model 2, the outcome 'excessive blood loss in the ICU' was defined as blood loss more than 1000 mL and measured

as total chest drain production from admission to the ICU until removal of the chest drains. This outcome of 1000 mL during ICU stay was also used as definition of excessive blood loss for the early postoperative period in several studies with cardiac surgery patients.[11-14] Therefore the same definition was chosen in this study.

However, in literature there are no generally used or accepted definitions for excessive intraoperative blood loss in cardiac surgery. In this study, patients with more than 1000 mL blood loss in the ICU period (excessive bleeders) comprised approximately 15% of the studied population.

In accordance, the same 15% upper limit percentage was used for the definition of excessive intraoperative blood loss in model 1 which resulted in blood loss of 1960 mL and was rounded up to 2000 mL.

For model 1, a logistic prediction model was developed using all seventeen predictors of the EuroSCORE model.[9] The EuroSCORE II model was introduced in the year 2011 and was therefore not used in this study. Table 1 shows the definition of all seventeen EuroSCORE variables. In the developed model, the variable age was categorized per 5 years above 60 years of age with the age ≤ 60 years as the reference group. For left ventricular ejection fraction categorical variables were used. The remaining EuroSCORE variables were dichotomous variables. This basic model 1 was then extended with pre-operative laboratory measurements collected on the day of admission, which was one day before surgery and included hemoglobin, hematocrit and thrombocyte count.

For model 2 again a logistic prediction model was developed using all seventeen predictors from the EuroSCORE including relevant intraoperative variables (i.e. cardiopulmonary bypass time (CPB time), cell saver use, transfusion of RBC, plasma, platelets and total transfusion, infusion of tranexamic acid, desmopressin, recombinant factor VIIa and fibrinogen concentrate). The selection of these intraoperative variables was based on the literature and clinical expertise.[2,15-18] The CPB time was a continuous variable while the remaining variables were dichotomous. This basic model 2 was then extended with intraoperative laboratory variables determined at the end of cardiopulmonary bypass (i.e. hemoglobin, hematocrit, thrombocyte count and end-CPB plasma fibrinogen concentration) to determine the added predictive value of these variables.

Table 1. EuroSCORE variables and definitions

Patient-related factors	
Age	per 5 years or part thereof over 60 years
Sex	female
Chronic pulmonary disease	long term use of bronchodilators or steroids for lung disease
Extracardiac arteriopathy	any one or more of the following: claudication, carotid occlusion or > 50% stenosis, previous or planned intervention on the abdominal aorta, limb arteries or carotids
Neurological dysfunction disease	severely affecting ambulation or day-to-day functioning
Previous cardiac surgery	requiring opening of the pericardium
Serum creatinine	>200 micromol/L preoperatively
Active endocarditis	patient still under antibiotic treatment for endocarditis at the time of surgery
Critical preoperative state	ventricular tachycardia or fibrillation or aborted sudden death, preoperative cardiac massage, preoperative ventilation before arrival in the anesthetic room, preoperative inotropic support, intra-aortic balloon counter-pulsation or preoperative acute renal failure (anuria or oliguria < 10 ml/hour)
Cardiac-related factors	
Unstable angina	rest angina requiring iv nitrates until arrival in the anesthetic room
LV dysfunction	moderate or LVEF 30-50% poor or LVEF < 30
Recent myocardial infarct	(< 90 days)
Pulmonary hypertension	systolic PA pressure > 60 mmHg
Operation-related factors	
Emergency	carried out on referral before the beginning of the next working day
Other than isolated CABG	major cardiac procedure other than or in addition to CABG
Surgery on thoracic aorta	for disorder of ascending, arch or descending aorta
Post-infarct septal rupture	

LV = left ventricle; LVEF = left ventricle ejection fraction; PA = pulmonary artery; CABG = coronary artery bypass graft

After initial development of both the basic and extended models we used internal validation to correct for overfitting or overoptimism in the estimated regression coefficients using bootstrap (200 samples).[19] Then, to assess the predictive performance of model 1 and model 2 and the extended models, discrimination (i.e. ROC (receiver operating characteristic) curve and Area Under the ROC (AUC)) and calibration (i.e. cali-

bration curve) was calculated. For model 1 and 2, the added discriminative value of the extended model was compared to the basic model using the differences in AUC's.[19,20]

In an additional analysis, the numerical EuroSCORE was related to the outcomes in this study. The EuroSCORE was divided in the "classic" division with three risk groups. The low risk group had EuroSCORE 0-2, the medium risk group had EuroSCORE 3-5 and the high risk group had EuroSCORE of 6 and above.[21,22] We also divided the numerical EuroSCORE above 6 to 6-8, 9-11, 12-14, 15-17 and above 18. The crude and adjusted association is shown in odds ratio with the 95% confidence interval. Multivariable regression analysis was used for the adjusted analysis. The study outcomes excessive intraoperative blood loss (≥ 2000 mL) was adjusted with the variables left ventricular hypertrophy, preoperative use of acetylsalicylic acid (ASA), clopidogrel, nadroparin, coumadins, CPB time, pre-operative hematocrit and pre-operative thrombocyte count. The study outcomes excessive postoperative blood loss (≥ 2000 mL) was adjusted with the variables left ventricular hypertrophy, preoperative use of ASA, clopidogrel, nadroparin, coumadins, CPB time, intraoperative hematocrit, intraoperative thrombocyte count and end-CPB plasma fibrinogen concentration.

Although very limited, some patients had missing values for one or more variables ranging from 0.1% (CPB time) to 3.9 % (Blood loss in ICU). Missing variables were imputed using a regression model with logistic regression for dichotomous variables and linear regression for continuous variables. The imputation model included all other variables including the outcome variables, as it has extensively been reported that imputation of missing covariate data should always be done with all available data including the outcome.[23-26] A p-value <0.05 was considered statistically significant. All analyses were performed using R 2.15.0 (R Foundation for Statistical Computing; www.R-project.org) and SPSS 21.0 (IBM SPSS Statistic, New York, USA). This study is described in accordance with the STROBE statement (www.strobe-statement.org).

RESULTS

In total 5490 patients were included in the study. Baseline patient and procedure characteristics and laboratory variables are depicted in table 2. There were

810 patients (15%) with intraoperative blood loss more than 2000 mL and 991 patients (18%) with more than 1000 mL postoperative blood loss.

Table 2. Baseline patient characteristics

	N = 5490
Age (years), median (IQR 25th; 75th)*	69 (61;76)
Gender, male (%)*	3788 (69%)
EuroSCORE, median (IQR 25th; 75th)	5 (3;8)
Comorbidity (%)	
COPD*	760 (14%)
Extracardiac arteriopathy*	569 (10%)
Unstable angina pectoris*	351 (6%)
Pulmonary hypertension*	136 (3%)
Left ventricular hypertrophy	1419 (26%)
Neurologic dysfunction/disease*	289 (5%)
Previous cardiac surgery procedure*	420 (8%)
Renal insufficiency (> 200 micromol/L preoperatively) *	117 (2%)
Active endocarditis*	91 (2%)
Critical preoperative state*	475 (9%)
Recent myocardial infarction*	775 (14%)
Pre-operative use of medication (%)	
ASA	3730 (68%)
Clopidogrel	1150 (21%)
LMWH	1217 (22%)
Warfarin	645 (12%)
LVEF (%)*	
< 30%	412 (7%)
30-50%	1698 %)
> 50%	3380 (62%)
Indication (%)	
Elective	5043 (92%)
Urgent	239 (4%)
Emergency*	208 (4%)
Type of surgery (%)*	
CABG only	3053 (56%)
Valve(s) only	1021 (18%)
CABG and valve(s)	975 (18%)
Aortic surgery	292 (5%)
Rest	149 (3%)
Post-infarct septal rupture (%)*	15 (0.3%)
CPB time in min., median (IQR 25th; 75th)	109 (79;159)

Table 2. Baseline patient characteristics (continued)

	N = 5490
Cell saver use (%)	3678 (67%)
Preoperative laboratory values, median (IQR 25^{th};75^{th})	
Hemoglobin, g/dl	13.9 (12.6;14.8)
Hematocrit, l/l	0.41 (0.38;0.43)
Thrombocyte count, 10^9/L	223 (185;265)
Laboratory values end of CPB median (IQR 25th;75th)	
Hemoglobin, g/dl	9.0 (8.1;10.0)
Hematocrit, l/l	0.26 (0.24;0.29)
Thrombocyte count, 10^9/L	138 (112;170)
Fibrinogen concentration, g/L	1.8 (1.6;2.2)

IQR = interquartile range; COPD = chronic obstructive pulmonary disease; ASA = acetylsalicylic acid; LMWH = low molecular weight heparin; LVEF = left ventricle ejection fraction
CABG = coronary artery bypass graft; CPB = cardiopulmonary bypass; * = EuroSCORE variables.

Model 1: EuroSCORE variables to predict excessive intraoperative blood loss

The first model (model 1), using the EuroSCORE variables to predict excessive intraoperative blood loss (≥ 2000 mL) resulted in a ROC area under the curve (AUC) value of 0.84 (95% confidence interval (CI): 0.83-0.86). Adding preoperative laboratory values of hemoglobin, hematocrit and thrombocyte count (extended model), resulted in a ROC AUC of 0.84 (95% CI: 0.82-0.86), figure 1 and table 3. In figure 2, the calibration plot of model 1 is shown and was identical to the calibration plot of the extended model 1. The calibration slope of model 1 was 0.98.

Model 2: EuroSCORE and intraoperative variables to predict excessive blood loss in the ICU

The second model (model 2), using the EuroSCORE variables and intraoperative variables (i.e. cardiopulmonary bypass time, cell saver use, RBC, plasma, platelets and total transfusion, tranexamic acid, desmopressin, recombinant factor VIIa and fibrinogen concentrate) to predict excessive early postoperative blood loss (≥ 1000 mL) resulted in a ROC AUC of 0.74 (95% CI 0.72-0.76). Adding laboratory variables measured at the end of CPB (i.e. hemoglobin, hematocrit, thrombocyte count and end-CPB plasma fibrinogen concentration) to the model resulted in a ROC AUC of 0.75 (95% CI 0.73-0.76), figure 3. In figure 4, the calibration plot of model 2 is shown and was again identical to the calibration plot of the extended model. The calibration slope of model 2 was 0.95.

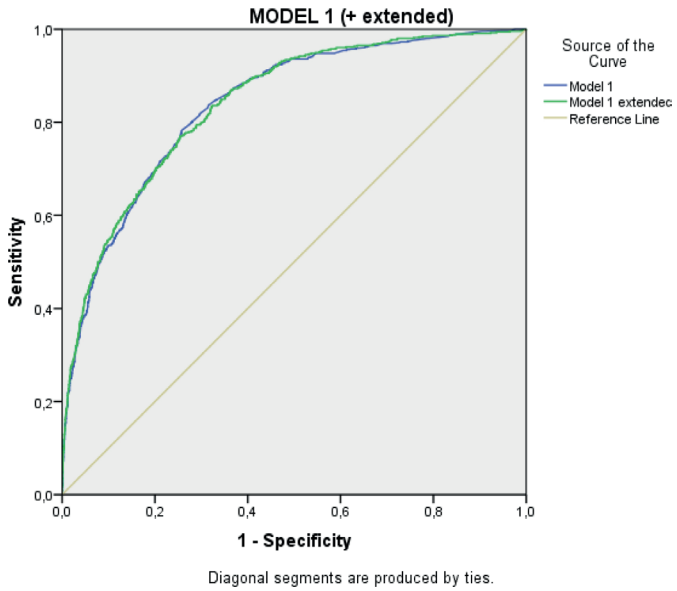


Figure 1. Figure 1. ROC curve of Model 1 and extended version
Blue line: Model 1. Green line: Model 1 extended. Beige line: Reference line

Table 3. ROC values of the developed models

	Excessive intraoperative blood loss (≥ 2000 mL)	AUC	95% CI
Model 1	EuroSCORE	0.84	0.83 - 0.86
Model 1 extended[a]	Model 1 and pre-operative variables	0.84	0.82 - 0.86
	Excessive postoperative blood loss (≥ 1000 mL)	AUC	95% CI
Model 2[b]	EuroSCORE and clinical variables	0.74	0.72 - 0.76
Model 2 extended[c]	Model 2 and end-CPB variables	0.75	0.73 - 0.76

ROC = receiver operating characteristic; AUC = area under curve; CI = confidence interval.
[a] preoperative variables were laboratory values of hemoglobin, hematocrit and thrombocyte count
[b] clinical variables were cardiopulmonary bypass time, cell saver use, RBC, plasma, platelets and total transfusion, tranexamic acid, desmopressin, recombinant factor VIIa and fibrinogen concentrate.
[c] end-CPB variables were hemoglobin, hematocrit, thrombocyte count and plasma fibrinogen concentration.

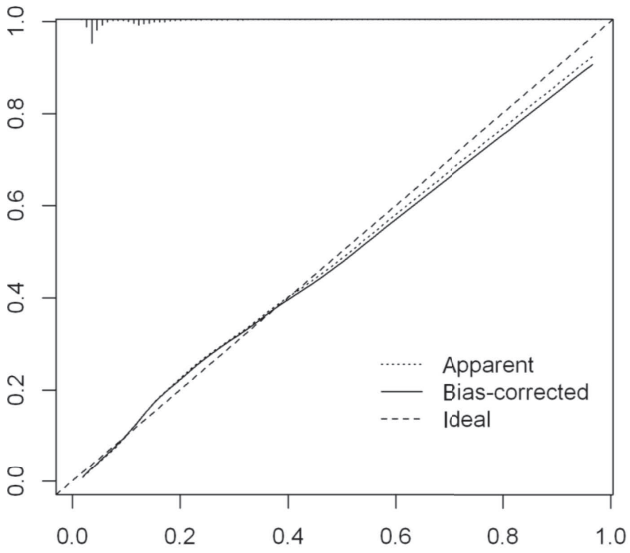


Figure 2. Calibration plot of Model 1 (calibration slope of 0.976)
Line
Dotted line: Apparent. Dashed line: Bias-corrected. Solid line: Ideal

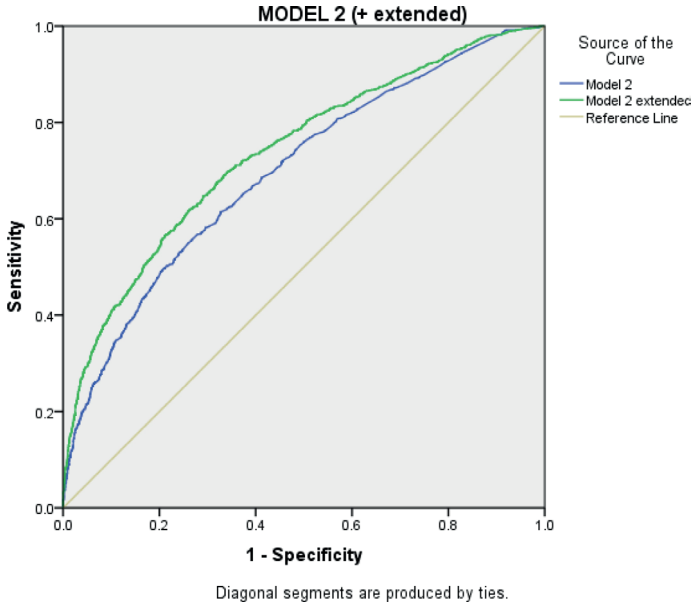


Figure 3. ROC curve of Model 2 and extended version
Blue line: Model 2. Green line: Model 2 extended. Beige line: Reference line

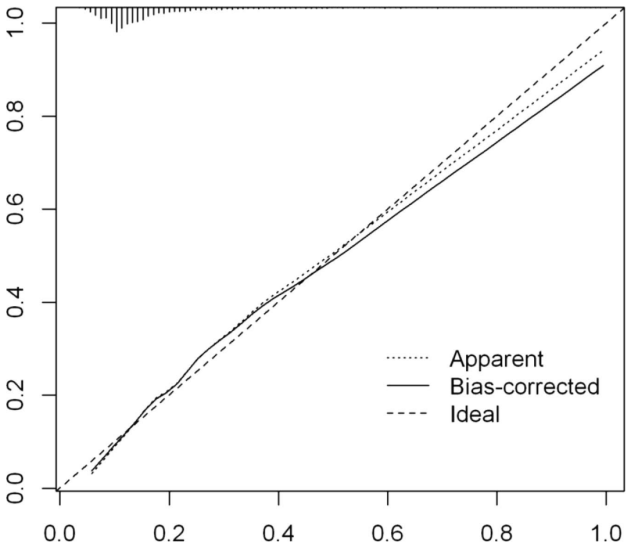


Figure 4. Calibration plot of Model 2 (calibration slope of 0.947)
Dotted line: Apparent. Dashed line: Bias-corrected. Solid line: Ideal

Additional analysis

In the additional analysis, the EuroSCORE of ≥ 6 (high risk group) was associated with excessive (≥ 2000 ml) intraoperative blood loss with OR 2.07 (95% CI; 1.37-3.15) and also with excessive postoperative blood loss with odd ratio of 1.54 (95% CI; 1.18-2.01). For the EuroSCORE above 6, with the defined subdivision 6-8, 9-11, 12-14, and above 15, there is a steady increase in the adjusted risk for excessive postoperative bleeding, (Table 4).

Table 4. EuroSCORE related to mean blood loss during surgery and during ICU

		Intraoperative blood loss ≥ 2000 mL			Postoperative blood loss ≥ 1000 mL		
EuroSCORE	Number of patients	Blood loss Mean (SD)	Crude (OR (CI))	Adjusted*(OR (CI))	Blood loss Mean (SD)	Crude (OR (CI))	Adjusted** (OR (CI))
Classic							
Low risk (0-2)	1076 (20%)	611 (562)	1	1	547 (418)	1	1
Medium risk (3-5)	1720 (31%)	874 (997)	2.43 (1.65-3.60)	1.15 (0.74-1.78)	600 (1092)	1.17 (0.91-1.49)	0.97 (0.74-1.27)
High risk (≥ 6)	2694 (49%)	1777 (2451)	10.13 (7.09-14.49)	2.07 (1.37-3.15)	857 (1105)	2.96 (2.39-3.67)	1.54 (1.18-2.01)
Subdivision of ≥ 6							
0-5	2796 (51%)	773 (865)	1	1	578 (895)	1	1
6-8	1586 (29%)	1398 (1930)	3.27 (2.65-4.04)	1.53 (1.19-1.96)	702 (731)	1.82 (1.53-2.16)	1.40 (1.16-1.71)
9-11	691 (13%)	2005 (2926)	6.65 (5.28-8.39)	2.43 (1.82-3.23)	946 (1313)	3.29 (2.69-4.02)	2.16 (1.71-2.74)
12-14	276 (5%)	2509 (2492)	12.64 (9.48-16.86)	4.27 (2.91-6.26)	1237 (1492)	5.28 (4.04-6.90)	3.06 (2.21-4.23)
≥ 15	141 (2%)	3487 (3626)	24.94 (17.17-36.22)	7.76 (4.66-12.92)	1414 (1912)	8.31(5.85-11.80)	4.88 (3.13-7.60)

SD: standard deviation, OR: odds ratio, CI: confidence interval.
* Adjusted for: Left ventricular hypertrophy, preoperative use of ASA, clopidogrel, nadroparin, coumadins, CPB time, pre-operative hematocrit and thrombocyte count.
** Adjusted for: Left ventricular hypertrophy, preoperative use of ASA, clopidogrel, nadroparin, coumadins, CPB time, intraoperative hematocrit, thrombocyte count and end-CPB plasma fibrinogen concentration.

DISCUSSION

With this study we aimed to predict excessive blood loss in cardiac surgery using the variables of the well-known EuroSCORE as the basis for the model. In this study, the EuroSCORE predictors had good performance in predicting the occurrence of excessive intraoperative blood loss in cardiac surgery. The addition of preoperative laboratory variables did not further improve the prediction model. To predict excessive blood loss in the early postoperative period, the performance of the EuroSCORE predictors in combination with relevant intraoperative predictors was lower. The addition of intraoperative laboratory measurements including end-CPB plasma fibrinogen concentration did not improve this model.

Although there are no known models to predict excessive blood loss during (i.e. intraoperative period) cardiac surgery, there are several models predicting transfusion of blood products during surgery, which is an outcome comparable to excessive blood loss, as bleeding and transfusion are closely related.[2-6]

In a study of Ranucci et al. five pre-operative predictors were used (i.e. age > 67 years, weight < 60 kg for females and < 85 kg for males, preoperative hematocrit, female gender and complex surgery) to predict the consumption of allogeneic blood products. This model had an AUC of 0.71 (95% CI: 0.68-0.72).[3] Karkouti et al. used 12 variables (CPB duration, preoperative hemoglobin, body surface area, nadir CPB hematocrit, previous sternotomy, preoperative shock, preoperative platelet count, urgency of surgery, age, surgeon, deep hypothermic circulatory arrest and type of procedure) to predict transfusion of five or more units of RBC in patients who underwent surgery with the use of CPB. This model had an AUC of 0.88.[4] In a study of Alghamdi et al., eight preoperative variables were used (i.e. preoperative hemoglobin, weight, female gender, age, non-elective procedure, preoperative creatinine, previous cardiac surgical procedure, and non-isolated procedure) to predict the outcome of exposure to blood transfusion in the operative and first postoperative days. This model had an AUC of 0.80.[5]

Until now, the EuroSCORE variables have not been incorporated in a model to predict excessive blood loss in cardiac surgery. Instead of developing yet another new prediction model, we choose to use a well-known and commonly used model

to predict excessive intraoperative and early postoperative blood loss in cardiac surgery. Compared to previously reported models, the EuroSCORE model performed very well, especially when predicting intraoperative blood loss.

Several clinical and laboratory variables are known to be associated with red blood cell transfusion, namely age, gender, hematocrit, serum creatinine, body weight, low left ventricular ejection fraction, emergency surgery, cardiopulmonary bypass time, redo surgery, and the individual surgeon.[27, 15-18, 28] Despite the association between these variables and red blood cell transfusion in cardiac surgery, including relevant laboratory variables did not improve the predictive ability of model 1 for excessive intraoperative blood loss. For plasma fibrinogen concentration both the preoperative and intra-operative concentrations are related to postoperative blood loss.[29-32] However, in model 2, the addition end-CPB plasma fibrinogen concentration did not improve the predictive ability of the model. There are limitations to this study. First, it is difficult to measure blood loss accurately during cardiac surgery. However, the method of blood loss measurement during cardiac surgery is daily routine for the surgical team and this method has not changed during the study period. Therefore, these blood loss measurements are as accurate as possible in this domain.

Second, laboratory plasma fibrinogen concentration was not available preoperatively. Addition of this variable might have improved the extended model 1. On the other hand, addition of laboratory plasma fibrinogen concentration did not improve model 2 either.

Another limitation to this study is that the performance of the prediction models was not measured in a different population of cardiac surgery patients (i.e. external validation). An external validation study is a necessary step for the implementation of this model in cardiac surgery procedures in other hospitals and even other countries.

This study has a number of strengths. First, the model is built with a large study population (5490 patients) over a four-year period and the data collection during the study period was performed prospectively which contributed to high quality data with a low percentage of missing data.

In conclusion, the EuroSCORE variables have proven to result in a solid and reliable prediction model for excessive intraoperative blood loss in cardiac surgery. This study also showed that the EuroSCORE variables complemented with known intraoperative predictors is not very robust in predicting excessive postoperative blood loss in the ICU. Furthermore, a higher numerical EuroSCORE is associated with increasing risk for excessive intraoperative and postoperative blood loss.

Authors' contributions

S.B. collected the data, initiated and designed the study, analysed the results and wrote the scientific paper.
J.A.H.d.G. initiated and designed the study, supported with statistic methodology, analysed the results, critically reviewed and wrote the scientific paper.
C.J.K. designed the study, analysed the results and critically reviewed the scientific paper.
A.J.S. analysed the results and critically reviewed the scientific paper.
K.G.M.M. designed the study, supported with statistic methodology, analysed the results, critically reviewed and wrote the scientific paper.
A.P.N. initiated and designed the study, analysed the results, critically reviewed and wrote the scientific paper.

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Chapter 5

The effects of plasma fibrinogen levels on blood loss and transfusion in cardiac surgery

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Submitted

ABSTRACT

Background: The association between end-cardiopulmonary bypass (CPB) plasma fibrinogen and postoperative bleeding and transfusion is unknown.

Study design and methods: A retrospective study of all cardiac surgery procedures in the years 2007-2010. The end-CPB plasma fibrinogen levels were categorized to: [< 1.5 g/L], [1.5 - < 2.0 g/L], [2.0 - < 2.5 g/L], [2.5 - < 3.0 g/L] and [≥ 3.0 g/L]. Excessive postoperative blood loss (in ICU) was defined as ≥ 1000 mL and postoperative transfusion (in ICU) as ≥ 1 unit of either red blood cells, fresh frozen plasma or platelet concentrate. Adverse events were also analyzed. Multivariable regression analysis was used.

Results: 4364 patients were included. End-CPB fibrinogen levels [2.5 - < 3.0 g/L] and [≥ 3.0g/L] were associated lower risk for ≥ 1000 mL ICU blood loss; odds ratios (OR) 0.66 (95% CI 0.45-0.98) and 0.58 (95% CI 0.36-0.92) respectively. End-CPB levels [< 1.5 g/L] and [1.5 - ≤ 2.0 g/L] were not associated with ≥ 1000 mL ICU blood loss with OR 1.32 (95% CI 0.98-1.76) and 1.06 (95% CI 0.83-1.76) respectively. Transfusion of blood products was not associated with end-CPB fibrinogen levels. End-CPB plasma fibrinogen category [< 1.5 g/L] was associated with increased in-hospital mortality OR 3.27 (1.10-9.79) and [> 3.0 g/L] was associated with lower risk for myocardial infarction, OR 0.28 (0.11-0.68).

Conclusion: Higher end-CPB plasma fibrinogen concentrations [≥ 2.5 g/L] reduced the risk for excessive postoperative blood loss in cardiac surgery. End-CPB levels were not associated with postoperative transfusion of blood products.

Keywords Transfusion, blood loss, fibrinogen, cardiac surgery

Plasma fibrinogen (factor I) plays an important role in hemostasis by acting as an endogenous substrate for fibrin formation, promoting clot formation and platelet aggregation by binding platelet glycoprotein IIb/IIIa receptors.[1,2] The average plasma fibrinogen level in humans is 2.0 to 4.5 g/L.[3,4] When major blood loss is substituted with red blood cells (RBC) and colloid fluids, plasma fibrinogen deficiency develops earlier than any other hemostatic abnormality.[5] This condition of coagulopathy is further worsened by hypothermia and acidosis.[6-8] Several studies in cardiac surgery have investigated the association between plasma fibrinogen levels and postoperative blood loss.[9-13] In one small study an inverse association between end-CPB plasma fibrinogen and postoperative blood loss was reported without mentioning which plasma fibrinogen level or range were associated with the risk for bleeding.[14] In cardiac surgery, the plasma fibrinogen concentration measured specifically at the end of cardiopulmonary bypass (end-CPB) is of special interest, as this is a suitable moment for targeted infusion of fibrinogen concentrate or other procoagulants as part of perioperative hemostasis management. Therefore, the association between end-CPB fibrinogen levels and postoperative blood loss remains unclear. The aim of this large single center cohort study was to determine the association between end-CPB plasma fibrinogen levels and post-operative blood loss (≥ 1000 mL) in cardiac surgery procedures. Secondary outcome was the association of end-CPB plasma fibrinogen levels with postoperative transfusion of allogeneic blood products (≥ 1 unit) and the occurrence of clinical adverse events.

MATERIALS AND METHODS

Study design and setting

This study was designed as a cohort study using existing data from a large teaching hospital (Isala Zwolle, The Netherlands). This study was approved by the institutional medical ethical committee, reference number 14.09118, Zwolle, The Netherlands.

Participants

Eligible were all patients undergoing cardiac surgery in the four-year period from 2007 to 2010. Excluded were patients in whom the variable of interest (plasma fibrinogen concentration measured at the end of cardiopulmonary bypass) was not available and patients who received the procoagulant fibrinogen concentrate either during surgery or in the intensive care unit (ICU).

Anesthetic management

During the entire study period, anesthetic management and surgical treatment was performed according to a standard procedure used in the Isala Zwolle in The Netherlands which has been described in detail previously.[15] In short, all patients underwent surgery through a median sternotomy. Myocardial protection during cardiopulmonary bypass was achieved with antegrade blood or crystalloid cardioplegia. One surgeon used the combination of retrograde and antegrade crystalloid cardioplegia for aortic valve surgery. CPB was managed using non-pulsatile flow applied by a centrifugal pump and pH management with the α-stat principle. Heparin was given to achieve an activated clotting time more than 480 seconds during CPB. After weaning from CPB, protamine was administered with ratio 1:1. The transfusion protocol in the ICU during the studied period was as follows; when blood loss in the ICU exceeded 100 mL in the first hour, protamine 25-50 mg was given. Additionally, if APTT was more than 35 seconds and INR more than 1.5, prothrombin complex concentrate was infused. When platelet count was below 75 x 10^9/L, one unit of platelets (five donors) was transfused. If bleeding persisted, tranexamic acid 2 grams followed by 1 gram/hour for five hours and/or desmopressin 30 µg could be infused if not given during surgery. For blood loss in the ICU exceeding 400 mL in 1 hour or exceeding 300 mL per hour for 2 hours or exceeding 200 mL per hour for 3 hours, 4 units RBC, 4 units of fresh frozen plasma (FFP) and 1 bag of platelets (5 donors) was transfused. After surgery, all patients were transferred to the ICU.

Variables

The primary outcome of this study was ICU blood loss defined as more than (≥) 1000 mL chest tube drain production measured from admission to the ICU until

removal of the chest drains. This outcome is in accordance with several studies with cardiac surgery patients in which excessive postoperative blood loss was defined as ICU blood loss of more than 1000 mL.[16-19] The secondary outcome was postoperative transfusion of one or more units of RBC, FFP or platelet concentrate (dichotomous outcome). Regarding the occurrence of clinical adverse events, the following data were collected within 30 days after surgery: in-hospital mortality, myocardial infarction, stroke, transient ischemic attack (TIA), renal injury or failure, re-thoracotomy and prolonged ventilation in the ICU (≥ 24 hours).[20,21] In table 4 the definitions of the adverse events are shown. Plasma fibrinogen concentration, hemoglobin levels and platelet count were measured at the end of cardiopulmonary bypass (end-CPB). Plasma fibrinogen was measured according to Clauss' (turbidometric) method.[22] Activated partial thromboplastin time (APTT) and prothrombin time (PT) were measured immediately at admission to the ICU. The end-CPB plasma fibrinogen concentrations were categorized to the following levels: [< 1.5 g/L], [1.5 - < 2.0 g/L], [2.0 - < 2.5 g/L], [2.5 - < 3.0 g/L] and [≥ 3.0 g/L]. The plasma fibrinogen concentration level [2.0 - < 2.5 g/L] was the reference level, as 2.0 g/L is the lower limit of the normal range 2.0-4.5 g/L.[3,4] Furthermore, plasma fibrinogen concentration above 2.0 g/L was suggested as necessary for optimizing the rate of clot formation and reducing transfusion.[16,23,24]

From admission until hospital discharge, all clinical data of patients undergoing cardiac surgery were prospectively registered in an electronic patient data management system. In the operating room, the attending anesthesiologist documented the patients' demographic data and intraoperative data on a dedicated form and also entered these variables in the electronic database. Clinical data during ICU stay were collected by the ICU medical staff. After the patient's discharge from the hospital, another member of the research team cross-checked all data collection forms to confirm accuracy of the data entry. The 30-day follow-up data was collected by sending the patient a questionnaire asking about his/her current health status. The referring physician was also contacted for further information.

Statistics

Mean and standard deviation (SD) was used for normally distributed continuous variables and median with interquartile range (IQR) of 25^{th} and 75^{th} was used for non-normally distributed continuous variables. Normality was determined with de Kolmogorov-Smirnov statistical test. Frequencies with percentages were used for categorical variables. For differences in categorical variables the Chi-squared test was used and data were presented as odds ratios (OR) with 95% confidence intervals (CI). For differences in continuous variables the independent sample t-test (for normally distributed variables) or Mann-Whitney U test (for non-normally distributed variables) was used.

The binary outcomes were analyzed using logistic regression analysis. In the multivariate model, the variables which were significantly related to the outcome with $p < 0.05$ were included. The variables used in this study were age (years), gender, body mass index (BMI kg/m^2), diabetes mellitus type 1&2, hypertension, left ventricular hypertrophy (LVH), acetylsalicylic acid (ASA) use, clopidogrel use, coumarin use, preoperative serum creatinine (µmol/L), end-CPB values of; hemoglobin (g/dL), platelet count (10^9/L), activated partial thromboplastin time (APTT, seconds) and prothrombin time (PT) and INR (international normalized ratio). Variables related to the procedure were cardiopulmonary bypass time (CPB time, minutes), cell saver use, previous cardiac surgery, indication of surgery (elective, urgent and emergent) and type of surgery (CABG only, valve only, CABG and valve, aortic procedures and 'other'). Although data capture was very high, some patients had missing values for one or more variables ranging from 0.1% (CPB time) to 3.3% (Blood loss in ICU). Missing variables were imputed with inclusion of all other variables including the outcome variables, as it has extensively been reported that imputation of missing covariate data should always be done with all available data including the outcome.[25-27] All analyses were performed with R 2.15.0 (R Foundation for Statistical Computing; www.R-project.org) and SPSS 21.0 (IBM SPSS Statistic, New York, USA).

RESULTS

In total 5490 patients underwent cardiac surgery during the four-year study period. In 494 patients (9%) no end-CPB plasma fibrinogen concentration was measured. There were 406 patients (7%) who received the procoagulant fibrinogen concentrate during surgery (after end-CPB plasma fibrinogen measurement) and 226 (4%) patients who received this procoagulant in the ICU period; these patients were excluded from the analysis. Eventually, 4364 patients (31% female) were included in the study for analysis. Most procedures were elective (94%) and isolated CABG comprised 59% of all procedures. Thoracic aortic surgery was performed in 4% of the total procedures. The mean CPB time was 125 minutes (SD 66 minutes). Mean end-CPB plasma fibrinogen concentration was 2.0 g/L (SD 0.6 g/L).

Table 1 depicts baseline characteristics of the study population and the crude univariate associations for the primary outcome excessive blood loss in the ICU (≥ 1000 mL). The total number of patients with excessive blood loss in the ICU comprised 596 (14%) patients. There was a crude association between end-CPB plasma fibrinogen concentration of < 1.5 g/L and excessive ICU blood loss with an odds ratio of 1.46 (95% CI; 1.13-1.89, p<0.01).

Table 2 shows the adjusted odds ratios and confidence intervals for the outcome excessive postoperative blood loss (≥ 1000 mL) after adjusting the variables gender, BMI, hypertension, ASA use, clopidogrel use, coumarin use, end-CPB platelet count, preoperative serum creatinine, APTT, PT, indication of surgery, cell saver, previous cardiac surgery and type of surgery. There was a significant association between end-CPB fibrinogen levels [2.5 - 3.0 g/L] and [≥ 3.0 g/L] and the outcome ≥ 1000 mL blood loss in the ICU with odds ratios (OR) of 0.66 (95% CI 0.45-0.98) and 0.58 (95% CI 0.36-0.92) respectively.

Table 1. Patient characteristics and crude univariable associations for excessive bleeding in the ICU

	Postoperative blood loss				
	< 1000 mL	≥ 1000 mL			
	N= 3768	N= 596	OR (95% CI)	P-value	Missing
Preoperative characteristics					
age (years), mean (SD)	68 (10)	68 (10)	1.00 (1.00-1.01)	0.44	0
female gender (%)	1186 (32%)	155 (26%)	0.77 (0.63-0.93)	0.01	0
BMI (kg/m^2), mean (SD)	28 (4)	27 (4)	0.94 (0.92-0.96)	<0.01	7 (0.2%)
diabetes mellitus type 1&2 (%)	856 (23%)	120 (20%)	0.86 (0.69-1.06)	0.16	0
hypertension (%)	1928 (51%)	274 (46%)	0.81 (0.68-0.97)	0.02	0
left ventricular hypertrophy (%)	944 (25%)	150 (25%)	1.01 (0.82-1.23)	0.95	0
serum creatinine (µmol/L), mean (SD)	93 (57)	103 (66)	1.00 (1.00-1.00)	<0.01	0
acetylsalicylic acid (%)	2689 (71%)	377 (63%)	0.69 (0.58-0.83)	<0.01	0
clopidogrel (%)	774 (21%)	149 (25%)	1.29 (1.05-1.58)	0.01	0
nadroparin (%)	847 (23%)	163 (27%)	1.30 (1.07-1.58)	0.01	
coumadins (%)	399 (11%)	84 (14%)	1.39 (1.08- 1.78)	0.01	0
previous cardiac surgery	193 (5%)	73 (12%)	2.59 (1.95-3.44)	<0.01	0
Procedure characteristics					
Elective	3605 (96%)	493 (83%)	0.22 (0.17-0.28)	<0.01	1 (0.1%)
Urgent	116 (3%)	53 (9%)	3.07 (2.19-4.31)	<0.01	0
Emergent	47 (1%)	50 (8%)	7.25 (4.82-10.91)	<0.01	0
CPB time in minutes, mean (SD)	119 (57)	162 (99)	1.01 (1.01-1.01)	<0.01	1 (0.1%)
cellsaver use (%)	2409 (64%)	447 (75%)	1.69 (1.39-2.06)	<0.01	0
Type of procedure					
CABG only	2289 (61%)	282 (47%)	0.58 (0.49-0.69)	<0.01	0
Valve only	734 (19%)	83 (14%)	0.67 (0.52-0.86)	<0.01	0
CABG and valve	596 (16%)	171 (29%)	2.14 (1.76-2.61)	<0.01	0
Aorta	110 (3%)	52 (9%)	3.18 (2.26-4.48)	<0.01	0
Other	39 (1%)	8 (1%)	1.30 (0.61-2.80)	0.50	0
Hematology					
End-CPB hemoglobin (g/dL), mean (SD)	9.1 (1.3)	9.0 (1.3)	0.95 (0.88-1.01)	0.10	14 (0.3%)
End-CPB platelet count (10^9/L), mean (SD)	150 (48)	137 (82)	0.99 (0.99-1.00)	<0.01	21 (0.5%)
ICU admission APTT (s), mean (SD)	30 (6)	32 (8)	1.04 (1.03-1.05)	<0.01	59 (1.4%)
ICU admission PT (INR), mean (SD)	1.2 (0.2)	1.3 (0.3)	5.31 (3.42-8.25)	<0.01	60 (1.4%)

End-CPB plasma fibrinogen concentration, g/L					
< 1.5	615 (16%)	131 (22%)	**1.46 (1.13-1.89)**	<0.01	0
1.5 - < 2.0	1622 (43%)	249 (42%)	1.05 (0.84-1.31)	0.66	0
2.0 - < 2.5 *	952 (25%)	139 (23%)	Reference	-	0
2.5 - < 3.0	356 (10%)	46 (8%)	0.89 (0.62-1.26)	0.50	0
≥ 3.0	223 (6%)	31 (5%)	0.95 (0.63-1.44)	0.82	0

OR; odds ratio, CI; confidence interval, BMI; body mass index, CPB; cardiopulmonary bypass, CABG; coronary artery bypass graft, ICU; intensive care unit, APTT; activated partial thromboplastin time, PT; partial thromboplastin time.
*: Reference category.

Table 2. Adjusted associations of end-CPB plasma fibrinogen levels with excessive postoperative blood loss and transfusion.

Fibrinogen concentration end-CPB (g/L)	Blood loss ICU ≥ 1000 mL	RBC transfusion	FFP transfusion	Platelets transfusion
< 1.5	1.32 (0.98-1.76)	0.91 (0.72-1.15)	1.34 (0.94-1.92)	1.22 (0.85-1.76)
1.5 - < 2.0	1.06 (0.83-1.76)	0.96 (0.80-1.16)	1.13 (0.84-1.53)	0.98 (0.72-1.35)
2.0 - < 2.5 *	1	1	1	1
2.5 - < 3.0	**0.66 (0.45-0.98)**	1.25 (0.96-1.63)	1.07 (0.69-1.68)	1.11 (0.68-1.79)
≥ 3.0	**0.58 (0.36-0.92)**	0.90 (0.65-1.24)	0.58 (0.32-1.08)	0.63 (0.34-1.24)

Odds ratio (OR) with 95% confidence interval. RBC; red blood cells, FFP; fresh frozen plasma.
Blood loss ≥ 1000 mL adjusted for; gender, BMI, hypertension, ASA use, clopidogrel use, coumarin use, end-CPB platelet count, preoperative serum creatinine, APTT, PT, indication of surgery, cell saver, previous cardiac surgery and type of surgery.
RBC transfusion adjusted for; age, gender, BMI, diabetes mellitus type 1&2, clopidogrel use, end-CPB levels of hemoglobin, APTT and PT, preoperative serum creatinine, indication of surgery, previous cardiac surgery, type of surgery and CPB time.
FFP transfusion adjusted for; BMI, hypertension, LVH, ASA use, coumarin use, end-CPB levels of hemoglobin, platelet count, APTT and PT, indication of surgery, cell saver use, previous cardiac surgery, type of surgery and CPB time.
Platelets transfusion adjusted for; age, BMI, diabetes type 1&2, hypertension, LVH, ASA use, end-CPB levels of hemoglobin, platelet count, APTT and PT, indication of surgery, cell saver use, previous cardiac surgery, type of surgery and CPB time
*: Reference category

Transfusion

In the postoperative ICU period, 1244 (29%) patients received one or more RBC transfusions, 363 (8%) patients received one or more FFP transfusions and 324 (7%) patients received one or more platelet transfusions. Table 2 shows the adjusted odds ratios and confidence intervals for the binary outcomes postoperative RBC, FFP and platelets transfusions. The variables in the regression model for each outcome are depicted in the legend of table 2. In the multivariable adjusted analysis there was no association between end-CPB plasma fibrinogen concentration and postoperative RBC, FFP or platelets transfusion.

Adverse events

Table 3 depicts the adjusted association between end-CPB fibrinogen concentration and the clinical adverse events. End-CPB plasma fibrinogen level [< 1.5 g/L] was associated with higher risk for in-hospital mortality with an OR of 3.27 (CI; 1.10 - 9.79). End-CPB plasma fibrinogen level of [2.5 - < 3.0 g/L] was associated with higher risk for renal injury or failure with an OR of 2.30 (CI; 1.28 - 4.12). End-CPB plasma fibrinogen [≥ 3.0 g/L] was associated with lower risk for myocardial infarction with OR of 0.28 (CI; 0.11-0.68) while the risk for prolonged mechanical ventilation was increased with an OR of 2.28 (CI; 1.24 - 4.19).

Table 3. Adjusted associations of end-CPB plasma fibrinogen levels with clinical adverse events.

Fibrinogen concentration end-CPB (g/L)	In-hospital mortality	Myocardial infarction	CVA or TIA	Renal injury or failure	Rethoracotomy	Prolonged ventilation
< 1.5	**3.27 (1.10-9.79)**	0.92 (0.60-1.41)	1.28 (0.58-2.83)	0.88 (0.45-1.72)	1.27 (0.80-2.02)	1.09 (0.62-1.91)
1.5 - < 2.0	1.24 (0.41-3.78)	1.06 (0.76-1.48)	0.51 (0.24-1.09)	1.24 (0.75-2.03)	0.98 (0.66-1.45)	0.84 (0.52-1.35
2.0 - < 2.5 *	1	1	1	1	1	1
2.5 - < 3.0	2.44 (0.65-9.11)	0.59 (0.33-1.013)	0.86 (0.31-2.44)	**2.30 (1.28-4.12)**	0.99 (0.56-1.74)	1.26 (0.68-2.34)
≥ 3.0	2.20 (0.52-9.40)	**0.28 (0.11-0.68)**	1.42 (0.53-3.82)	1.49 (0.72-3.06)	1.32 (0.71-2.44)	**2.28 (1.24-4.19)**

Adjusted for: age, gender, BMI, hypertension, diabetes mellitus type 1 & 2, ASA use, clopidogrel use, coumarin use, end-CPB levels of hemoglobin, platelet count, APTT and PT, preoperative serum creatinine, cell saver use, indication of surgery, previous cardiac surgery, type of surgery and CPB time

Table 4: Clinical adverse events and definitions

In-hospital mortality	All-cause mortality within 30 days after the initial cardiac surgery procedure
Myocardial infarction	CKMB value ≥120 U/L (five times upper reference limit) plus a peak CKMB/CK ratio >10%, or pathological new Q waves on a postoperative electrocardiogram
CVA	New motor or sensory deficit of the central nervous system or an unexplained coma status lasting ≥ 24 hours
TIA	Neurological dysfunction from focal temporary cerebral ischemia lasting < 24 hours, no cerebral infarction
Renal injury/failure	Renal injury and failure was defined by the RIFLE criteria
Rethoracotomy	Re-thoracotomy within 30 days after initial cardiac surgery
Prolonged ventilation	Mechanical ventilation after initial surgery lasting longer than 24 hours in the ICU period

DISCUSSION

The main finding of this large cohort study was that end-CPB plasma fibrinogen levels of [2.5 - < 3.0 g/L] and [≥ 3.0 g/L] reduced the risk for excessive postoperative blood loss (≥ 1000 mL) in the ICU. There was no end-CPB plasma fibrinogen level related to increased risk for excessive postoperative blood loss. End-CPB plasma fibrinogen levels were not associated with postoperative transfusion of RBC, FFP or platelet concentrate. Regarding clinical adverse events, end-CPB plasma fibrinogen levels of [< 1.5 g/L] were associated with increased risk for in-hospital mortality, while higher plasma fibrinogen levels of [2.5 - < 3.0 g/L] were associated with increased risk for renal insufficiency or failure. End-CPB plasma fibrinogen levels [≥ 3.0 g/L] were associated with reduced the risk for myocardial infarction and increased risk for prolonged mechanical ventilation (≥ 24 hours) in the ICU.

The results of this study differ from those reported by Waldén et al. who investigated the association between preoperative fibrinogen plasma concentrations and excessive blood loss and RBC transfusion in cardiac surgery. [12] They observed that a preoperative plasma fibrinogen concentration lower than 2.5 g/L (critical level) was associated with excessive ICU blood loss. In contrast, in the current study there was no critical plasma fibrinogen level determined for excessive ICU blood

loss. Moreover, plasma fibrinogen levels ≥ 2.5 g/L at end-CPB reduced the risk for excessive postoperative blood loss.

There was no association between end-CPB fibrinogen levels and transfusion of any type of blood product. One possible explanation might be that, as blood loss and transfusion is related, in this study end-CPB plasma fibrinogen levels were not associated with excessive ICU blood loss and consequently did not increase the risk for transfusion of any of blood product. In addition, there was a restricted use of blood product in our hospital.[28]

In several studies, infusion of fibrinogen concentrate resulted in reduced blood loss and transfusion of allogeneic blood products. Rahe-Meyer et al investigated in a randomized, placebo-controlled trial the effects of fibrinogen infusion on post-operative transfusion in the highly complex procedure of thoracic aorta surgery. In this study, 10 patients with a mean (SD) plasma fibrinogen concentration of 2.2 (0.6) g/L, received a mean (SD) fibrinogen concentrate dose of 5.7 (0.7) g which led to an upper normal level plasma fibrinogen concentration of 3.6 g/L. In this study significant reductions in 24-hour postoperative bleeding and transfusion requirements were reached.[29] In a study of Ranucci et al. higher plasma fibrinogen levels of 3.7 (IQR 3.3;4.1) g/L after infusion of fibrinogen concentrate also led to reductions is postoperative blood loss and transfusion.[30] In these studies, high normal levels of plasma fibrinogen were reached after infusion of fibrinogen concentrate. The current study shows that plasma fibrinogen levels of [2.5 - < 3.0 g/L] might prevent excessive postoperative blood loss. This level is above the recommended plasma fibrinogen concentration in recent guidelines. In earlier guidelines, the critical plasma fibrinogen level in acquired hypofibrinogenemia was set at < 1.0 g/L.[31,32] In more recent guidelines this critical level for plasma fibrinogen was raised to 1.5 to 2.0 g/L.[33-35] However, the critical levels in these guidelines were determined for a variety of surgical procedures including trauma and pediatric surgery and not specifically for cardiac surgery. On the other hand, high normal end-CPB plasma fibrinogen levels might pose a thrombo-embolic risk. Although two recent studies mentioned a low risk of adverse events for low dose fibrinogen concentrate infusions, the effects of high-normal levels of plasma fibrinogen concentration on the occurrence of (thrombo-embolic) adverse events remains unknown.[36,37]

There are some limitations to this study. First, this study was a retrospective study which may introduce selection bias or recall bias. However, the hospital had a stringent perioperative data collection protocol, where data from each patient were prospectively registered in a patient data management system during both the surgery and ICU period.

Second, patients without end-CPB measurement of plasma fibrinogen concentrations were excluded from the analysis. Most patients in whom end-CPB plasma fibrinogen concentrations had not been measured underwent low-risk cardiac surgery; of the 494 patients who were excluded, 228 (46%) patients underwent off-pump CABG and 87 (18%) patients were in the rest (remaining procedures) group of which 60% underwent arrhythmia surgery. In these procedures (off-pump CABG and rhythm surgery) no CPB was used. In order to relate specifically the end-CPB plasma fibrinogen concentrations to the study outcomes, only patients with end-CPB plasma fibrinogen were included. Finally, patients who had received the procoagulant fibrinogen concentrate were also excluded from the analysis. As this procoagulant is given after the measurement of plasma fibrinogen concentration end-CPB, the associated with the study outcomes may be distorted and/or diluted. In order to relate specifically plasma fibrinogen concentrations to postoperative blood loss, postoperative transfusion and the occurrence of clinical adverse events, any exogenous intervention with concentrated fibrinogen was excluded. This may have led to exclusion of many of the actively bleeding patients. However, this study reports on a large cohort of patients (n= 4364) that includes various types of cardiac surgical procedures and varying levels of acuity (elective, urgent or emergent).

In conclusion, end-CPB plasma fibrinogen concentrations of [2.5 to < 3.0 g/L] and [≥ 3.0 g/L] were associated with a reduced the risk for excessive postoperative blood loss in the ICU. End-CPB plasma fibrinogen levels were not associated with excessive postoperative blood loss in the ICU. End-CPB plasma fibrinogen concentrations were not associated with postoperative RBC, FFP or platelets transfusion in a 'restrictive' transfusion practice setting. Regarding adverse clinical events, low end-CPB fibrinogen levels [< 1.5 g/L] were associated with in-hospital mortality, which may be an epiphenomenon or causal. Higher end-CPB plasma fibrinogen levels [2.5 - < 3.0 g/L] might increase the risk for renal injury or failure, while on

the other hand the risk for myocardial infarction appeared to be reduced with [≥ 3.0 g/L]. The clinical relevance of these observations needs to be confirmed in prospective studies.

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ATTRIBUTION FOR THE WORK

S.B. initiated and designed the study, analysed the results and wrote the scientific paper.

J.A.H.d.G. initiated and designed the study, supported with statistic methodology, analysed the results, critically reviewed and wrote the scientific paper.

C.J.K. designed the study, analysed the results and critically reviewed the scientific paper.

A.J.S. designed the study and critically reviewed the scientific paper.

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Chapter 6

Effect of fibrinogen concentrate on perioperative blood loss among patients with coagulopathic bleeding during high-risk cardiac surgery: a randomized clinical trial

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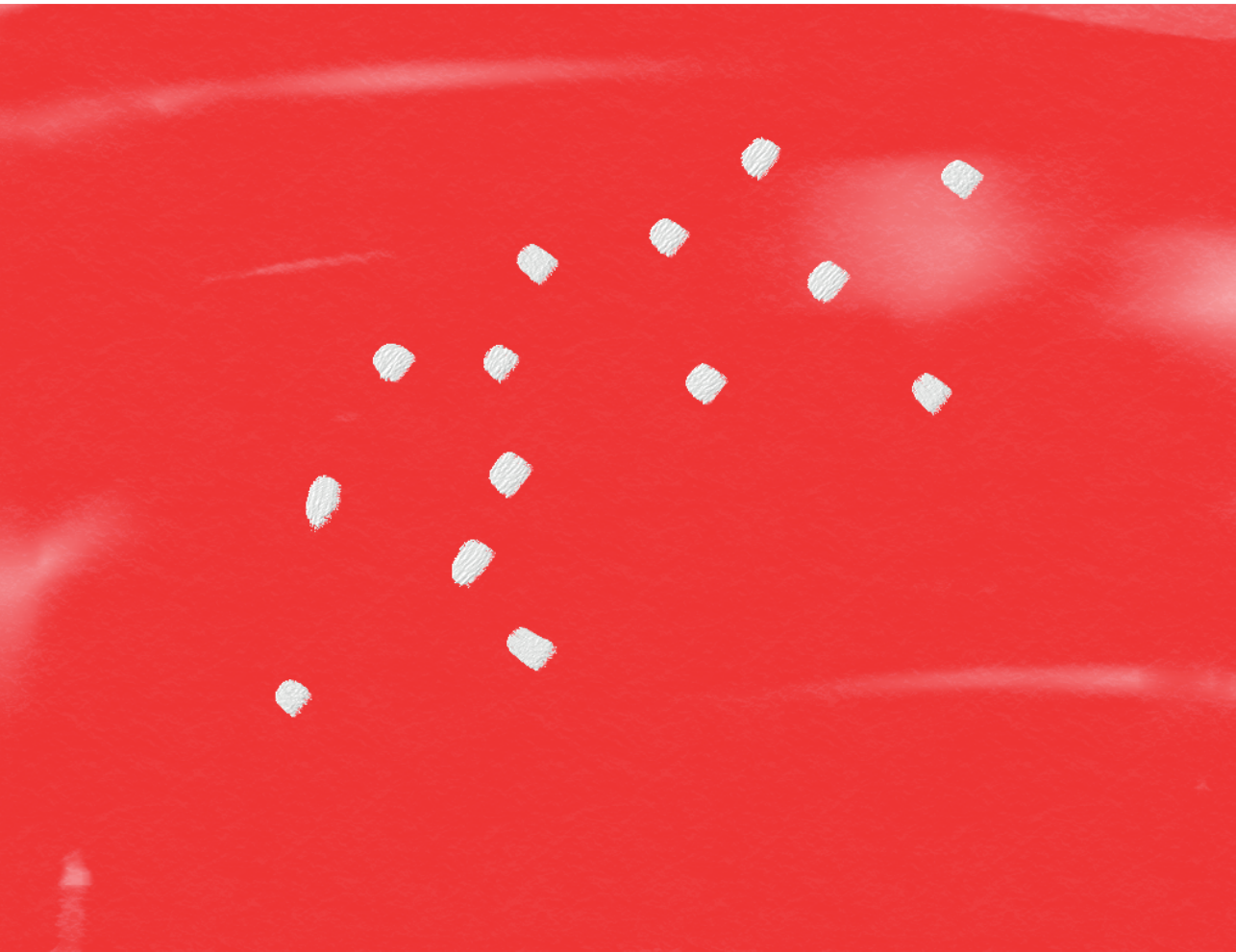

ABSTRACT

Importance: Fibrinogen concentrate might partly restore coagulation defects and reduce perioperative bleeding.

Objective: To determine whether fibrinogen concentrate infusion (targeted plasma level 2.5 g/L) in high-risk cardiac surgery patients with coagulopathic bleeding reduces perioperative blood loss.

Design, setting, and participants: In this randomized, placebo-controlled, double-blind clinical trial conducted in Isala Zwolle, The Netherlands. Patients undergoing elective, high-risk cardiac surgery (i.e. combined CABG and valve(s), multiple valves, aortic root, ascending aorta or aortic arch surgery) with coagulopathic bleeding (blood volume between 60 and 250 mL suctioned from the thoracic cavity in a period of 5 minutes) were randomized to receive either fibrinogen concentrate or placebo.

Interventions: Fibrinogen concentrate (n=60) or placebo (n=60), intravenous route, single-dose infusion based on formula, targeted at post-infusion plasma fibrinogen level of 2.5 g/L.

Main outcomes and measures: Primary outcome was blood loss in mL between intervention (infusion of study medication after removal of CPB) and closure of chest.
Secondary outcome was blood loss (mL) measured upon admission in the ICU as chest tube drainage volume collected after 1, 3, 6, 12 and 24 hours. Data was collection between February 2011 and January 2015.

Results: Among 120 patients (mean (SD) age; 71 (10) years, 37 (31%) females) included in the study, CABG with valves comprised 72% of procedures, mean (SD) cardiopulmonary bypass time was 200 (83) minutes. For the primary outcome,

median blood loss in the fibrinogen group was 50mL (IQR 29;100) compared to 70mL (IQR 33;145) in the control group (p= 0.190).
For the secondary outcome, cumulative median ICU blood loss within 24 hours in the fibrinogen group was 570 mL (IQR 390;730) compared to 690 mL (IQR 440;1090) in the control group (p= 0.047). There were 6 cases of stroke or transient ischemic attack (4 in fibrinogen group), 4 myocardial infarctions (3 in fibrinogen group) and 2 cases of mortality (fibrinogen group).

Conclusions and relevance: Among patients with coagulopathic bleeding during high-risk cardiac surgery, administration of fibrinogen concentrate, compared with placebo, resulted in no significant difference in the amount of perioperative blood loss.

Excessive bleeding is one of the most common complications in cardiac surgery with a minority of patients (15% to 20%) consuming more than 80% of all blood products.[1] As each unit of red blood cells transfused is associated with increased risk for an adverse outcome, a more judicious use of blood products is necessary.[2-5] To further reduce the use of allogeneic blood products in cardiac surgery, coagulation factor replacement therapies have been considered as substitutes. Plasma fibrinogen (factor I) plays a key role in hemostasis by acting as an endogenous substrate for fibrin formation, promoting clot formation and platelet aggregation by binding platelet glycoprotein IIb/IIIa receptors.[6,7] The procoagulant fibrinogen concentrate is increasingly used in surgical patients with coagulopathic bleeding, a condition in which the blood's ability to clot is impaired, refractory to conventional hemostasis treatment.[8]

To our knowledge, only two studies have investigated the use of fibrinogen concentrate in cardiac surgery in a randomized clinical trial. One study had a small patient population with total 60 patients and the other study had a first-line fibrinogen concentrate infusion based on thromboelastometry parameters and not based on ongoing coagulopathic bleeding.[9,10] As a result, it is still debated what the effect of fibrinogen concentrate is on blood loss and transfusion in cardiac surgery.

The aim of this study was to determine whether fibrinogen concentrate infusion targeted at plasma fibrinogen level of 2.5 g/L in high-risk cardiac surgery patients with coagulopathic bleeding, reduced perioperative blood loss.

METHODS

Study design and study population

This study was a randomized, placebo-controlled and double-blind clinical trial of fibrinogen concentrate (Haemocomplettan® P, CSL Behring, Marburg, Germany) versus placebo for the treatment of coagulopathic bleeding during high-risk cardiac surgery. The study was conducted in the Isala Zwolle, a large university-affiliated teaching hospital in The Netherlands. The study protocol was approved on August 23, 2010 by the institutional medical ethical committee and was conducted in ac-

cordance with the principles of the Declaration of Helsinki and the International Conference on Harmonisation of Good Clinical Practice guidelines. Recruitment of patients was between February 2011 and December 2014. On February 2011 the protocol was amended with minor changes.

Eligible for study participation were patients aged 18 years or older who underwent elective high-risk cardiac surgery defined as combined CABG and valve(s), multiple valves, aortic root, ascending aorta or aortic arch surgery. Specific exclusion criteria were proof or suspicion of a congenital or acquired coagulation disorder, any stroke or myocardial infarction in the 2 months preceding surgery, manifest venous or arterial thrombosis, clopidogrel use in the 5 days preceding surgery, GPIIb/IIIa receptor antagonist use in the 2 days preceding surgery and INR (international normalized ratio) of >1.4 in patients who used coumarins. Eligible patients were informed and asked for signed informed consent one week prior to surgery date with an information letter approved by the institutions' medical ethical committee. One day prior to surgery, trained research nurses screened the eligible patients for participation in the study.

The study data were prospectively collected starting 1 day prior to surgery and ending at 30-day follow-up with collection of information on adverse events by sending a fill-in form questioning the clinical condition and if necessary by contacting the referring physician. The referring physician was contacted in case the patient did not return the follow-up fill-in form after it was sent for the third time. When the patient had documented an event in the fill-in form, the referring physician was also contacted for more details of the event.

During the entire study period, anaesthetic management and surgical treatment was performed according to the same standard procedures as described previously.[11] Antifibrinolytic prophylaxis was given to all study participants (i.e. both fibrinogen and placebo group) as part of the surgical procedure. After the infusion of study medication, the criteria for transfusion were based on a transfusion protocol introduced at the Isala Zwolle in the year 2009, specifically designed for cardiac surgery procedures.[12] In short, one unit of red blood cells (RBC) was administered when hemoglobin (Hb) level was lower than 8.5 g/dL. When plasma loss was more than one or two liters, two or four units of fresh frozen plasma

(FFP) were transfused respectively. A platelet count of less than 80 x 10^9/L was transfused with one unit of platelet concentrate (five donors). The transfusion protocol in the ICU was as follows. For blood loss in the ICU exceeding 400 mL in 1 hour or exceeding 300 mL per hour for 2 hours or exceeding 200 mL per hour for 3 hours, 4 units RBC, 4 units FFP and 1 unit of platelet concentrate (five donors) was transfused. The attending surgeon was also informed. For a patient with platelet count below 75x10^9/L, 1 unit of platelet concentrate (five donors) was transfused.

Intervention

Reconstitution of fibrinogen concentrate and placebo were carried out under aseptic conditions by trained personnel of the research team. Solutions that were cloudy or contained residues (deposits or particles) were not used. Fibrinogen concentrate was diluted in 50 mL of sterile water (room temperature). For placebo, Albumin (Albuman, 200 g/l, Sanquin CLB, Amsterdam, The Netherlands) was diluted with NaCl 0.9% (room temperature) and contained 2 g in a 50 mL syringe. All study medication was administered through an 18 Gauge peripheral intravenous line in the brachial vein. After ending CPB and after a period of surgical hemostasis, patients were randomized to either fibrinogen concentrate (fibrinogen group) or placebo (control group) only if clinically relevant coagulopathic bleeding was experienced. Clinically relevant coagulopathic bleeding was determined by a "5-minute bleeding volume test" by suctioning of blood from the thoracic cavity (pleura and pericardium) for a period of 5 minutes. Volumes below 60 mL were considered "dry" and thus had "no coagulopathic bleeding"; these participants were excluded from the study. Volumes above 250 mL were considered "surgical bleeding" for which participants underwent additional surgical hemostasis followed by repetition of the 5-minute bleeding volume test. Volumes between 60 and 250 mL were diagnosed as clinically relevant coagulopathic bleeding. This method was also used in previous studies for determining clinically relevant coagulopathic bleeding.[13,14] Before the "5-minute bleeding volume test", the following clinical conditions were required to standardize the hemostatic condition of every study participant: activated clotting time (ACT) < 140 seconds, body temperature > 36°C, blood pH > 7.30 and hemoglobin (Hb) > 8.5 g/dL (or > 5.3 mmol/L) or hematocrit (Ht) > 25%. In

case the "5-minute bleeding volume test" was positive for coagulopathic bleeding, patients were randomized to receive fibrinogen concentrate or matching placebo with a dose which was calculated for both groups based upon plasma fibrinogen levels at the end of CPB, measured with Clauss' method (turbidometric assay).[15] The post-infusion target plasma fibrinogen concentration was 2.5 g/L. This dosing regimen was based on available in vitro and in vivo literature.[13,16-20] The time-point "intervention" was defined as the moment of infusion of study medication (fibrinogen concentrate or placebo), after removal of CPB. The formula used for dosing of fibrinogen concentrate was:

(2.5 − [plasma fibrinogen level end-CPB, g/L]) x 0.07 x (1-Ht on CPB) x body weight (kg) = whole grams fibrinogen concentrate to be dosed.

This formula was based on a formula using thromboelastometry variables.[13,14,21] In this trial, the plasma fibrinogen concentrations instead of thromboelastometry variables were used to calculate the target post-infusion plasma fibrinogen concentrations. Therefore, a modification of the existing formula was needed, based on circulating plasma volume calculated by weight and corrected for hematocrit as a safety margin.

Mode of administration was single-dose intravenous infusion. The post-infusion plasma fibrinogen concentration was determined immediately after admission to the ICU and no additional doses of fibrinogen concentrate were given during the study. The post-infusion plasma fibrinogen concentrations were blinded (censored, not shown in laboratory results) for all personnel until official unblinding of the trial. Patients with end-CPB plasma fibrinogen levels above 2.5 g/L, thus creating a negative formula outcome, were not randomized to receive fibrinogen concentrate or placebo.

Study outcomes

The primary outcome of this study was blood loss (mL) measured between intervention (i.e. infusion of study medication after removal of CPB) and closure of the chest at the end of surgery. This period is relatively short compared to the whole surgery period as the intervention is at the end of surgery. The blood loss was measured using a separate collector with an accurate scale (mL) connected to

the cell saver. Reading of the collector was based on consensus by two personnel present in the operating room. All observers were blinded to the study drug assignment.

As the period of the primary outcome (i.e. blood loss between infusion of the study medication and closure of the chest was) was relatively short, blood loss (mL) measured upon admission in the ICU as chest tube drainage volume collected after 1, 3, 6, 12 and 24 hours was measured as secondary outcome.

Further exploratory outcomes assessed were the proportion of patients transfused and units transfused with RBC, FFP, platelet concentrate and any blood product administered between intervention and closure of chest and between intervention and 24 hours thereafter and units of procoagulants and antifibrinolytics (tranexamic acid, desmopressin, prothrombin complex concentrate, recombinant factor VIIa and fibrinogen concentrate) given in peri- and postoperative period.

To evaluate safety and tolerability of fibrinogen concentrate, the following exploratory variables regarding clinical adverse events were collected: in-hospital mortality (30 days), myocardial infarction (30 days), cerebrovascular accident (CVA) or transient ischemic attack (TIA) (30 days), renal insufficiency or failure (30 days), venous thromboembolism (30 days), pulmonary embolism (30 days), allergic or other systemic reaction to study medication (30 days), sternal or wound (venectomy site) infections (30 days) and rethoracotomy (within 5 days of initial surgery). As an additional safety measure, thrombosis in the lower extremity was diagnosed with compression ultrasound testing 1 day prior to surgery and at day 3 after surgery. The exact definitions of the clinical adverse events are shown in online supplementary table 1.[22,23]

Sample size calculation

The sample size was calculated based on total intraoperative blood loss during cardiac surgery (i.e. from start of surgery to closure of the chest) of retrospective analysis of Isala Zwolle data. The median total intraoperative blood loss (including blood loss as measured with the cell saver) was 2200 mL.

It was expected that this could be reduced by about 40% to 1350 mL. The 40% reduction in blood loss was based on earlier studies with porcine models and more

recent studies with cardiac surgery procedures.[13,14,24,25] Based on these criteria and using a power of 80% and an overall level of significance of 0.05, 53 patients were required in each group, making 106 patients in total. Taking into account potential loss to follow-up, 120 participants were included in the study.

Randomization and blinding

The participants were randomized with a web-based randomization protocol using unstratified fixed block size of 4. The order of blocks was also randomized. Much attention was given to ensure strict blinding during the randomization process, the infusion of study medication (placebo or fibrinogen concentrate), the follow-up period, during data collection and during the data analysis. The medical team involved in the entire surgical process (i.e. in the operating room, at the ICU and at the ward) were blinded for the allocated treatment. The following measures were taken to ensure blinding of the treatment allocation; only the research team had access to a separate room, using a personal badge for entrance, to access the web-based randomization protocol and to prepare the study medication. To avoid noticing and identifying the treatment allocation based on the volume, temperature, color and viscosity of the infused study medication, the prepared number of syringes for both treatment groups were calculated using the same dosing formula and were delivered in amber colored syringes of 50 mL (50 mL, Luer-Lok™ BD Plastipak™ Spain) and diluted albumin was used as placebo to equal the protein load (2 g in 50mL). In addition, in the first 24 hours after infusion of study medication, the hematology measurements of plasma fibrinogen concentration were censored until official unblinding of the trial, at the final stage of the statistical analysis.

Statistical analysis

Mean and standard deviation was used for normally distributed continuous variables and median with interquartile range (IQR) was used for non-normally distributed continuous variables. Normality was determined with de Kolmogorov-Smirnov statistical test. Frequencies with percentages were used for categorical variables. Non-normally distributed primary and secondary blood loss outcomes were log-transformed (i.e. log 10) and both were presented using medians with

interquartile ranges (IQR). For estimating differences between the fibrinogen group and the control group, univariate linear regression analysis was used with log-transformed blood loss as dependent variable and treatment as the only (dichotomous) covariate. Given the small size of the study and possible remaining differences between groups after randomization a pre-planned multivariable linear regression analysis was performed to adjust for the primary outcome using the variables age, gender, EuroSCORE (numerical),[26] lowest core temperature and cardiopulmonary bypass time.

Between-group comparisons of the secondary outcome, blood loss at the ICU, were conducted using a mixed model for repeated measurements. The outcome was the amount of blood loss in each interval, which was log-transformed because of the skewness in values. The models included group (i.e. fibrinogen or placebo) and time as fixed categorical covariates, as well as the group × time interactions. An unstructured covariance matrix was used to account for the correlation of blood loss between time points within individuals as it provided the best model fit according to Akaike's information criteria. The results of this secondary outcome were based on between-group comparisons at 1, 3, 6, 12 and 24 hours after admission on the ICU, estimated with the appropriate contrasts from the mixed model. No adjustment for multiple comparisons was performed. As exploratory analyses, differences in proportion of patients transfused between the fibrinogen and placebo groups were described, as well as units of transfusion between intervention and 24 hours after using medians with 10^{th} and 90^{th} percentiles. In addition, differences in use of procoagulants and antifibrinolytics and clinical adverse event occurrence between the two groups were described. P-values < 0.05 were considered statistically significant. Missing data on the primary outcome are assumed missing completely at random and were excluded from the analysis. Missing values on the secondary outcome (i.e. blood loss at ICU) will be handled automatically within the mixed model for repeated measurements.

After 50% of the outcomes were collected, a planned interim analysis of efficacy on blinded data was performed by the Data and Safety Monitoring Board. The O'Brien-Fleming type criterion was used to control the overall Type I error. The interim analysis was done at a two-sided α-level of 0.003 (0.3%) and the final analysis was done at a level of 0.049 (4.9%).

All analyses were performed using R 2.15.0 (R Foundation for Statistical Computing; www.R-project.org) and SPSS 21.0 (IBM SPSS Statistic, New York, USA). The statistical analysis plan (SAP) is available as a supplementary file.

RESULTS

In Figure 1, the flow of study participants is shown. Data collection started in February 2011. Follow-up of all patients was completed in January 2015. During the study period, 647 patients that underwent elective high-risk cardiac surgery were found eligible to participate in the trial. After initial screening, 203 patients were willing to participate and gave informed consent. During surgery, 73 patients who had no coagulopathic bleeding, 7 patients who had major surgical complications and 3 patients in which no 5-minute bleeding volume test was performed were excluded. There were 120 patients who were diagnosed with coagulopathic bleeding after removal of CPB, and were randomized to placebo or fibrinogen concentrate. There were 5 patients with missing data for the primary outcome; 2 in the fibrinogen group and 3 in the control group. These participants were deleted from the analysis of the primary endpoint. The 30-day follow-up data regarding clinical adverse events was complete for all 120 patients with two patients for whom adverse events data was collected after one year. In august 2013, an interim analysis was performed on all outcomes (primary, secondary and exploratory/safety outcomes). No statistically significant differences were measured. The Data and Safety Monitoring Board (DSMB) concluded that the trial should continue. Among 120 patients (mean (SD) age; 71 (10) years, 37 (31%) females) included in the study, the most common operation was CABG with valves (72%) and the mean (SD) cardiopulmonary bypass time was 200 (83) minutes. Table 1 shows the baseline characteristics for each group. In the fibrinogen group, the mean infused dose of fibrinogen concentrate was 3.1 (95% CI 2.7-3.5) gram which resulted in a mean plasma fibrinogen concentration after intervention (at arrival in the ICU) of 2.3 (95% CI 2.2-2.4) g/L. In the placebo group, the mean plasma fibrinogen concen-

tration at arrival in the ICU was 1.7 (95% CI 1.6-1.8) g/L. Figure 2 depicts the plasma fibrinogen levels during the first three days after surgery.

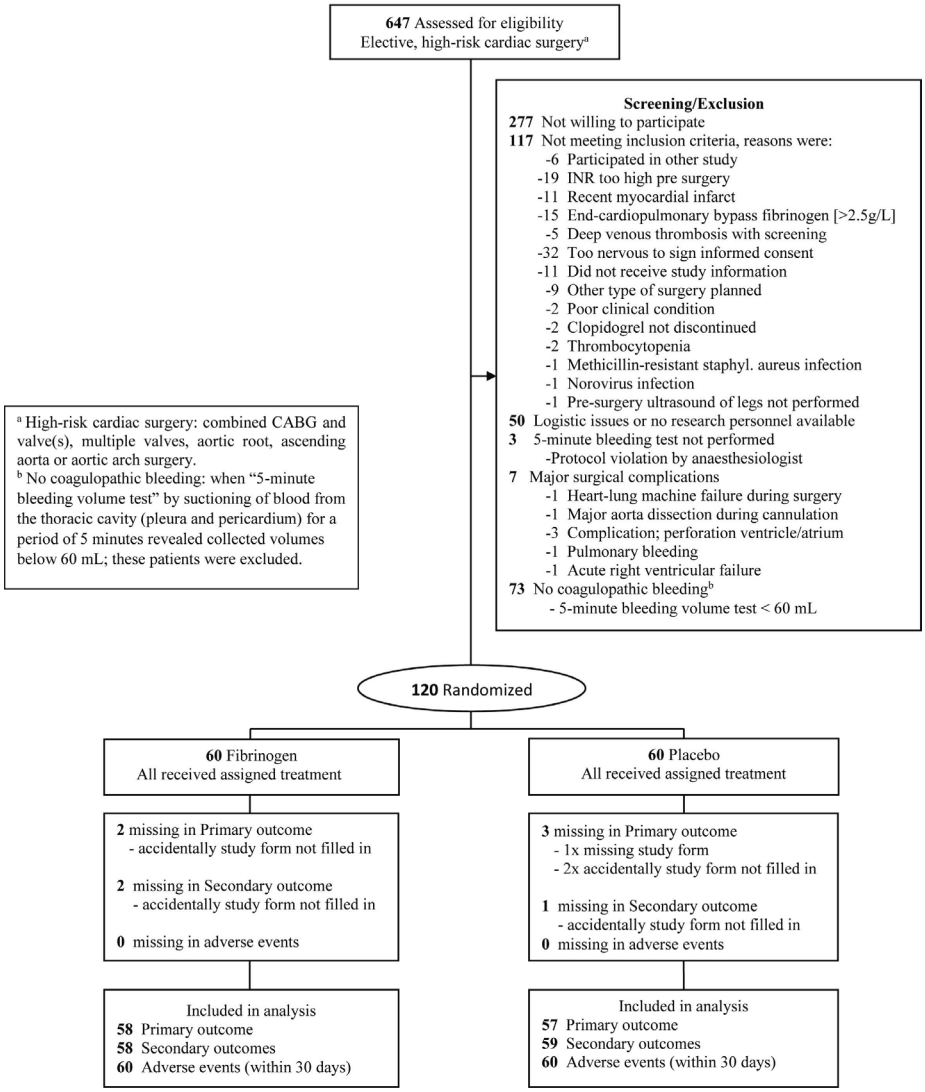

Figure 1. Flow diagram of progress through phases of a randomized trial comparing fibrinogen concentrate vs placebo for coagulopathic bleeding among patients undergoing high-risk cardiac surgery

Table 1. Baseline and Patient Characteristics

	Fibrinogen (n = 60)	Control (n = 60)
Age, mean (SD), y	70 (10)	72 (10)
Female gender, No. (%)	21 (35%)	16 (27%)
Body surface area in m^2, (SD)	1.96 (0.19)	1.99 (0.22)
EuroSCORE, mean (SD)*	7 (3)	7 (2)
Diabetes type 1 or 2, No. (%)	16 (27%)	19 (32%)
Hypertension, No. (%)	38 (63%)	41 (68%)
Left ventricular hypertrophy, No. (%)	27 (45%)	32 (53%)
Preoperative use of anticoagulants, No. (%)		
Aspirin	31 (52%)	33 (55%)
Clopidogrel	0 (0%)	1 (2%)
Nadroparin	8 (13%)	6 (10%)
Coumarines	16 (27%)	10 (17%)
Left ventricular ejection fraction, No. (%)		
<30%	9 (15%)	5 (8%)
30-50%	18 (30%)	10 (17%)
>50%	33 (55%)	45 (75%)
Previous cardiac surgery, No. (%)	5 (8%)	4 (7%)
Type of surgery, No. (%)		
CABG and valve(s)	43 (72%)	43 (72%)
Valves only	6 (10%)	7 (11%)
Thoracic aorta surgery	11 (18%)	10 (17%)
Preoperative data, mean (SD)		
Hemoglobin, g/dL	14.3 (1.7)	13.9 (1.4)
Hematocrit, %	0.43 (0.05)	0.41 (0.04)
Platelet count, 10^9/L	222 (78)	220 (53)
end-CPB data, before study medication, mean (SD)		
Hemoglobin, g/dL	9.2 (1.3)	9.2 (1.3)
Hematocrit, l/l	0.27 (0.04)	0.27 (0.04)
Platelet count, 10^9/L	131 (37)	142 (39)
Plasma fibrinogen concentration, g/L	1.7 (0.4)	1.8 (0.3)
5-minutes bleeding volume in mL, median (IQR)	75 (66;100)	80 (70;100)
Transfusion during CPB, mean (SD)		
RBC units	0.95 (2.02)	0.61 (1.00)
FFP units	0.03 (0.17)	0.00 (0.00)
Transfusion of autologue blood in mL, median (IQR)	500 (313;650)	450 (350;638)
Lowest core temperature in °C, median (IQR)	30 (28;32)	30 (28;32)
CPB time in minutes, mean (SD)	209 (94)	192 (71)

IQR; interquartile range (25^{th}; 75^{th}), SD; standard deviation, CABG; coronary artery bypasss graft, CPB; cardiopulmonary bypass, OR; operating room, RBC; red blood cells, FFP; fresh frozen plasma. *EuroSCORE (European System for Cardiac Operative Risk Evaluation) is a risk model for calculating the risk of death after a heart operation using 17 items relating to the patient, state of the heart and the proposed operation. EuroSCORE categorizes risk of death in low risk (EuroSCORE 0-2), intermediate risk (EuroSCORE 3-5), high risk (EuroSCORE ≥ 6).

Primary outcome

There was no significant difference with respect to the primary outcome of this study (i.e. blood loss between intervention and closure of chest at the end of surgery) between the fibrinogen group (median 50 mL; IQR 29;100 mL) and the placebo group (median 70 mL; IQR 33;145), (p=0.190), see Table 2. The duration of primary outcome collection was 4.2 (SD 14.7) minutes in the fibrinogen group and 8.7 (SD 13.0) minutes in the control group. After adjustment for predefined variables (i.e. age, gender, EuroSCORE, lowest core temperature and cardiopulmonary bypass time) this difference in blood loss remained not significant (p=0.09).

Secondary outcomes

Based on our mixed model for repeated measurements cumulative blood loss in the first 24 hours in the ICU was significantly lower for the fibrinogen group compared to the control group with median 570mL (IQR 390;730) against 690mL (IQR 400;1090), p=0.047.

Exploratory outcomes

Table 3 depicts the results of exploratory information of transfusion outcomes. Although the descriptive analyses of the number of patients and the number of units transfused suggest fewer transfusion in the fibrinogen group than in the control group, this study did not have enough power to formally test these differences. The same holds for the data on procoagulants and antifibrinolytics use and on the occurrence of clinical adverse events.

Table 2. Primary and Secondary Outcomes of the Study

	Median (IQR)			
Primary outcome	Fibrinogen (n = 58)	Control (n = 57)	Absolute difference (95% CI)	*P* Value
Blood loss between intervention and chest closure in mL	50 (29;100)	70 (33;145)	20 (-13;35)*	0.190
	Median (IQR)			
Secondary outcome	Fibrinogen (n = 58)	Control (n = 59)	Absolute difference (95% CI)	P Value
Blood loss in the ICU per time interval in mL starting from admission (i.e. time on ICU=0 hours)				
0 up to1 hour on the ICU	70 (35;130)	90 (46;149)		
1 up to 3 hours on the ICU	80 (50;156)	110 (40;220)		
3 up to 6 hours on the ICU	100 (54;169)	110 (60;208)		
6 up to12 hours on the ICU	110 (80;160)	125 (83;224)		
12up to 24 hours on the ICU	130 (80;180)	160 (90;270)		
Cumulative 24h blood loss	570 (390;730)	690 (400;1090)	120 (-45;355)*	0.047**

IQR; interquartile range 25^{th};75^{th}
Time point "intervention" is defined as moment of infusion of study medication.
*95% confidence interval of difference in medians is based on a non-parametric bootstrap procedure (10,000 bootstraps with replacement)
***P* value is based on the constructed mixed model for repeated measurements.

Table 3. Exploratory Information on Transfusion Outcomes

	Fibrinogen	Control
	(n = 60)	(n = 60)
Number of patients transfused between intervention and chest closure		
RBC	0	3 (5%)
FFP	0	1 (2%)
Platelets	2 (3%)	2 (3%)
Any transfusion	2 (3%)	4 (7%)
Number of patients transfused between intervention and 24 hours after		
RBC	10 (17%)	20 (33%)
FFP	9 (15%)	13 (22%)
Platelets	9 (15%)	13 (22%)
Any transfusion	20 (33%)	23 (38%)
Transfusion in units between intervention and 24 hours after	**Median (10^{th}-90^{th})**	
RBC transfusion units	0 (0;1)	0 (0;4)
FFP transfusion units	0 (0;2)	0 (0;4)
Platelets transfusion units	0 (0;1)	0 (0;1)
Any transfusion units	0 (0;2)	0 (0;8)

RBC; red blood cells, FFP; fresh frozen plasma.
Time point "intervention" is defined as moment of infusion of study medication.

In the ICU period six patients (10%) in the control group against 1 (2%) in the fibrinogen group received the procoagulant fibrinogen concentrate. Tranexamic acid was additionally given in 15 (25%) patients of the control group and 9 (15%) patients of the fibrinogen group. Prothrombin complex concentrate was given to 10 (17%) patients in the control group against 4 (7%) patients in the fibrinogen group (see Online supplementary table 2).

Table 4 depicts the data on number of clinical adverse events during the trial. More events were observed in the fibrinogen group: 4 cases against 2 for stroke or TIA, 3 cases against 1 for myocardial infarction and 2 cases against 0 for mortality. No patient had deep venous thrombosis with compression ultrasound of both legs on the third postoperative day. In four patients, the compression ultrasound was not performed; information was obtained with 30-day follow-up.

Table 4. Clinical adverse events, No. of events in studied population

	Fibrinogen	Control
	(n = 60)	(n = 60)
In-hospital mortality	2	0
Stroke	4	1
TIA	0	1
Myocardial infarction	3	1
Renal insufficiency / failure	3	2
Thromboembolism	0	0
Allergic reaction	0	0
Infections	3	2
Rethoracotomy	4	5

TIA; transient ischemic attack

In online supplementary table 3 data on all clinical adverse events that occurred during the study are displayed, with information on allocated treatment, infused dose of fibrinogen concentrate, fibrinogen plasma concentrations before and after intervention and the time of occurrence of the clinical adverse event in days after surgery.

DISCUSSION

The main finding of this study was that targeted fibrinogen concentrate infusion in high-risk cardiac surgery did not significantly reduce perioperative blood loss. Targeted fibrinogen concentrate infusion in high-risk cardiac surgery did, however, significantly reduce 24-hour blood loss in the ICU. More adverse events within 30 days were observed in the fibrinogen group: 4 cases against 2 for stroke or TIA, 3 cases against 1 for myocardial infarction and 2 cases against 0 for mortality.

To our knowledge, this study is one of the first to investigate the effects of fibrinogen concentrate on perioperative blood loss in cardiac surgery procedures and therefore comparison with other studies is difficult. However, in one other study only reduction in units of any blood product transfusion was measured 24

hours after infusion of fibrinogen concentrate in cardiac surgery patients.[9] In the current study, there was a reduction in blood loss in the period 24 hours after infusion of fibrinogen concentrate. Furthermore, in the previous study, after 45 days of follow-up, one patient in the fibrinogen group had a myocardial infarction, two patients in the control group had a cerebral hemorrhage or infarction and four patients in the placebo group died against one in the fibrinogen group. The dose of infused fibrinogen concentrate in that study was a median of 8 grams which resulted in a mean post-intervention plasma fibrinogen concentration of 2.6 (SD 0.5) g/L.

In the current study, the median dose of fibrinogen concentrate infusion in the fibrinogen group was 3 (IQR 2; 4) gram resulting in a mean post-intervention plasma fibrinogen concentration (at arrival in the ICU) of 2.3 (95% CI 2.2-2.4) g/L, which is lower compared to the previous study.[9]

In the current study, a steady rise in plasma fibrinogen concentrations was observed after surgery in both the fibrinogen group as well as the control group. As depicted in Figure 2, the fibrinogen group had a slightly higher level between time points “end-CPB” and “24 hours after infusion”. At the time point “24 hours after infusion” the mean plasma fibrinogen concentrations were nearly equal with 3.4 g/L for the fibrinogen group and 3.1 g/L for the control group. In the previous study, the plasma fibrinogen concentration 1 day after surgery was 3.4 g/L for the fibrinogen group and 3.3 g/L for the control group which is comparable to this study.[9] The rapid rise in plasma fibrinogen concentrations after surgery in both the fibrinogen group and the control group is caused by upregulated fibrinogen synthesis in the liver and can occur in both study groups as part of the acute phase response after tissue injury.[27-30]

In this study, blood loss measured as both the primary and secondary outcomes was less than the historical intraoperative blood loss on which the sample size of this study was based. For the primary outcome this can be explained by the fact that the period between infusion of study medication and closure of chest (the time frame for determination of primary blood loss), was relatively short. For the secondary outcome a 120 mL difference in cumulative 24 hours ICU blood loss can not be considered a clinically important difference. Furthermore, the significant

p-value for the secondary endpoint might be due to multiple comparison, but a true effect cannot be ruled out.

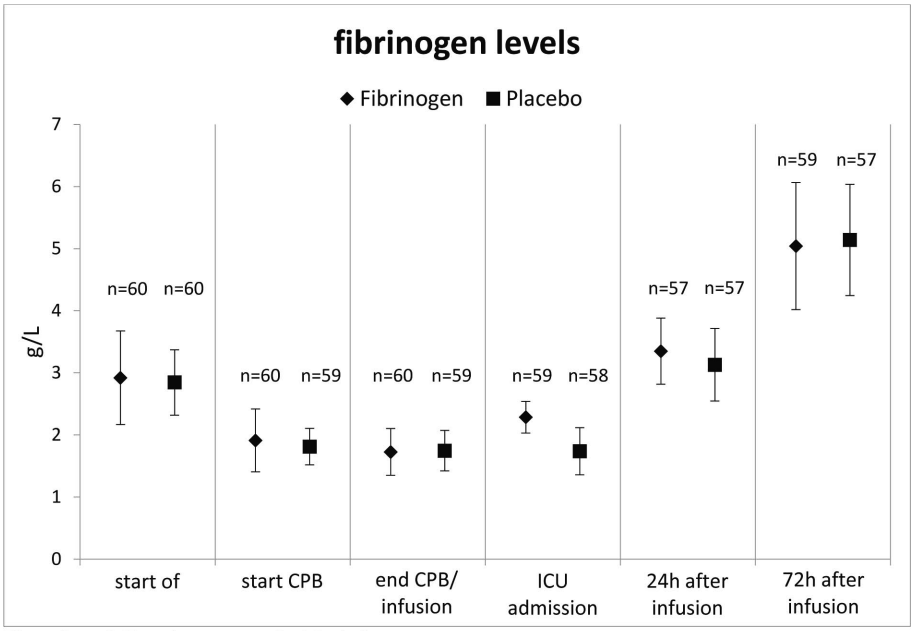

Figure legend: Error bars are standard deviations

Figure 2. Mean Plasma Fibrinogen Levels During the Study
Figure legend: Error bars are standard deviations

In general, this study had a high risk patient population; patients were of old age, had more often diabetes mellitus and had long cardiopulmonary bypass times, which might explain the larger total number of adverse events observed.[31] It is worth mentioning that all patients in this study who suffered a stroke had a post-intervention plasma fibrinogen concentration below the targeted 2.5 g/L. In the literature, approximately 45% of perioperative strokes occur within the first 24 hours after surgery and are mostly caused by manipulations of the heart and aorta with dislodgement of atherosclerotic debris from the aorta.[32-35] This might also have caused the observed first-day strokes. In this trial, routine systematic perioperative screening by echocardiography (TEE, modified TEE and/or epi-aortic scanning) on the severity of aortic atherosclerosis was not performed.[36,37] However, this study

was not powered to assess statistical differences in adverse events between the treatment and control group, and therefore no conclusive statements can be made on whether the adverse events were caused by the intervention. In addition, the effect of interaction of fibrinogen concentrate with other pro-thrombotic agents is unknown and may be harmful. See online supplementary table 3 for more details of the adverse events.

The study has some limitations. Firstly, despite randomization, inequalities between the two groups occurred. Therefore, a multivariable analysis was performed to reduce the effect of these baseline differences on our results. Secondly, although fibrinogen concentrate infusion was allowed only in the study population, it was still infused in the ICU period in 6 patients (10%) in the control group and 1 patient (2%) in the fibrinogen group (online supplementary table 2). The cross-over of these patients may have reduced the differences in outcomes between the groups.

Finally, this study was a single center study which may raise concerns regarding external validity of the results. On the other hand, this single center coordinated study had the advantage of reduced variability in the conduct of the study and adherence to the study protocol with a dedicated and well-trained team.

CONCLUSION

Among patients with coagulopathic bleeding during high-risk cardiac surgery, administration of fibrinogen concentrate, compared with placebo, resulted in no significant difference in the amount of perioperative blood loss

Registrations:

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- EudraCT reference number 2009-018086-12
- Institutional Medical Ethical Committee of Isala Zwolle reference number METC 10.0662
- National registry CCMO reference number NL32188.075.10

– The full trial protocol is available as an online supplementary file. The corresponding author can also be contacted.

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– Süleyman Bilecen and Joris A.H. de Groot had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. The remaining authors (Cor J. Kalkman, Alexander J. Spanjersberg, George J. Brandon Bravo Bruinsma, Karel G.M. Moons) had, upon request, full access to all study data.
– None of the authors have financial interests, activities or relationships to declare.
– The interim analysis was performed by Dr. Paul Westers of the Julius Center for Health Sciences and Primary Care. University Medical Center Utrecht, The Netherlands.
– Süleyman Bilecen and Joris A.H. de Groot were responsible for the data analysis described in this manuscript.
– CSL Behring sponsored this study and donated the bottles of study medication.
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Supplement 1. Definitions of Clinical Adverse Events Within 30 Days After Initial Surgery

In-hospital mortality	Death of any cause within 30 days after initial surgery.
Stroke	Within 30 days, new motor or sensory deficit with its origin in the central nervous system or an unexplained coma status lasting longer than 24 hours. Diagnosis by consulting neurologist or confirmed by positive finding on CT or MRI.
TIA	Within 30 days, brief episode of neurological dysfunction resulting from focal temporary cerebral ischemia lasting less than 24 hours, not associated with cerebral infarction. Diagnosis by a consulting neurologist.
Myocardial infarction	Within 30 days, myocardial specific creatine kinase (CKMB) >180 U/L (7.5 times upper reference limit) plus a peak CKMB/CK ratio >10%, or pathological new Q waves or new left bundle branch block on a postoperative electrocardiogram or angiographically documented new graft or native coronary artery occlusion, or imaging evidence of new loss of viable myocardium.[22]
Renal injury	Within 30 days, increase in postoperative serum creatinine of at least two times the preoperative value or a decrease in glomerular filtration rate (GFR) of more than 50%. [23]
Renal failure	Within 30 days, increase in postoperative serum creatinine of at least three times the preoperative value and a decrease in GFR of at least 75% or a serum creatinine >354μmol/L associated with an acute increase of serum creatinine of at least 50μmol/l.[23]
Venous thrombosis	Within 30 days, thrombosis in the lower extremity diagnosed with compression ultrasound testing prior to surgery and at day 3 after surgery.
Pulmonary embolism	Within 30 days, pulmonary embolism diagnosed with a spiral CT.
Allergic reaction	Within 30 days, allergic or other systemic reaction to study medication.
Infections	Within 30 days, infection of venectomy site (arm or leg) and sternal infection.
Rethoracotomy	Proportion of subjects that receive a follow-on surgery to correct unacceptable bleeding within 5 days of last suture.

Supplement 2. Procoagulants and Antifibrinolytics use During Surgery and ICU

	Fibrinogen	Control
	(n = 60)	(n = 60)
During surgery, patients receiving, No. (%):		
Tranexamic acid	59 (98%)	60 (100%)
Desmopressin	42 (70%)	41 (68%)
Prothrombin complex concentrate	5 (8%)	2 (3%)
Recombinant factor VIIa	0 (0%)	0 (0%)
During ICU period, patients receiving, No. (%):		
Protamine	3 (5%)	8 (13%)
Tranexamic acid	9 (15%)	15 (25%)
Desmopressin	3 (5%)	8 (13%)
Prothrombin complex concentrate	4 (7%)	10 (17%)
Fibrinogen concentrate	1 (2%)	6 (10%)
Recombinant factor VIIa	0 (0%)	0 (0%)

Supplement 3. Data on All Clinical Adverse Events that Occurred During the Study, With Allocated Treatment, Infused Dose, Fibrinogen Plasma Concentrations and Time of Event in Days after Surgery

Participant	Medication	Pre-infusion	Infusion	Post-infusion	Clinical adverse events						
		[fibrinogen g/L][a]	dose (g)[b]	[fibrinogen g/L][c]	Mortality	Stroke	TIA	MI	RI	Infections	Rethoracotomy
H-012	fibrinogen	1.6	3	2.4	day +10	day +1		day +1	day +2		day 0
H-161	fibrinogen	0.8	6	1.7	day +5	day +1			day +2		day 0
H-047	placebo	0.5	7	0.6		day 0					
H-140	fibrinogen	1.6	4	2.3		day +1				day +30	
H-155	fibrinogen	2.4	0	2.3		day +6					
H-252	placebo	2.0	2	1.9			day +30				
H-048	fibrinogen	1.5	3	2.1				day +1			
H-065	fibrinogen	2.0	2	2.4				day 0		day +5	day 0
H-022	placebo	1.9	3	2.0				day +1			
H-007	placebo	1.7	3	1.1					day +19		day +1
H-108	placebo	1.9	3	2.2					day +1		
H-112	fibrinogen	1.8	3	2.5					day +5		
H-129	fibrinogen	2.2	1	2.5						day 0	
H-139	placebo	1.5	6	1.2						day +15	
H-244	placebo	2.0	2	2.2						day +17	
H-166	placebo	1.4	4	1.5							day 0
H-239	fibrinogen	1.4	5	1.7							day 0
H-078	placebo	1.6	4	1.6							day 0
H-170	placebo	1.5	4	1.4							day 0
H-107	placebo	1.4	5	1.4							day 0

End-CPB; end-cardiopulmonary bypass, TIA; transient ischemic attack, MI; myocardial infarction, RI; renal insufficiency or failure.

[a] Plasma fibrinogen concentration at end-CPB.

[b] Infusion of study medication after removal of CPB. For placebo matched number of syringes is infused.

[c] Plasma fibrinogen concentration at ICU admission.

Chapter
6

Chapter 7

Summary and General Discussion

Excessive bleeding is a common complication in cardiac surgery, and is most frequently due to insufficient surgical hemostasis or impairment of the coagulation system; treatment with allogeneic blood products is often necessary. However, nowadays the majority of patients undergoing cardiac surgery will not receive allogenic blood products. Approximately 20% of patients consume more than 80% of all products in cardiac surgery.[1] Excessive bleeding and transfusion of blood products is associated with several risks including early and late mortality, increased risk for infections, prolonged hospital stay and decrease of long term quality of life.[2-5] In addition, transfusion of blood products is costly. Next to the bleeding potential brought about by the large wound area intrinsic to cardiac surgery, cardiopulmonary bypass (CPB) is associated with temporary dysfunction of the hemostatic and fibrinolytic systems.[6-9] The result is platelet dysfunction, coagulation factor activation, depletion and fibrinolysis.[10] At the start of this thesis project the anaesthesiologists in Isala Zwolle used the CBO National Blood Transfusion Guideline in their daily practice; a generic blood management guideline used by various medical specialties. However, there was a strong need for a transfusion protocol specifically designed for cardiac surgery procedures with attention to timing and dosing of blood products, procoagulant and antifibrinolytic medication.

In the same year (2006), the Isala Zwolle started to infuse a potentially effective procoagulant, fibrinogen concentrate (coagulation factor I) in cardiac surgery patients with excessive bleeding refractory to conventional procoagulant and antifibrinolytic medication. Procoagulant fibrinogen is a heat-treated, virus inactivated, lyophilized fibrinogen powder made from pooled human plasma and does not require blood type matching.

Plasma fibrinogen plays an important role in hemostasis by acting as an endogenous substrate for fibrin formation, promoting clot formation and platelet aggregation by binding platelet glycoprotein IIb/IIIa receptors.[11,12]

The procoagulant fibrinogen concentrate is labelled for congenital hypofibrinogenemia and acquired hypofibrinogenemia, which can occur in cardiac surgery as fibrinogen concentrate is the first coagulation factor to reach critically low plasma concentrations in major bleeding.[13]

However, at the start of this thesis project there was no published randomized clinical trial (RCT) with sufficient statistical power to reach definitive conclusions on the effectiveness of fibrinogen concentrate on blood loss and transfusion in the domain of cardiac surgery. With the known risks and costs associated with blood loss and attendant transfusion of allogeneic blood products, a more judicious use of blood products was aimed at. This aspiration to optimize hemostasis in cardiac surgery procedures formed the basis for the work described in this thesis.

The studies described in this thesis were designed to provide more evidence for the effects of fibrinogen levels on blood loss in cardiac surgery. We hypothesized that plasma fibrinogen played an important role in coagulation and that infusion of the procoagulant fibrinogen concentrate might reduce blood loss and transfusion in cardiac surgery procedures.

In order to obtain the evidence to support or disprove our hypothesis, several necessary steps had to be taken, which are described in Chapters 2, 3, 4 and 5, in the work-up to the trial described in Chapter 6.

In *Chapter 2* a large retrospective cohort of high-risk cardiac surgery procedures revealed that administering fibrinogen concentrate did not reduce postoperative blood loss and transfusion. There was also no increase in clinical adverse events such as 30-day mortality, myocardial infarction, stroke, renal insufficiency or failure, infections and prolonged mechanical ventilation. At the time of this study, there were only three studies that had investigated the use of fibrinogen concentrate in cardiac surgery procedures.[14-16] In a pilot study of Karlsson et al. the infusion of fibrinogen concentrate was prophylactic. Twenty elective coronary artery bypass graft (CABG) patients were randomized to either prophylactic infusion of 2 g fibrinogen concentrate or no infusion which was the control group. For the fibrinogen group the mean preoperative plasma fibrinogen concentration had risen from 2.9 g/L to 3.5g/L.[14] As the average plasma fibrinogen level in humans is 2.0 to 4.5 g/L,[17,18] the decision of Karlsson et al. to design a study with prophylactic infusion of fibrinogen concentrate (in all patients) to reach such high plasma fibrinogen levels before the procedure even started and before any evidence of excessive coagulopathic bleeding, can be considered risky. In the latest study of Karlsson et

al., which was a randomized trial, prophylactic infusion of fibrinogen concentrate was ineffective in reducing postoperative bleeding in cardiac surgery patients.[19]

Rahe-Meyer et al. chose a more pragmatic approach in their two studies by infusing fibrinogen concentrate after removal of cardiopulmonary bypass. This timepoint during surgery is pivotal for hemostasis management and is more suitable for pharmacological intervention, as heparin is reversed after CPB removal.[15,16] In the study described in Chapter 2, we found no association between the use of fibrinogen concentrate and the occurrence of thromboembolic events, an observation which was in line with studies on the safety of fibrinogen concentrate at that time.[18,20,21] A major limitation of our study was the retrospective, non-randomized design. Differences in baseline characteristics likely have caused confounding by indication. Although we used propensity scored matching as a method for (pseudo) randomisation, it remains a challenge to correct for confounding factors in non-randomized intervention studies. The results of this study were important, however, to guide the design of the randomized clinical trial described in Chapter 6. We hypothesized that the lack of effect of fibrinogen concentrate on blood loss and transfusion could be attributed to the relatively low dose of fibrinogen concentrate given, with 60% of the patients in the fibrinogen group given 1 or 2 gram of fibrinogen concentrate, while in two other studies a mean of 5.7 grams and 7.8 grams of fibrinogen concentrate was infused.[15,16]

We also recognized that the timing of fibrinogen concentrate might be crucial in hemostasis management. Based on all the previously published work and our own observations, we chose the period after removal of CPB and surgical hemostasis as the optimal time for administration of fibrinogen concentrate.

In *Chapter 4*, we developed a prediction model using the widely used EuroSCORE variables to predict intraoperative and postoperative blood loss in cardiac surgery. In the past there have been several prediction models developed that predicted the risk of postoperative transfusion in cardiac surgery,[22-25] but there were no studies available that could predict blood loss during or after surgery. For the first time, the EuroSCORE variables were used to predict blood loss during and after cardiac surgery. The EuroSCORE variables proved to be very solid and accurate in predicting intraoperative blood loss with good calibration and a discrimination (an area under

the curve (AUC)) of 0.84. For the postoperative period the discriminative ability was lower (AUC 0.74). In the Isala Zwolle, EuroSCORE variables were routinely collected and recorded in a perioperative database. This data collection was prospective and real-time. Our new prediction model might thus easily be incorporated in a software program or app for daily clinical use in cardiac surgery. Before surgery, two risk evaluations can then be delivered: predicted in-hospital mortality, which is the purpose for which the EuroSCORE was originally developed, and the risk for intraoperative and postoperative blood loss. Such quantitative risk estimates allow for appropriate preoperative preparation and timely institution of preventive measures. This study also had its limitations. The prediction model for blood loss has not yet been externally validated in a different patient population. In addition, measuring blood loss correctly during cardiac surgery is a major challenge. For the estimation of intraoperative blood loss, we used the following formula:

> total blood loss during surgery = (plasma-loss in cell saver x 1.66) + blood loss in gauzes.

We assumed the mean hematocrit to be 40% and the plasma content to be 60%. In addition, we could calculate whole blood loss by multiplying plasma loss measured with the cell saver by multiplying with 1/0.6 = 1.66. This calculation of intraoperative blood loss in cardiac surgery is not a generally accepted method, as blood suctioned with the cell saver is in part auto-transfused to the patient as erythrocytes. However, this processed auto-transfused blood has higher hematocrit values and is deficient on coagulation factors and platelets. A disadvantage of this blood loss calculation is overestimation of intraoperative blood loss values for cardiac surgery patients. In our opinion, the calculated method is a more standardized method which also includes loss of plasma coagulation factor and platelets during surgery and should be considered for all studies related to this subject. For postoperative blood loss, actual drainage volumes measured in the ICU were used without modification by a formula.

IMPLEMENTING A NEW TRANSFUSION PROTOCOL

In *Chapter 3*, we describe the next step in the process to the randomized clinical trial (described in Chapter 6), namely the implementation of a cardiac surgery-specific transfusion protocol in order to systematically direct the use of blood products and pro-hemostatic medication to prevent excessive transfusion of blood products and reduce the number of transfused patients. The new 'cardiac surgery-specific' transfusion protocol resulted in fewer patients transfused with red blood cells (RBC) and fresh frozen plasma (FFP) and a lower risk for myocardial infarction. The reductions in the amount of transfusion between groups were not significant due to the already restrictive transfusion practice in Isala Zwolle. Although the new cardiac surgery-specific transfusion protocol was not a major departure from daily practice, adherence to the new protocol remained a challenge in the first year of introduction. Such problems with implementation are known from other studies.[26,27]

We had reason to believe that a restrictive transfusion protocol compared to a liberal protocol, was safe regarding the occurrence of clinical adverse events.[28-30] In our study, a restrictive transfusion protocol even proved to reduce the incidence of myocardial infarction.

One important limitation in this study was that during the studied period, the use of cell savers and procoagulant medication had increased, which might have contributed to reductions in transfusion of blood products apart from the new protocol. The successful implementation of this new transfusion protocol was an important milestone in the work-up to the clinical trial. This protocol was introduced in January 1st, 2009, while the inclusion of the first patient of the trial was on February 10, 2011. In this two-year period, this transfusion protocol had become the standard in Isala Zwolle. The importance of adherence to a transfusion or study protocol was shown by the REPLACE trial.[31] In that study, fibrinogen concentrate was associated with increased allogeneic blood product transfusion in the setting of aortic surgery with CPB, which was an unexpected finding and contrary to all previous studies on this topic. That study had a multicenter design with relatively few patients enrolled in each of the 34 centers in 11 countries including Canada and

Japan. The authors speculated that variations in adherence to the study protocol, which ranged from 48% to 100% in participating centers, may have influenced the study outcomes. The results of the REPLACE trial are nonetheless interesting and should be taken into account in future research on this topic.

THE RANDOMIZED CLINICAL TRIAL

In *Chapter 5*, we found that low end cardiopulmonary bypass (end-CPB) plasma fibrinogen levels were related to postoperative blood loss and transfusion. The purpose of this study was to determine the critical plasma fibrinogen levels for excessive bleeding. Interestingly, we were unable to define a low critical fibrinogen level that resulted in excessive bleeding, which is in contrast with the study of Waldén et al. in which preoperative fibrinogen levels lower than 2.5 g/L were related to excessive ICU blood loss. However, in our study, end-CPB plasma fibrinogen levels higher than ≥ 2.5 g/L reduced the risk for excessive postoperative bleeding. This gave us valuable information, since there was no published dose finding study available at the time. We considered it safe to set the target fibrinogen concentration at this level (2.5 g/L) in the randomized clinical trial, since it corresponded with the lower levels of fibrinogen observed in clinical practice, but was slightly higher than the recommended levels in current guidelines which is between 1.5 and 2.0 g/L.[32-34]

Chapter 6 describes the randomized clinical trial. The results of this trial suggest that targeted fibrinogen concentrate infusion does not reduce intraoperative blood loss. However, postoperative blood loss at 24 hours after surgery was reduced significantly. Also, after 24 hours less patients had received postoperative RBC transfusion.

The results of our trial contrast with the REPLACE trial.[31] This latter study had approximately the same number of patients included in multiple centers, included high-risk aortic surgery procedures with CPB and used a very similar test to determine the presence of coagulopathic bleeding (5-minute bleeding volume test). In contrast to our trial, their primary outcome was the number of units of alloge-

neic blood products administered during the first 24 hours after infusion of study medication. In REPLACE, the infusion of fibrinogen concentrate (6.3 g on average), resulted in an unexpected increase of allogeneic blood products. As stated earlier, lack of adherence to the study protocol and variations in local practice may have resulted in the increase of transfusion in the fibrinogen group.

We observed more cases of stroke in the fibrinogen group compared to the placebo group. Of the five stroke cases in our study, four patients had their stroke within 24 hours after surgery of which three had received fibrinogen concentrate and one placebo. The fifth case of stroke was on day 6 and was in the intervention group but this patient had not received fibrinogen concentrate. All cases of stroke had a post-infusion plasma fibrinogen concentration below the targeted 2.5 g/L. In literature, approximately 45% of the perioperative strokes occur within the first day after surgery and are mostly caused by manipulations of the heart and aorta with dislodgement of atherosclerotic debris from the aorta.[35-38]

In our trial routine systematic perioperative screening by echocardiography (TEE, modified TEE and/or epi-aortic scanning) on the severity of aortic atherosclerosis was not performed.[39,40]

Although our intervention group was on average a higher risk group, it is not directly clear whether fibrinogen concentrate infusion may have contributed to the occurrence of thromboembolic events in the intervention group. It must be emphasized that this study was not powered to assess statistical differences in adverse events between the fibrinogen and the control group.

Our trial had some limitations. In retrospect, the primary outcome was too close in time after administration of the study drug. During the design of the study, it was expected that the surgeon would not close the chest if there was on-going coagulopathic bleeding. However, it became clear very soon that in practice the surgeons continued closing the chest of the patient. The stated reason was that by design the surgeon had to be sure that surgical hemostasis was completed before randomization; he therefore had no means to further improve the bleeding condition after randomization and left further hemostasis management to the anesthesiologist. This resulted in a very short time between administration of the study drug and assessment of the primary outcome. Consequently, we observed very low blood

loss for the primary outcome. This raises the question about the clinical relevance of the chosen primary endpoint. It would have been highly undesirable to amend the primary outcome of an ongoing RCT after discovery of this phenomenon. If that had been done, the trial would have suffered serious credibility issues. Fortunately, the secondary outcomes of our trial provide the relevant answers regarding 24 h blood loss, i.e., a clinically relevant statistically significant reduction in blood loss and reduced red blood cell transfusion.

A challenging aspect during the trial was the increased focus on hemostasis by the surgeon. This phenomenon is called the "Hawthorne effect" and has been defined as research participants' alteration of behaviour when (knowing to be) observed.[41-43] The cardiac surgeon was the observed participant in the operating room, while the rest of the team was the observer, which might have put pressure on the surgeon to improve surgical hemostasis. In addition, the 5-minute bleeding volume test at the end of cardiopulmonary bypass, might have created an unintended rivalry between surgeons to have more patients without coagulopathic bleeding during the 5-minute bleeding volume test. This effect can be noticed in the difference between the first and second year of the trial. In the first year of the trial 17 patients had no coagulopathic bleeding ("dry surgical field") after the 5-minute bleeding volume test. In the year hereafter, with the same patient load, 29 patients had no coagulopathic bleeding. This nearly doubling of excluded patients might be due to extensive and meticulous surgical attention. The research team noticed and accepted this phenomenon in the first year of the trial. Further improvement of skills and more attention to hemostasis is a normal and expected response in the continuous search for improvement by the skilled surgical team.

An important challenge during the design of our trial was to determine a valid way for measuring the presence of coagulopathic bleeding in cardiac surgery procedures. One method - as used by the group of Rahe-Meyer et al. - is the 5-minute bleeding volume test. In this test the blood collected in the thoracic cavity and pericardial space in a period of 5 minutes is used to determine the presence of coagulopathic bleeding.[15,16,31] A cutoff point of 60 mL in 5 minutes for the diagnosis of coagulopathic bleeding was used. We chose to adopt this level in order to achieve

comparability between studies. However, the 60 mL cutoff was an arbitrarily chosen level and not based on evidence.

CLINICAL RELEVANCE

The studies in this thesis show that a specific tailor-made transfusion protocol in cardiac surgery is an important first step for optimizing transfusion in cardiac surgery. In addition, the development of this transfusion protocol was necessary to allow the conduct of our pragmatic randomized clinical trial. If optimization of coagulation in cardiac surgery is the aim, a cardiac surgery-specific transfusion protocol should be implemented and adhered to. This should be the basis for further refinement of hemostasis management.

Our randomized trial showed that administration of fibrinogen concentrate was able to significantly reduce 24 h postoperative blood loss. In addition, postoperative 24 hours RBC transfusion was reduced by 75%, FFP transfusion by 50%, platelets transfusion by 40% and transfusion of any blood product by 60%. These are clinically important outcomes. This trial was unique in using end-CPB plasma fibrinogen levels as clinical starting point instead of Point of Care (thromboelastometry) based algorithms as used by other studies. Hereby making this method accessible to other centers and countries who do not have access to a thromboelastometry device.

Although our trial was not powered for analyzing the incidence of potential clinical (thromboembolic) events, the distribution of the events is interesting, in particular the occurrence of stroke and transient ischemic attack. However, in a detailed analysis of these events, the post-infusion fibrinogen levels were within normal range and below targeted levels, while one patient randomized to fibrinogen concentrate had not even received fibrinogen concentrate. These events were likely caused in the context of high risk cardiac surgery procedures combined with a population with certain risk factors. Furthermore, the hypercoagulable state in the days after surgery might have played an important and causal role in the occurrence in some of these clinical events.

In the REPLACE trial,[31] in which (twice) higher doses of fibrinogen concentrate were infused, the thromboembolic events were higher in the placebo group, contrary to the results in our trial.

Nonetheless, fibrinogen concentrate should be infused with caution given the uncertainties concerning the safety of its use. Certainly, follow-up is needed for all cardiac surgery patients, especially for patients receiving fibrinogen concentrate. A prophylactic infusion of fibrinogen concentrate is not recommended. Fibrinogen concentrate infusion should only be considered in cardiac surgery procedures where ongoing coagulopathic bleeding is caused by acquired hypofibrinogenemia. Until we know more about the risk/benefit ratio of exogenous fibrinogen concentrate in this setting, a target post-infusion plasma fibrinogen level of 2.5 g/L can be recommended. The clincial efficacy of even lower target fibrinogen levels should be investigated in a dedicated dose-finding trial.

FUTURE RESEARCH

With the positive results on clinically relevant outcomes such as a significant reduction in post-operative bleeding and transfusion of red blood cells, focus should be shifted to clinical safety.

The aim of a future study should be to assess both efficacy and safety of fibrinogen concentrate in cardiac surgery and such a study should be powered for the analysis of thromboembolic events. Obviously, the sample size will need to be much higher to allow solid inferences on safety and to arrive at a reliable estimate of the risk/benefit ratio.

This future trial should include all cardiac surgery procedures, because excessive bleeding may also occur in low-risk cardiac surgery. A multicenter trial design is preferred in a future trial. All participating centers should have implemented a cardiac surgery-specific transfusion protocol at least 1 year before the start of this new trial. Adherence to the transfusion protocol should be assessed continuously during that first year of introduction and also during the conduct of the trial.

In addition, the diagnosis of clinically relevant coagulopathic bleeding is of importance. Coagulopathic bleeding should be determined with a quantitative method, but possibly combined with a visual method to estimate the presence of coagulopathy. Preferably, the diagnosis of coagulopathic bleeding should be made by more than one person and based on consensus. Furthermore, an improved method for determining coagulopathic bleeding will reduce the Hawthorne Effect which will invariably occur in this kind of studies. The dosage of fibrinogen concentrate should be based on the formula used in this thesis in Chapter 6. The primary outcome of this trial should focus first on safety with effectiveness as secondary endpoint. The moment of intervention of fibrinogen concentrate should be the starting point (time = 0) of data collection. From here on, 12 hours, 24 hours or longer periods for collection of outcome data can be chosen. Follow-up data on clinical safety should extend to at least 1 year.

Future clinical research should also focus on the hypercoagulable state which is present in the days after cardiac surgery. Thrombin generation and interaction with fibrinogen are important topics, as both can be reduced with coagulopathy caused by dilution. The role of platelets should be included in the study as platelet function is also an important part of coagulation management.

CONCLUDING REMARKS

The main question of this thesis was: What is the role of fibrinogen in blood loss and transfusion in cardiac surgery? From our studies we learnt that plasma fibrinogen levels can modulate bleeding and reduce postoperative blood loss with plasma levels higher than 2.5 g/L. Fibrinogen concentrate infusion targeted at post-infusion plasma levels of 2.5 g/L is able to reduce postoperative blood loss and transfusion of allogeneic blood products. However, more data are needed on the clinical safety and effectiveness of perioperative administration of fibrinogen concentrate. Finally, a cardiac surgery-specific transfusion protocol, that is generally accepted and adhered to, is a solid and necessary basis for further research on and refinement of hemostasis management during cardiac surgery.

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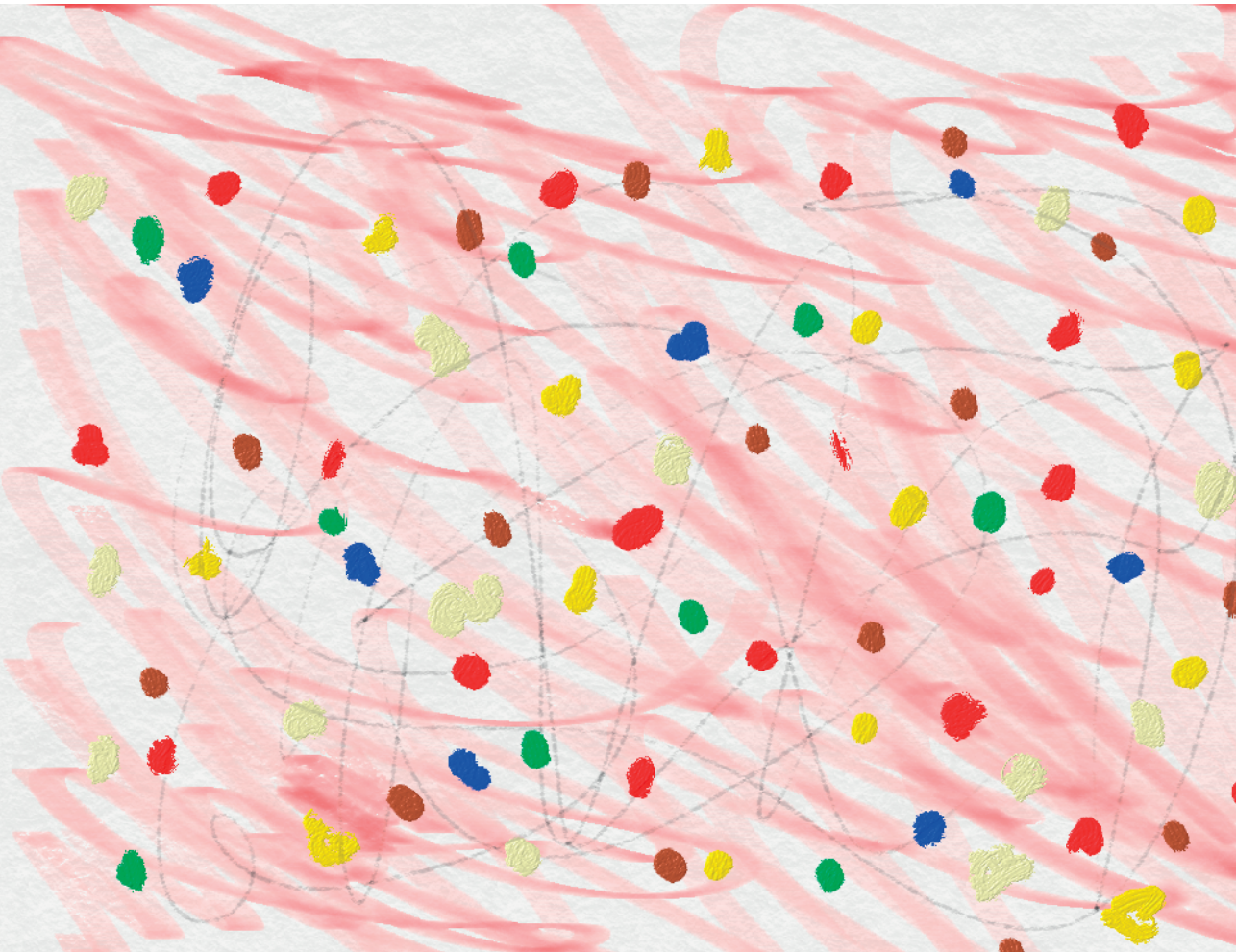
Chapter 8

Summary in Dutch
Samenvatting in het Nederlands



In de hartchirurgie is overmatig bloedverlies, als gevolg van een chirurgische bloeding of een stollingsstoornis, een veel voorkomende complicatie. Overmatig bloedverlies leidt vaak tot transfusie van bloedproducten. Maar een klein deel (tot 20%) van de hartchirurgie patiënten, verbruikt meer dan 80% van de bloedproducten. Transfusie van bloedproducten kan noodzakelijk zijn in de hartchirurgie. Echter, er zijn ook negatieve kanten aan transfusie zoals verhoogde kans op overlijden na de operatie, verhoogde kans op infecties, verlengde opnameduur en vermindering van kwaliteit van leven. Het is van belang dat er goed nagedacht wordt over transfusie, waarbij de voor- en nadelen afgewogen worden.

Er zijn verschillende methoden om transfusie tijdens hartchirurgie te verminderen. Het gebruik van medicatie die de stolling bevorderen is een belangrijke methode om het bloedverlies en de hieraan gerelateerde transfusie van bloedproducten te verminderen. Een van die medicijnen is geconcentreerd fibrinogeen. Fibrinogeen is een plasma eiwit, ook wel stollingsfactor I genoemd. Het is een belangrijke stollingsfactor. Dit plasma eiwit wordt uit humaan (menselijk) plasma gewonnen en in gedroogde en geconcentreerde vorm in ampullen geleverd als medicijn voor klinisch gebruik. Dit middel wordt wereldwijd in toenemende mate gebruikt bij verschillende chirurgische ingrepen waarbij de patiënt veel bloed verloren heeft en de stolling niet op gang komt door een lage plasma fibrinogeen concentratie. Met het medicijn geconcentreerd fibrinogeen kan men de lage plasma concentraties aanvullen om de stolling van het bloed op gang te brengen. Ondanks toename in het gebruik van het medicijn geconcentreerd fibrinogeen, was er bij de start van dit onderzoeksproject geen duidelijkheid over het effect ervan op bloedverlies en transfusie.

In de Isala Zwolle werd dit medicijn eind 2006 voor het eerst gebruikt bij patiënten die een hartoperatie ondergingen. Er was destijds een sterke behoefte aan meer bewijs voor het effect en de mogelijke bijeffecten van geconcentreerd fibrinogeen in patiënten die een hartoperatie ondergingen. Deze behoefte naar meer bewijs aangaande het gebruik van geconcentreerd fibrinogeen, heeft geleid tot de start van het onderzoeksproject welke beschreven is in dit proefschrift. Dit onderzoeksproject is in een aantal stappen opgedeeld en zal per hoofdstuk aan u worden gepresenteerd.

Hoofdstuk 2 beschrijft een retrospectieve analyse (analyse van voorgaande jaren 2007 t/m 2010) met gegevens van patiënten die een complexe hartchirurgie ondergingen in Isala Zwolle. Met deze eerste analyse is er gekeken of geconcentreerd fibrinogeen enig effect heeft gehad op bloedverlies en transfusie na de operatie. Er is ook gekeken naar klinische bijwerkingen zoals hartinfarct, herseninfarct, nierfalen, infecties en overlijden. Uit deze studie is naar voren gekomen dat geconcentreerd fibrinogeen geen effect heeft op bloedverlies en transfusie na de hartoperatie. Geconcentreerd fibrinogeen had tevens geen effect op klinische bijwerkingen. Ondanks dat er een statistische correctie werd verricht om pseudo-randomisatie te verkrijgen, is het analyseren van bestaande data niet de meest geschikte methode om effectiviteit van een medicijn te bepalen. Dit was tevens een belangrijke beperking van deze studie.

Hoofdstuk 3 beschrijft de introductie van een specifiek voor hartchirurgie ontworpen nieuwe transfusie protocol (richtlijn). Er werd in deze studie onderzocht of een nieuwe transfusie protocol enig invloed had op transfusie van bloedproducten en klinische uitkomsten zoals hartinfarct, herseninfarct, nierfalen, infecties en overlijden. Belangrijkste uitkomst van deze studie was dat het nieuwe transfusie protocol heeft geleid tot minder patiënten met plasma en rode bloedcellen transfusie. Tevens was het risico op een hartinfarct lager in deze groep. Dit hoofdstuk was belangrijk voor de succesvolle afronding van de gerandomiseerde studie welke beschreven is in hoofdstuk 6.

Hoofdstuk 4 beschrijft de prestatie van een model, dat is gebaseerd op de EuroSCORE variabelen, om het bloedverlies tijdens en na een hartchirurgie te voorspellen. Vroegtijdig voorspellen van “de bloedende patiënt” heeft voordelen voor het chirurgisch en anesthesiologisch team; men kan van techniek veranderen en/of de juiste maatregelen treffen om zo effectief mogelijk de stolling bij een patiënt te optimaliseren. De EuroSCORE is ontwikkeld om het risico op overlijden bij een hartoperatie te voorspellen. In dit hoofdstuk wordt de EuroSCORE gebruikt om het bloedverlies tijdens (model 1) en na (model 2) de hartoperatie te voorspellen. De EuroSCORE heeft 17 variabelen welke automatisch verzameld worden in het

digitale data system van Isala Zwolle. De belangrijkste bevinding van dit hoofdstuk was dat de EuroSCORE het bloedverlies tijdens de hartoperatie zeer goed kon voorspellen. EuroSCORE kon in mindere mate ook het bloedverlies na de hartoperatie voorspellen. Het toevoegen van laboratoriumwaardes verbeterde de prestatie van beide modellen niet.

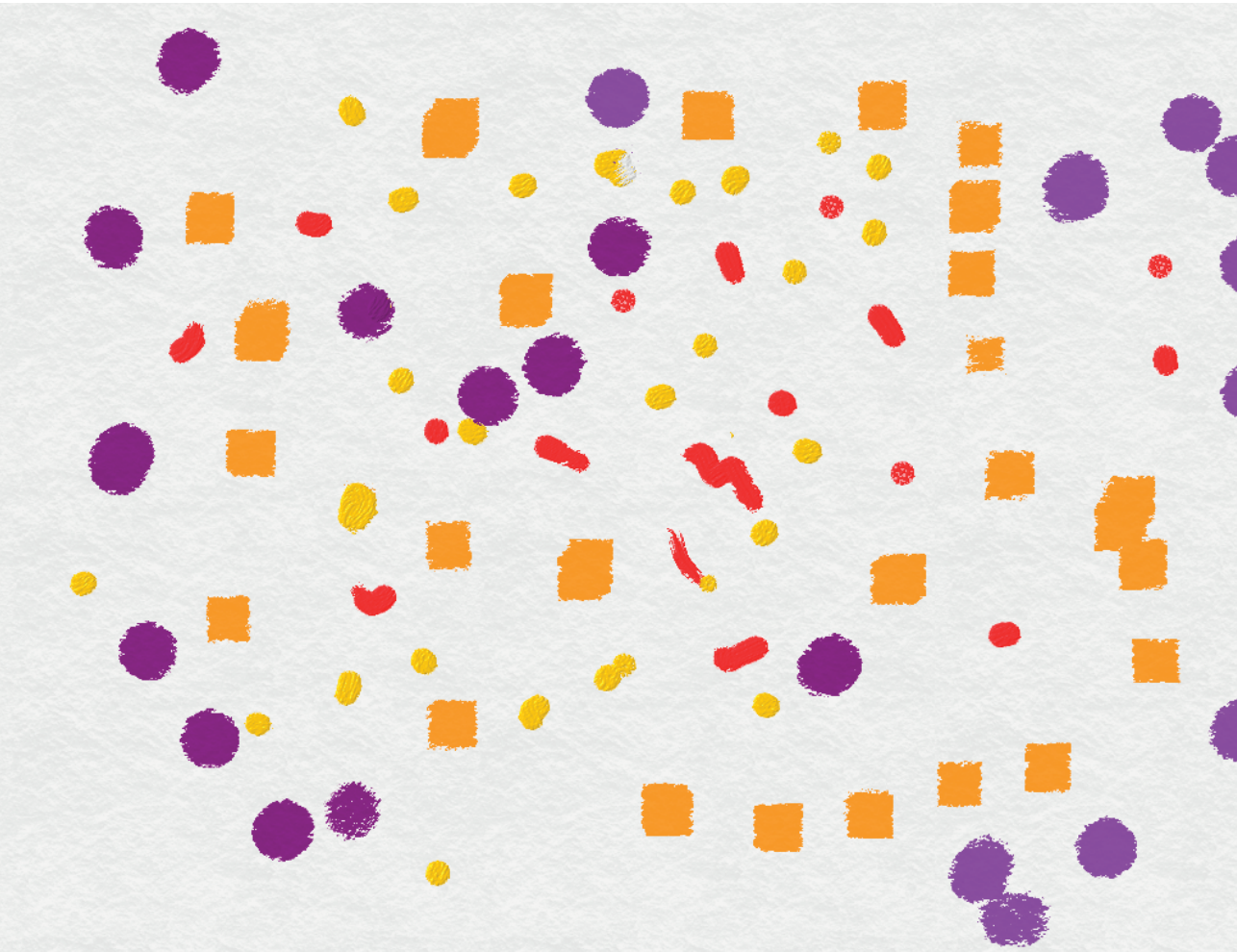
Hoofdstuk 5 beschrijft de relatie tussen plasma fibrinogeen waardes en bloedverlies na een hartoperatie. Met dit onderzoek werd bepaald welke plasma fibrinogeen waardes bloedverlies veroorzaakten en welke waardes bloedverlies verminderden. In een groot aantal patiënten werd er gekeken naar de relatie tussen plasma fibrinogeen gemeten aan het eind van de hartlongmachine en het bloedverlies na een hartoperatie. Juist het moment aan het eind van de hartlongmachine is van grootste belang voor het chirurgisch en anesthesiologisch team. Dit moment is meest geschikt voor het geven van medicijnen, waaronder geconcentreerd fibrinogeen. Belangrijkste bevinding van deze studie was dat de plasma fibrinogeen concentratie boven 2.5 g/L, gemeten aan het eind van de hartlongmachine, het risico op overmatig bloedverlies verminderde tijdens hartchirurgie. In dit hoofdstuk werd de streef-concentratie voor plasma fibrinogeen bepaald welke nodig was voor de gerandomiseerde studie beschreven in hoofdstuk 6.

Hoofdstuk 6 beschrijft de gerandomiseerde studie waarvan de voorzet deels gegeven is in de voorgaande hoofdstukken. In dit hoofdstuk werden patiënten die hoog risico hartchirurgie ondergingen en tijdens de operatie stollingsstoornissen kregen, gerandomiseerd (loting) naar placebo (nep medicijn) of geconcentreerd fibrinogeen (echt medicijn). Deze studie was dubbelblind (zowel arts als de patiënt wisten niet wat toegediend werd). Of de patiënt een belangrijke stollingsstoornis had werd bepaald middels een “5 minuten bloedingstest” waarbij de chirurg de borstholte in 5 minuten met een zuiger uitzoog. Indien patiënt een belangrijke stollingsstoornis had, werd geconcentreerd fibrinogeen of placebo toegediend. De dosis werd bepaald met behulp van een formule zodat na infusie de plasma fibrinogeen concentratie 2.5 g/L benaderde. Bij placebo was deze stijging uiteraard niet aanwezig. Belangrijkste uitkomstmaten waren bloedverlies tussen de

tijdsperiode infusie van studiemedicatie na verwijderen van de hartlongmachine en het sluiten van de borstkast (in feite het einde van de operatie). Tevens was het bloedverlies in de Intensive Care in de eerste 24 uur van belang. Er is ook gekeken naar klinische bijwerkingen zoals overlijden in het ziekenhuis, hartinfarct, hersen-infarct, nierfalen en infecties. Belangrijkste bevinding was dat geconcentreerd fibrinogeen het bloedverlies gemeten tussen infusie van studiemedicatie en sluiten van de borstkast niet verminderde. Er was wel een vermindering van het 24 uurs bloedverlies op de Intensive Care. Bij de patiënten die geconcentreerd fibrinogeen kregen was het aantal gevallen met een herseninfarct hoger. Bij nadere analyse van deze gebeurtenissen kwam niet duidelijk naar voren dat geconcentreerd fi-brinogeen de oorzaak was van de herseninfarcten. Tevens moet gemeld worden dat deze studie niet genoeg patiënten had om überhaupt een uitspraak te kunnen doen over de veiligheid van het medicijn geconcentreerd fibrinogeen. Er is meer onderzoek nodig met een groot aantal patiënten zodat er ook een uitspraak over de veiligheid gedaan kan worden.

Tot die tijd, zal bij het gebruik van geconcentreerd fibrinogeen, een afweging gemaakt moeten worden tussen de beoogde voordelige effecten en de mogelijke nadelige effecten van het middel.

Acknowledgments | Dankwoord



Dit proefschrift is tot stand gekomen met de hulp en ondersteuning van velen. Zonder een van u te vergeten, wil ik u allen bedanken.

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List of publications

Bilecen S, de Groot JA, Kalkman CJ, Spanjersberg AJ, Moons KG, Nierich AP. Effectiveness of a cardiac surgery-specific transfusion protocol. Transfusion. 2014 Mar;54(3):708-16.

Bilecen S, Peelen LM, Kalkman CJ, Spanjersberg AJ, Moons KG, Nierich AP. Fibrinogen concentrate therapy in complex cardiac surgery. J Cardiothorac Vasc Anesth. 2013 Feb;27(1):12-7.

Bilecen S, de Groot JAH, Kalkman CJ, Spanjersberg AJ, Moons KGM, Nierich AP. Predicting blood loss in cardiac surgery: A new application of the EuroSCORE. submitted.

Bilecen S, de Groot JAH, Kalkman CJ, Spanjersberg AJ, Moons KGM, Nierich AP. The effects of plasma fibrinogen levels on blood loss and transfusion in cardiac surgery. submitted.

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Meijssen S, Derksen RJ, Bilecen S, Erkelens DW, Cabezas MC. In vivo modulation of plasma free fatty acids in patients with familial combined hyperlipidemia using lipid-lowering medication. J Clin Endocrinol Metab. 2002 Apr;87(4):1576-80.

Meijssen S, Cabezas MC, Ballieux CG, Derksen RJ, Bilecen S, Erkelens DW. Insulin mediated inhibition of hormone sensitive lipase activity in vivo in relation to endogenous catecholamines in healthy subjects. J Clin Endocrinol Metab. 2001 Sep;86(9):4193-7.

Curriculum vitae

21-10-1976	Born in the city of Utrecht, The Netherlands
1989 – 1996	Atheneum, Niels Stensen College, Utrecht
1996 – 1997	Universiteit van Antwerpen, Faculteit Geneeskunde
1997 – 2003	Universiteit Utrecht, Faculteit Geneeskunde
2004 – 2005	Resident Cardiology Isala Zwolle
2006 – 2013	Resident Anesthesiology, University Medical Center Utrecht
2008 – 2012	Universiteit Utrecht, Master's Clinical Epidemiology (MSc)
2014 – present	Staff Anesthesiology / Pain specialist

Presentations:

2012	EACTA 2012	Poster presentation: Fibrinogen concentrate complex cardiac surgery.
2012	NVA Anesthesiologendagen	Oral presentation: Use of fibrinogen concentrate in cardiac surgery; it's effectiveness and safety. A retrospective study.
2011	Wetenschapsdag Isala klinieken	Oral presentation: Fibrinogeen in de hartchirurgie.
2009	ASA	meeting New Orleans Oral Presentation: Fibrinogen concentrate and blood loss in complex cardiac surgery.
2008	10th annual meeting of the Dutch	Poster presentation: Society of Cardiac Anesthesiologist Coagulation and lysis optimization in thoracic surgery.
2007	NVA Anesthesiologendagen	Oral presentation: Lithium; proposal for perioperative management.
2000	UMC Utrecht Wetenschapsdag	Poster presentation: Insulin mediated inhibition of hormone sensitive lipase activity in vivo in relation to endogenous catecholamines in healthy subjects. S. Bilecen, S. Meijssen, M. Castro Cabezas, C.G.M. Ballieux, R.J. Derksen, D.W. Erkelens.