Enhanced hemostasis management strategies in cardiac surgery

Meesters, M.I.

2017

document version
Publisher's PDF, also known as Version of record

citation for published version (APA)
Meesters, M. I. (2017). *Enhanced hemostasis management strategies in cardiac surgery*.

Enhanced hemostasis management strategies in cardiac surgery

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Enhanced hemostasis management strategies in cardiac surgery
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VRIJE UNIVERSITEIT

Enhanced hemostasis management strategies in cardiac surgery

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad Doctor aan
de Vrije Universiteit Amsterdam,
op gezag van de rector magnificus
prof.dr. V. Subramaniam,
in het openbaar te verdedigen
ten overstaan van de promotiecommissie
van de Faculteit der Geneeskunde
op donderdag 7 december 2017 om 11.45 uur
in de aula van de universiteit,
De Boelelaan 1105

door
Michael Isaäc Meesters
geboren te Amsterdam

PART I. Prevention of coagulopathy

PART II. Prediction of postoperative bleeding

PART III. Monitoring coagulopathy

PART IV. Treatment of coagulopathy

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ABBREVIATIONS

ACT	Activated Clotting Time
AF	Atrial Fibrillation
AKI	Acute Kidney Injury
ANH	Acute Normovolemic Hemodilution
ANOVA	Analysis of Variance
APTEM	Thromboelastometry extrinsic coagulation assay in the addition of Aprotinin
ASA	American Society Anesthesiology (classification)
AT	Antithrombin
AoX	Aortic Clamp Time
AUC	Area Under Curve
aPTT	Activated Partial Thromboplastin Time
BMI	Body Mass Index
CABG	Coronary Artery Bypass Graft
CAT	Calibrated Automated Thrombography
CC	Coaguchek; Point-of-care PT
CD	Conventional Dosing
CPB	Cardiopulmonary Bypass
CONSORT	Consolidated Standards of Reporting Trials
CT	Clotting Time
CTI	Corn Trypsin Inhibitor
CVA	Cerebral Vascular Accident
DOAC	Direct Oral Anticoagulant
ECC	Extracorporeal Circulation
ECMO	Extracorporeal Membrane Oxygenator
ELISA	Enzyme-Linked Immuno Sorbent Assay
EPO	Erythropoietin
EXTEM	Thromboelastometry extrinsic coagulation assay
FFP	Fresh Frozen Plasma
FIBTEM	Thromboelastometry fibrinogen assay
GP	Glycoprotein
Hb	Hemoglobin concentration
HEPTEM	Thromboelastometry intrinsic coagulation assay in the addition of heparinase
HIT	Heparin Induced Thrombocytopenia
HLM	Heart Lung Machine

ICU	Intensive Care Unit
IH-ratio	Intem:Heptem ratio
INTEM	Thromboelastometry intrinsic coagulation assay
INR	International Normalized Ratio
IQR	Interquartile range
IU	International Units
IABP	Intra-aortic balloon pump
LAB	Laboratory
LoA	Level of Agreement
LVAD	Left Ventricle Assist Device
MCF	Maximum Clot Firmness
MCL	Medical Center Leeuwarden
MEA	Multiple Electrode Aggregometry
METc	Medisch Ethische Toetsingscommissie
NATEM	Non-activated Thromboelastometry assay
PBM	Patient Blood Management
PCC	Prothrombin Complex Concentrate
PF4	Platelet Factor 4
PK	Pharmacokinetic
POC	Point-Of-Care
PRBC	Packed Red Blood Cell
PT	Prothrombin Time
RCT	Randomized Controlled Trial
ROTEM	Rotational Thromboelastometry
SD	Standard Deviation
SE	Standard Error
TEG	Thromboelastography
TEM	(Rotational) Thromboelastometry
TF	Tissue Factor
TIA	Transient Ischemic Attack
TRALI	Transfusion Related Acute Lung Injury
TXA	Tranexamic Acid
ROTEM	Rotational Thromboelastometry (= TEM)
VUmc	VU University Medical Center
vWF	von Willebrand Factor

Chapter 1

General introduction

HEMOSTASIS IN CARDIAC SURGERY

Hemostasis during cardiopulmonary bypass

During cardiac surgery with cardiopulmonary bypass (CPB), the coagulation system faces major alterations. First, as blood will flow through the large surface of the highly thrombogenic tubing of the heart lung machine, patients have to be anticoagulated in order to prevent consumptive coagulopathy and the formation of (micro) thrombi. Second, patients undergo major surgical trauma including sternotomy, venesection, arterial and venous cannulation, leading to hemostasis activation. Third, anticoagulation has to be reversed after weaning from cardiopulmonary bypass to restore normal clot formation. Fourth, multiple perioperative factors, such as acidosis, hypocalcemia, hypothermia and hemodilution may adversely affect hemostasis. The combination of these factors explain why major blood loss due to postoperative coagulopathy is common after cardiac surgery. [1]

In this context, the present thesis describes new methods for optimization of hemostasis management during cardiac surgery with cardiopulmonary bypass. In the first part of the introduction, the hemostasis system and the influences of cardiac surgery with cardiopulmonary bypass are reviewed. Hereafter, prevention, prediction, monitoring and treatment approaches for hemostatic disturbances in cardiac surgery will be discussed.
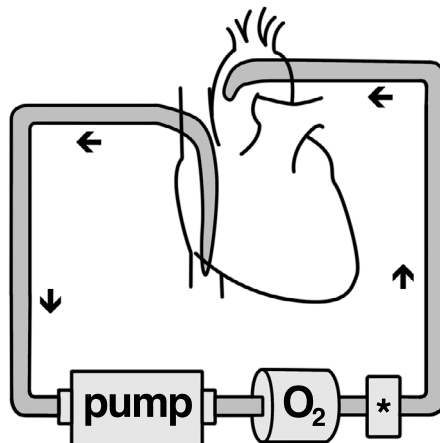

Figure 1.   Schematic overview of the heart lung machine. Blood is drained from the right atrium or central venous vessels, oxygenated, filtered (*) and pumped into the aorta.

Current view on hemostasis

A thorough understanding of hemostasis is necessary for optimal coagulation management. Here we outline the general concept of hemostasis. Hemostasis can be described as the complex interaction between the vascular wall, thrombocytes and clotting factors. The traditional concept of hemostasis divides the clotting system in two sequences: the formation of a platelet plug followed by the coagulation 'waterfall' cascade, which dates back to 1964. [2,3] Although, this approach supports the interaction between clotting factors and is in line with the classical laboratory coagulation tests it does not represent in vivo hemostasis. A novel view on hemostasis is the 'cell-based model of hemostasis', which incorporates endothelium, platelets and clotting factors into one concept. [4] This model hypothesizes that hemostasis should not be regarded as a cascade but continuously takes place on cell surfaces in the following three overlapping stages:

1) Initiation of hemostasis occurs on tissue factor (TF) bearing cells, including fibroblasts, macrophages, mononuclear cells, outside the vascular endothelium. This is a continuous low-grade process in the absence of vascular damage, which is active in an equilibrium with a process counteracting coagulation.

2) Amplification results from vascular damage leading to contact between the TF bearing extravascular cells and large hemostatic intravascular components, as factor VIII and von Willebrand factor (vWf). In this phase, a platelet plug is formed, while thrombocytes and cofactors are prepared for major thrombin generation.

3) Propagation is the generation of large amounts of thrombin on the platelet surface due to an abundance of procoagulatory factors that outweigh inhibitors of the coagulation. In the final step thrombin in formed which results in the cleavage of fibrinogen to fibrin which polymerizes within the platelet plug to form a solid clot.

Besides platelets, the endothelium and coagulation factors as prerequisites of adequate hemostasis, blood temperature, pH and calcium levels also need to be within normal ranges as they serve as cofactors for adequate hemostasis. [5] Hypothermia has been found to prolong the initiation phase, whereas hypocalcemia inhibits normal clot formation. [6] Acidemia prolongs the propagation phase, and insufficient organ perfusion with metabolic acidosis can therefore deteriorate hemostasis, linking hemodynamics and hemostasis. [7] This lethal triad, consisting of acidosis, hypothermia and hypocalcemia, is particularly important in the case of ongoing hemorrhage as blood loss leads to hypovolemic

shock with subsequent acidosis. Moreover, massive blood transfusion can cause hypocalcemia leading to a downward spiral of ongoing bleeding due to coagulopathy leading to further bleeding.

1

intrinsic
XII
  XI
    IX + VIII **extrinsic**
      VII + TF
      ↑ **X**
     V ← **II**
        I
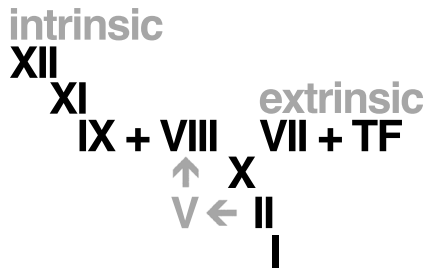
Figure 2. Classical coagulation cascade: the intrinsic and extrinsic pathway.

It has been suggested that erythrocytes as main oxygen carriers within the circulation are also involved in hemostasis, as they get trapped in the fibrin network (figure 3), thereby assisting in the formation of a solid blood clot. However, evidence for this phenomenon from in vitro – and clinical studies is limited. [8-11] A recent meta-analysis reported no differences in bleeding or thrombotic complications between liberal and restrictive red blood cell transfusion thresholds. [12] This suggests that aiming for a higher hemoglobin level in the bleeding patient does not improve hemostasis. On the other hand, adequate hemoglobin levels may contribute to normal oxygen delivery to the tissues, thereby preventing organ hypoxia and academia, which is favorable for the hemostatic system.

Another, important component of the hemostatic system is the ability to breakdown fibrin polymers after restoration of vascular damage, the so-called fibrinolytic pathway. [13] The fibrin network is cleaved by plasmin, which is formed by the activation of plasminogen. Pathologic (hyper)fibrinolysis resulting in abundant fibrin degradation has been observed in surgical wounds, and might play a role in persistent bleeding after cardiac surgery. [14,15] In most cardiosurgical procedures (hyper)fibrinolysis is counteracted by the administration of antifibrinolytics.

Hemostasis in cardiac surgery is further complicated by the preoperative use of platelet inhibitors and anticoagulants. These drugs are widely used in cardiac patients to prevent cardiovascular accidents as recommended by the European Society of Cardiology (ESC). [16] Acetylsalic acid is advised to be continued during the perioperative period, whereas P2Y12 inhibitors (i.e. clopidogrel, prasugrel and ticagrelor) should be discontinued 5-7 days prior to cardiac surgery. [17,18]

Increasing evidence suggests that the continuation of preoperative platelet inhibition reduces the postoperative risk on ischemic events, such as myocardial infarction. [19] Therefore, the increasing preoperative use of platelet inhibitors leads to an additional risk of bleeding. [20-22]
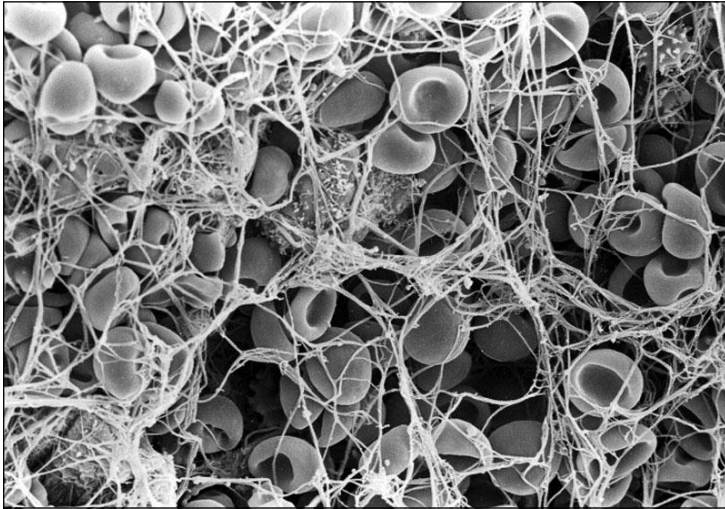
Figure 3. A scanning electron micrograph image of a blood clot that shows red blood corpuscles and fibrin. Credit: David Gregory and Debbie Marshall / Wellcome Images.

Other anticoagulants, such as heparins, vitamin-K-antagonists and direct oral anticoagulants (DOACs) are used in cardiac patients for the prevention of clot formation in atrial fibrillation, venous thromboembolism and artificial cardiac valves. [23,24] While heparins and vitamin-K-antagonists can be rapidly antagonized by protamine and prothrombin complex concentrate, respectively, most DOACs have no specific antagonist available. When postponement of surgery is impossible, unantagonized anticoagulants and long acting platelet inhibitors may impair patient hemostasis in emergency situations.

Cardiopulmonary bypass-related effects on hemostasis
The cardiopulmonary bypass circuit, used during most forms of cardiac surgery, exerts major effects on hemostasis. First, the extracorporeal circulation circuit is primed with fluids, causing significant dilution of coagulation factors. [25,26] Second, during cardiopulmonary bypass, blood is exposed to the non-biological,

negatively charged surface of the tubing, thereby activating the coagulation cascade. This causes fibrin formation and consumptive coagulopathy. [27,28] Third, contact activation during cardiopulmonary bypass leads to initiation of the fibrinolytic system, which might cause rapid clot breakdown and exaggeration of coagulopathy after CPB. [13]

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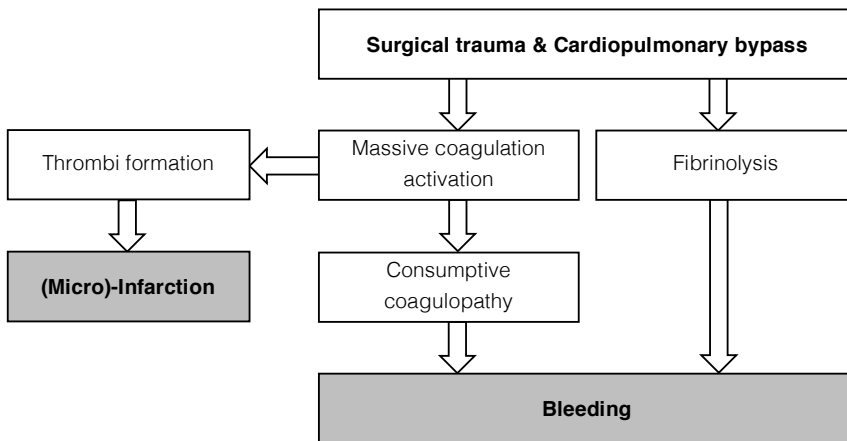
Figure 4. The effect of surgical trauma and cardiopulmonary bypass on hemostasis.

Heparin anticoagulation

In order to reduce massive hemostatic activation during cardiopulmonary bypass, patients are usually fully anticoagulated prior to extracorporeal circulation with unfractioned heparin. Heparinoids are physiologically present on the vascular endothelium to prevent occult thrombin formation. Heparins bind to antithrombin (AT), a plasma protein that inhibits the activated clotting factors II, VII, IX, X and XI. By administration of a high dose of heparin, the risk of thrombin formation and consumptive coagulopathy is decreased.

Some patients have a reduced AT level, which decreases the effect of heparin and requires increased heparin dosage for the desired anticoagulant effect. This phenomenon is called 'heparin resistance' and can be congenital (1:3000 patients) or acquired due to kidney disease (increased AT loss), malnutrition or reduced liver function (reduced synthesis). The clinically most relevant cause of 'heparin resistance' is due to heparin pretreatment and the use of cardiac support devices (IABP, LVAD, ECMO), which may cause a consumption of antithrombin. Unfractioned heparin consists of a mix of a short and long chained molecules, which have a different affinity for thrombin and factor Xa. The ratio in chain lengths

varies among batches and suppliers, leading to a varied anticoagulant effect. Additionally, heparin binds to the glycocalyx of the endothelium and plasma proteins affecting its biological availability and half-life. The clearance of heparin reduces with increasing heparin concentrations due to a rapidly saturated clearance by macrophages and endothelium in addition to the slower elimination by renal clearance.

Because of the variable effect of heparin, the level of effective anticoagulation is monitored using the activated clotting time (ACT). The ACT measures the time until clot formation in whole blood after the addition of a contact activator, activating the intrinsic coagulation cascade. The contact activator is used as a surrogate for the surface of the HLM. However, the ACT has major limitations as it does not correlate with the heparin concentration and the optimal ACT for adequate anticoagulation is unknown, resulting in varied ACT targets among practices. [29,30] Although an ACT greater than 400 – 480 seconds is commonly thought to reflect adequate anticoagulation, thrombin formation is still present. Finally, the ACT is prolonged in the case of protamine overdosing, this clinical relevance will be discussed later.

Heparin
Variable chain length
[AT] dependent
Binding to non-AT surfaces
Pleiotrophic effects (mainly IIa and Xa)
Dose dependent half-time
Monitoring difficulty (ACT)
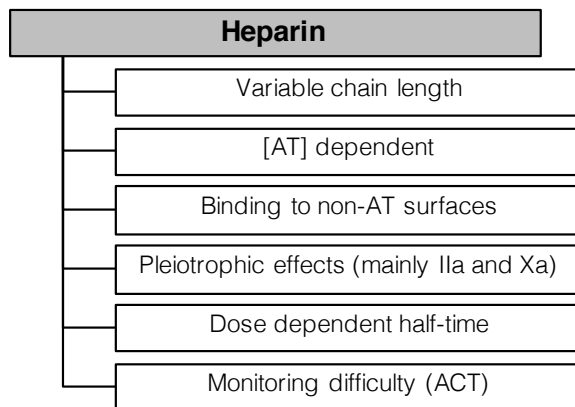
Figure 5.   Causes of the variable effect of heparin among different cases.

Other methods to prevent coagulopathy

Besides the use of heparin, further attempts have been made to reduce the massive hemostatic activation during bypass, such as the use of cell salvage. Blood sucked from the surgical field is highly activated due to its contact with the extravascular surface. [31-33] When this activated blood is directly pumped into the heart lung machine, it will attribute to consumptive coagulopathy. However,

when pericardial blood is washed by the cell saver, coagulopathic components are removed leading to less hemostatic activation. Still, large amounts of cellsaver blood transfusion (> 1 liter) may cause dilution of coagulation factors due to the retransfusion of erythrocytes without plasma. This might explain why the use of a cell saver reduces allogenic red blood cell transfusion, but not overall outcome. [34]

Another attempt to reduce hemostatic activation during extracorporeal circulation is by heparin coating of the heart lung machine. Also, the surface of certain extracorporeal circuits has been modified to prevent protein binding and complement and coagulation activation. The use of these circuits has resulted in improved outcome, although the effect size is limited. [35,36] Reduction of the volume of the extracorporeal circuit may further reduce hemostatic activation surface area. These miniaturized CPB circuits have indeed proven to improve hemostasis, most likely due to the relatively lower level of hemodilution and contact activation. [37] Finally, the avoidance of exposure to an extracorporeal circuit by performing off-pump surgery reduces blood transfusion compared to on-pump surgery. [38,39]

Cardiopulmonary bypass
Heparin
Hemodilution
Surface activation
Fibrinolysis
Pericardial shed blood
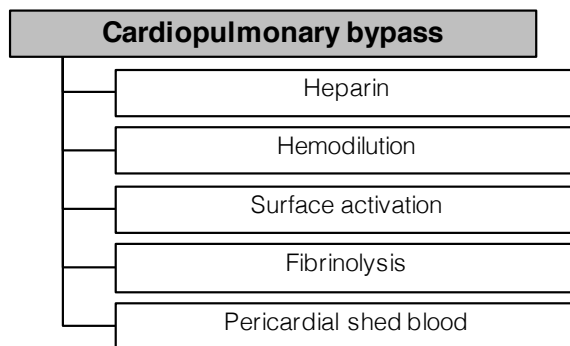
Figure 6. Hemostatic complicating factors with the use of cardiopulmonary bypass.

Bleeding

Due to all the above-mentioned risks for coagulopathy, major bleeding is common after cardiac surgery but highly variable among centers, ranging from 0.4% to 16.3%. [1,40-43] Still, the definition of massive blood loss is highly variable among studies. Dyke et al. attempted to develop a universal definition for major perioperative bleeding. [46] They based their definition on several measures including the amount of postoperative chest tube drainage, blood product transfusion (erythrocytes, platelets, plasma), factor concentrate administration

(prothrombin complex concentrate, fibrinogen concentrate), delayed sternal closure and surgical reexploration for bleeding. Nevertheless, postoperative blood loss remains highly variable among practices and therefore massive blood loss should be seen in the light of the average amount of blood loss per center. The definition of Dyke et al. should be reserved for research purposes and benchmarking among centers.

Massive bleeding is associated with adverse outcome. [44,45] Ongoing hemorrhage risks the patient for rethoracotomy to control the bleeding, which is an independent predictor for mortality. [47-49] Besides the risk for rethoracotomy, bleeding leads to anemia. Pre- and postoperative anemia is also associated with adverse outcome. [50,51] However, not only anemia, also blood product transfusion is independently associated with short - and long term mortality. [52,53] Even transfusion of small amounts of blood are associated with reduced outcome. [54-61]

Blood transfusion can cause immune suppression, thereby increasing the risk of infection and cancer recurrence. [62] Also, although rare in current practice, blood product administration can trigger transfusion reactions and transmission of infections. Transfusion further risks patients for Transfusion Related Acute Lung Injury (TRALI), hemochromatosis, acute kidney injury (AKI), atrial fibrillation (AF), respiratory failure, neurological accidents, myocardial infarctions and rethoracotomy. [53,63,64] This has led to the design and execution of large randomized controlled trials investigating the optimal transfusion trigger for red blood cells in order to reduce blood product exposure. [65-67]
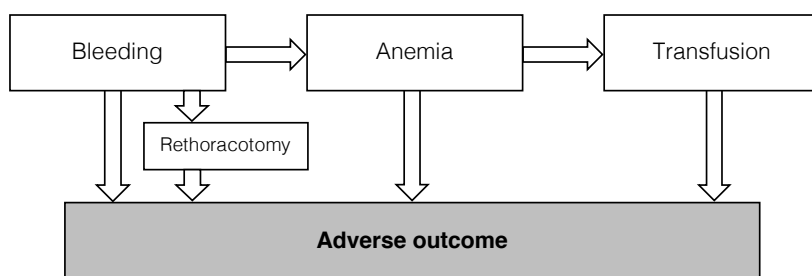
Figure 7.   The relationship between bleeding, rethoracotomy, anemia, transfusion and outcome.

Besides these consequences, blood transfusion in cardiac surgery has a major burden on the total blood product consumption as up to 20% of all blood transfusion occur in cardiac surgery. [68] Although this statement dates back

15 years, recent reports still show absence of a reduction in blood transfusion due to surgery in higher risk patients, an increase in the complexity of surgery and the lack of implementation of improved management strategies. [69,70] The high transfusion rate brings major costs due to the utilization of blood products and the subsequent adverse outcome of transfusion. [71] As bleeding, anemia and transfusion are associated with adverse outcome the main focus should be to prevent, predict, monitor and promptly treat blood loss in order to improve outcome. This method is multimodal and commonly referred to as 'Patient blood management'. This thesis focusses on several aspects of this approach.
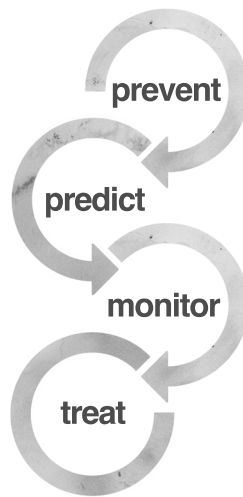
Figure 8. The aspects of patient blood management.

PREVENTION OF BLEEDING

The first step in patient blood management should be the prevention of coagulopathy, bleeding and transfusion. Many attempts have been made to prevent coagulopathy during and after cardiac surgery. Common practice is the use of antifibrinolytics to prevent massive fibrinolysis caused by the extracorporeal circulation and surgical wound. Formerly, aprotinin was used for this purpose with pleiotropic positive effects on hemostasis; inhibition of fibrinolysis, thrombin, platelets and activated protein C, preventing consumptive coagulopathy during bypass. [72,73] However, due to safety concerns aprotinin has been withdrawn from the market in 2007 as it was associated with kidney failure and increased mortality while it was superior in hemostatic optimization. [74,75] Although, aprotinin has recently been readmitted to the market its use has been replaced by tranexamic acid and ε-aminocapron acid.

A hot topic in the prevention and treatment of bleeding in cardiac surgery is the use of fibrinogen concentrate. Fibrinogen (coagulation factor I) is converted to fibrin by thrombin and the key element in clot formation. Additionally, fibrinogen is one of the first elements to cease critically in the case of bleeding. [76] Preoperative fibrinogen levels are inversely associated with postoperative bleeding in cardiac surgery, but correction of preoperative fibrinogen level to > 2.5 g l^{-1} is not recommended based on the current evidence. [77-79]

As stated before, inadequate tissue perfusion during major bleeding can cause acidosis, which impairs clot formation and contributes to further bleeding. [7] Therefore, adequate tissue perfusion is important for the prevention of (further) bleeding after cardiac surgery. However, excessive fluid administration to revert shock causes dilution of coagulation factors, which also impairs clot formation. Although the evidence for goal directed fluid management in cardiac surgery to reduce bleeding is lacking, reducing the prime volume and infusion volume (and thereby reducing hemodilution) has been proven to improve outcome. [37] If reducing the cardioplegia volume affects clinical outcome remains controversial, still it reduces hemodilution and might improve hemostasis. [80] Besides the prevention of fluid overload, the choice of the infused fluid and prime volume is important. Colloids preserve plasma volume and tissue perfusion more than crystalloids. Yet, colloids are associated with hemostatic impairment when compared to (balanced) crystalloids. [81-85]

The exposure to allogenic blood products could be prevented by autologous blood (pre)donation of blood prior to elective cardiac surgery. Although robust studies are lacking, there is some evidence that predonation of blood might reduce allogenic blood product exposure in cardiac surgery. [86-89] However,

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the safety, criteria and indication for predonation have not yet been clarified. An alternative to preoperative autologous blood predonation is acute normovolemic hemodilution. A certain amount of patients' blood is drawn prior to CPB and retransfused after cardiopulmonary bypass. This method is better investigated and shows a reduction of bleeding and transfusion after cardiac surgery. [90] Likewise, there is a specific method for harvesting patients' thrombocytes before surgery by 'acute preoperative platelet pheresis'. Although a reduction in blood product transfusion was seen, the evidence for this technique is scarce, outdated and of poor quality. [91]

A more logical way to prevent transfusion is to optimize preoperative hemoglobin levels in order to prevent anemia-related blood transfusion. Only a limited number of studies investigated the preoperative treatment of anemia in cardiac surgery. [92,93] The use of erythropoietin (EPO) with and without iron supplementation contributes to a reduction in transfusion requirements. [94-96] There is insufficient data to advice on the preoperative red cell transfusion to prevent anemia and transfusion requirements. [97]

PREDICTION OF BLEEDING

Several risk factors for bleeding in cardiac surgery have been identified. Patient related risk factors include advanced age, diabetes mellitus, preoperative low fibrinogen level, dual antiplatelet use, female gender, a low body mass index (BMI) and impaired left ventricular function. [77,98,99] Surgery related risk factors are emergency surgery, more complex cardiac surgery, a long duration of extracorporeal circulation and the cardiac surgeon. [99] In attempts to facilitate the preoperative prediction of need for transfusion, specific risk assessment scores have been developed in order to identify patients at high risk for bleeding. [100-104] Besides these clinical parameters several studies have investigated the predictive value of point-of-care (POC) hemostasis monitors for postoperative bleeding. To evaluate if rotational thromboelastometry can predict which patients will excessively bleed in the postoperative period we conducted a systematic review of the literature in addition to the data of our own center in **chapter 5.**

Other POC devices evaluate the aggregation capacity of thrombocytes after activation by different activators, also known as platelet aggregometry or multiple electrode aggregometry (MEA). Platelet function testing is found to be a good predictor of postoperative blood loss. [105,106] Still, the question remains what degree of platelet inhibition is still safe to perform surgery and when and how platelet inhibition should be treated in order to prevent excessive bleeding. [107]

MONITORING AND TREATMENT OF COAGULOPATHY

There are several causes of bleeding after cardiac surgery as summarized in figure 9. [108] Bleeding can be caused by insufficient reversal of heparin, leading to residual anticoagulation. Other causes of reduced thrombin formation can be the loss or dilution of coagulation factors, including fibrinogen. Circumstantial support for clot formation might be inadequate such as hypothermia, academia or hypocalcemia, as mentioned earlier. Thrombocytes might be deficient or dysfunctioning due to the cardiopulmonary bypass or preoperative continued antiplatelet therapy. Furthermore, clots may be broken down rapidly after formation or there might be a macrovascular (surgical) cause of bleeding. Here we will discuss the possible non-surgical treatment options for bleeding after cardiac surgery.
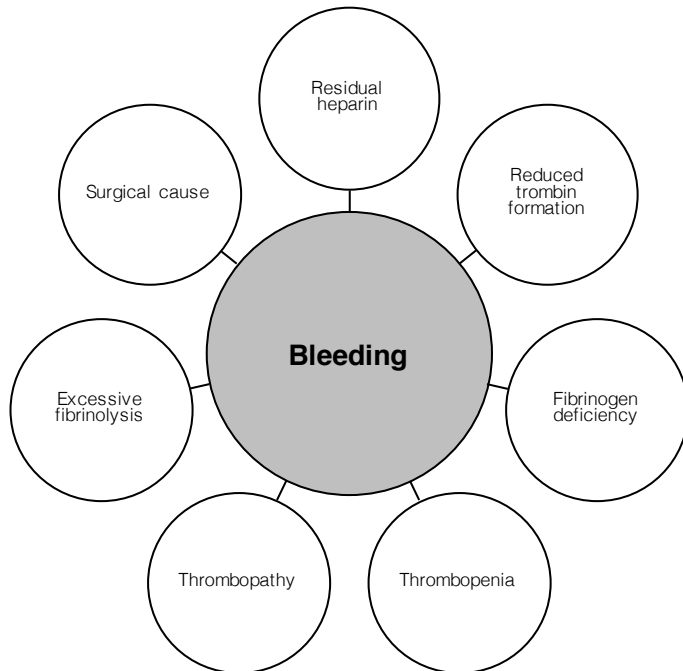

Figure 9.   Postoperative causes of bleeding (Based on: Ranucci M. Hemostatic and thrombotic issues in cardiac surgery. Semin Thromb Hemost; 2015 Feb;41(1):84–90.)

The first step in the optimization of hemostasis after cardiopulmonary bypass is the reversal of heparin with protamine. Although protamine has been used

for over 50 years, still no optimal dosing regime has been established and the adequate reversal ratio is unknown. [109,110] For many years it was thought that overdosing of protamine was harmless. However, the last decades it has come apparent that *in vitro* protamine has anticoagulant properties and preventing an excess of protamine might reduce bleeding. [111] In **chapter 2** we reviewed the literature for the (side)effects of protamine, the evidence for optimal protamine dosing and suggested directions for future research. In order to investigate if protamine impairs hemostasis in the clinical setting we conducted a randomized controlled trial to investigate the effect of high protamine dosing on hemostasis and outcome compared to low protamine dosing, as shown in **chapter 3**. Hereafter, we evaluated if a pharmacokinetic model for protamine dosing leads to more adequate protamine dosing and prevents a protamine excess (**chapter 4**). After (adequate) reversal of heparin by protamine patients may show signs of excessive microvascular bleeding for which hemostatic treatment is required. Classical laboratory coagulation tests (aPTT and PT) have long turnaround times and the time from blood withdrawal till the test results can be up to 45 minutes. However, as mentioned before, a bleeding patient needs prompt treatment to prevent adverse outcome. A method to gain rapid insight in patients' coagulation status is the use of thromboelastometry. [112] Whole blood is placed in a plastic cup and coagulation activators are added. A pin is placed in the cup which oscillates back and forth and the resistance of the oscillations are measured. This method assesses the viscoelastometric properties of whole blood and thereby several aspects of hemostasis are evaluated. The increase of the viscosity due to clot formation results in an increased resistance of the oscillations which correspond with the formation of a blood clot. The time until clot formation (e.g. clotting time (CT)) represents the time until the first increase in resistance, as shown in figure 10. The CT is mainly influenced by the amount of clotting factors, comparable to the classical coagulation testing (aPTT and PT). The amplification phase of hemostasis is represented by the alpha angle or clot formation time (CFT), which represents the speed of the clot formation. The maximum resistance of the oscillations is related to the clot thickness and corresponds with the platelet and fibrinogen part of the clot, represented by the maximum clot formation (MCF) respectively. Furthermore, there are specific tests which evaluate; the fibrinogen part of the clot (by inactivating the platelets; FIBTEM), the hemostatic activity with (INTEM) and without the heparin effect (by the addition of heparinase; HEPTEM) and without the fibrinolytic activity (by the addition of aprotinin; APTEM).
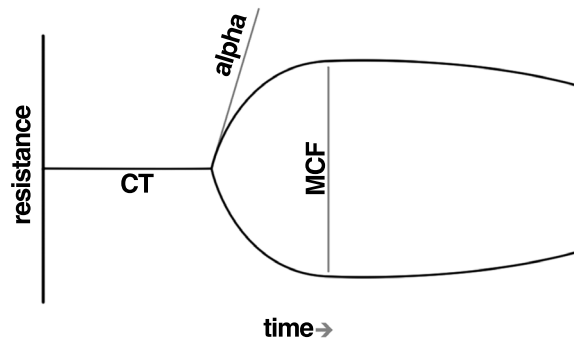
Figure 10. Thromboelastometry trace, CT = clotting time, MCF = maximum clot firmness. (copyright M I Meesters 2017)

Performing thromboelastometry gives rapidly a lot of information about the different aspects of hemostasis and indicates which components are deficient. As we found that the heparinase thromboelastometry assay (HEPTEM) showed inconsistencies before and after cardiac surgery we evaluated the value of the HEPTEM test in **chapter 6**. Furthermore, we investigated the influence of storage time of the blood sample on the non-activated thromboelastometry assay (NATEM) in the citrated blood in **chapter 7**.

Thromboelastometry has gained rapid popularity in the last decades without many proper studies investigating its effect on clinical practice. Most studies had a retrospective design and/or implementation of thromboelastometry was accompanied by the change of practice from fresh frozen plasma (FFP) to factor concentrates for coagulation factor replenishment. [113] Therefor it is difficult to assess if improvement of practice was caused by the implementation of thromboelastometry or the use of factor concentrates. Furthermore, the purchase of a thromboelastometry device creates more awareness for hemostatic treatment and can bias the study results. In **chapter 10** we evaluated if the decision to transfuse fresh frozen plasma based on the clinical view of the attending anesthesiologist to transfuse FFP is in line with the findings of thromboelastometry. Besides thromboelastometry there are other devices which rapidly evaluate the clotting capacity and might guide hemostasis treatment practice, i.e. sonoclot, tromboelastography (TEG), point-of-care fibrinogen monitors, etcetera. In **chapter 8** we evaluated the accuracy of a point-of-care prothrombin time (PT) meter 3 minutes after protamine reversal after cardiac surgery and found a large discrepancy between the POC and laboratory PT. In order to assess if this discrepancy persisted over time we conducted another investigation to assess if

6 or 10 minutes after protamine reversal the device would give accurate results, as shown in **chapter 9**.

When specific coagulation deficiencies have been identified, several therapeutic options are available depending on the deficient component. In the case of a lack of coagulation factors fresh frozen plasma or prothrombin complex concentrate (PCC) may be transfused. PCC contains coagulation factors II, VII, IX, X, which are thought to be the critical coagulation factors for clot formation. PCC further contains the natural anticoagulants protein C and S. The advantage of PCC is the small volume and lack of risk for blood transfusion related events which can occur in FFP transfusion. Furthermore, PCC is rapid available compared to FFP which has to be brought from the blood bank and thawed before administration. Additionally, the concentration of coagulation factors is low in FFP and its large volume risks the patient for fluid overload. Still, PCC is more expensive and some clinicians argue that FFP contains all clotting factors and is therefore superior to PCC in restoring hemostasis. However, this argument is refuted by current evidence. [114] A possible advantage of FFP is the preservation of the glycocalyx as shown in the preclinical setting. [115,116] Furthermore, there is insufficient safety data on the use of PCC. As PCC was developed for the reversal of anticoagulation by vitamin K antagonists PCC is off-label used for bleeding management. [117,118] Currently there is no evidence for the advantage of PCC over FFP. [119] However, the evidence for FFP transfusion after cardiac surgery itself is insufficient. [120]

A component of fresh frozen plasma is fibrinogen, the concentration is very low and large quantities need to be transfused in order to raise the plasma fibrinogen level. [121] A more effective way to supplement fibrinogen is by administration of fibrinogen concentrate. [122] Recently, many studies have been published on the use of fibrinogen and its use seems an effective method to reduce bleeding in the patient with hypofibrinogemia. [123-127] Again, its safety profile has not yet been fully elucidated. [128] In **chapter 11**, we comment on the most recent and one of the largest trials on fibrinogen supplementation in cardiac surgery. [124]

A less known coagulation factor is factor XIII, which is activated by thrombin. Factor XIII is responsible for the cross linking of fibrin and stabilizes the fibrin network. When this factor is low or deficient the stability of the clot is reduced. Cardiopulmonary bypass causes a deficiency of this factor and therefor factor XIII has been targeted to reduce postoperative bleeding. [26,129] Still, only one study investigated the effect of recombinant factor XIII on postoperative bleeding in cardiac surgery. They found that it is reasonable to administrate f.XIII in the case of postoperative bleeding when the plasma activity of factor XIII is below 70%. [130]

Besides coagulation factor deficiency, thrombocytopenia and thrombocytopathy are causes of bleeding after surgery. The number of platelets can easily be assessed by laboratory testing and thromboelastometry, with results after approximately 20 and 15 minutes. Thrombopenia is treated by platelet transfusion and according to most guidelines indicated when thrombocytes are below $50\text{-}100*10^9$ L^{-1}. [131] Platelet function is more difficult to assess and recently it has become possible to measure the clotting capacity at the point of care, as mentioned before. However, the consequences of the results from platelet aggregometry have not fully been elucidated in current literature. [107] A possible treatment strategy for the treatment of platelet dysfunction is the administration of desmopressin. However, when given as general treatment for bleeding only a small reduction in postoperative blood loss is found. [132] Further studies need to elucidate if desmopressin might have a greater advantage in the thrombopathic and vWF deficient patient. [133]

As a last resort treatment option for massive coagulopathic bleeding activated factor VII may be considered. However, as this might cause excessive clotting, including in the coronaries, brain and other end organs its use should be saved for patients in which all other treatment options fail. [134]

1

PATIENT BLOOD MANAGEMENT

As described, the multimodal approach to reduce bleeding and improve outcome is highly diverse and complex. Figure 11 gives a condensed overview of the current management possibilities in cardiac surgery, the themes of this thesis are marked grey. Although each intervention may have a small impact on clinical outcome the overall benefit of the blood management bundle leads to improved outcome. [20,135]
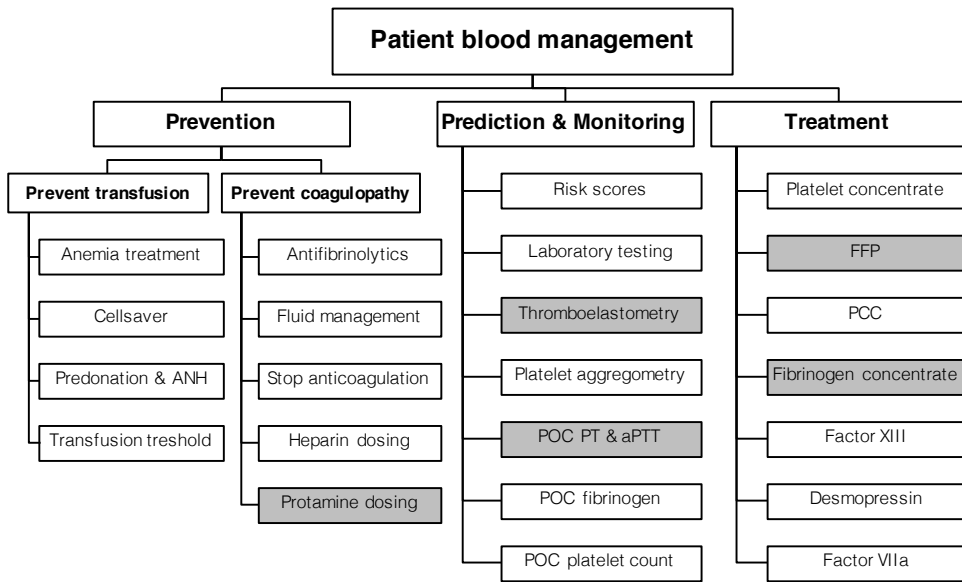

Figure 11. Multimodal patient blood management in cardiac surgery. Grey marked boxes = themes of this thesis. ANH = acute normovolemic hemodilution, aPTT = activated partial thromboplastin time, FFP = fresh frozen plasma, PCC = prothrombin complex concentrate, POC = point-of-care, PT = prothrombin time.

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Prevention of coagulopathy

part

Chapter 2

Procoagulant and anticoagulant effects of protamine in cardiac surgery

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ABSTRACT

Neutralisation of systemic anticoagulation with heparin in cardiac surgery with cardiopulmonary bypass requires protamine administration. If adequately dosed, protamine accurately neutralises heparin and reduces the risk of postoperative bleeding. However, as its anticoagulant properties are particularly exerted in the absence of heparin, overdosing of protamine may contribute to excessive bleeding and increased transfusion requirements. This review describes the mechanisms underlying the anticoagulant properties and side effects of protamine, the impact of protamine dosing on the activated clotting time (ACT) and point-of-care viscoelastic test results, and explains distinct protamine dosing strategies in relation to haemostatic activation and postoperative bleeding.

INTRODUCTION

Protamines are small, nuclear, basic, arginine-rich, positive charged proteins with similarities to histones that are involved in the compact folding and stabilization of DNA in the sperm head. Protamines are required for normal sperm function in most organisms. [1] Protamine was primarily isolated from salmon fish sperm (salmine), but is now increasingly produced through recombinant biotechnology. Protamine is available in a sulphate and chloride formulation, with the latter being the most resistant to breakdown by peptidases.

In the first half of the 20th century, protamine was mostly used in insulin preparations to prolong the hypoglycaemic action of insulin. This resulted in the development of the effective crystalline neutral protamine Hagedorn insulin. [2] The haemostatic effects of protamine are first described in the beginning of the 20th century, [3,4] and in 1937 it was shown that protamine neutralises the anticoagulant effects of heparin in plasma and intact organisms [5] and humans. [6] While topical application of protamine on bleeding wounds in dogs did not influence haemostasis, it was also found that adding increasing quantities of protamine sulphate to fresh whole blood prolonged the clotting time. [7] These anticoagulant effects of protamine were appointed to interference with the conversion from prothrombin to thrombin. [8,9] From these studies, it became apparent that the dosing of protamine is crucial in the shift towards an anticoagulant effect. [7] In the years that followed, the anticoagulant action of protamine was noticed, but there was more emphasis on heparin and the heparin-neutralising potency of protamine and the concomitant side effects. This resulted in an imbalance between Pubmed publications focusing on heparin or protamine in cardiac surgery, although the attention for heparin also decreased over the last decades (Figure 1).

While protamine is indispensable in cardiac surgery with cardiopulmonary bypass and off-pump cardiac surgery, the ignorance of the anticoagulant properties of excessive protamine may result in prolonged bleeding and increased transfusion requirements, and increasing attention for adequate dosing is therefore warranted. The objective of this review was to present an overview of the anticoagulant properties and side-effects of protamine, the evidence for the unfavourable effects of protamine overdosing on patient haemostasis, and suggestions for optimization of protamine dosing for heparin neutralisation.
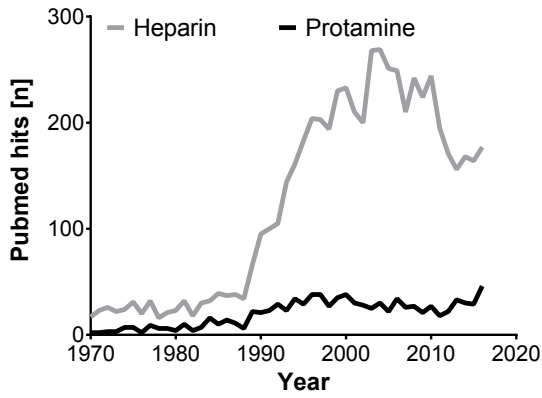
Figure 1. Pubmed hits for [Heparin] and [Cardiac Surgery] (gray line) and [Protamine] and [Cardiac Surgery] (black line). *Results of January 1 2017.*

MECHANISM OF ACTION

Pharmacodynamics

Protamine is a highly positive charged peptide consisting of about 32 amino acids, and neutralises the effect of heparin through electrostatic binding between the cationic arginine groups of protamine and the anionic heparin. Therefore, the empirical dosing of protamine is 1 IU for 1 IU heparin (1:1). The resulting neutral protamine-heparin salt aggregates are clearly visible as white suspension, and are formed within seconds. [10] In parallel, the binding of protamine to heparin dissociates the antithrombin-heparin complex, leading to recovery of original antithrombin activity. [11-14] The neutralisation of heparin by protamine is further influenced by platelet factor 4 (PF4), a heparin-binding protein excreted by activated platelets. PF4 is complimentary to protamine regarding heparin neutralisation.15 In case of vessel wall damage, PF4 overcomes the anticoagulation effect of the endothelium. During extracorporeal circulation, PF4 release contributes to the stability of the protamine-heparin complex. [15]

Pharmacokinetics

Protamine has a relatively short half-life of about 10 minutes in healthy volunteers in the absence of heparin administration. [16] Protamine has a rapid onset of action, with a heparin-neutralising effect within 5 minutes. In patients undergoing cardiac surgery with CPB, 250 mg of protamine sulphate administered as infusion over 5 minutes in the presence of heparin showed a clearance of protamine of 1.4 L min^{-1} and a half-life between 1.9 and 18 minutes. [17] Although there is almost no insight in the metabolism of the neutral protamine-heparin salt complex, the protamine-heparin complex may lead to antibody formation and side effects which are described below.

2

ANTICOAGULANT PROPERTIES OF PROTAMINE

While protamine primarily neutralises heparin, protamine has anticoagulant properties that are attributed to an interaction with platelet function, interference with coagulation factors and stimulation of clot breakdown. The effects of protamine on these different functions in the haemostasis system are summarized in figure 2.
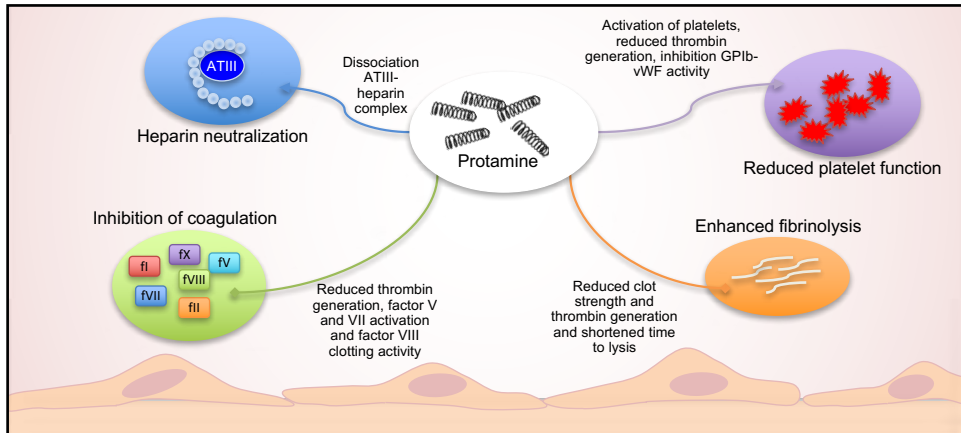
Figure 2. Overview of the haemostatic actions of protamine. Protamine not only neutralises heparin but also interferes with platelet function, the generation and activation of coagulation factors and potentiates clot lysis. ATIII = antithrombin, GP1b = glycoprotein Ib; vWF = von Willebrand Factor.

Interaction with platelet function

The effect of protamine on platelet function was first described in 1949, showing thrombocytopenia after protamine exposure. [18] Although this effect is nowadays ascribed to protamine-heparin complex antibody formation, protamine also directly activates platelets and reduces the response of platelets to thrombin. [19-21] Barstad and colleagues further showed that the glycoprotein Ib interaction with surface-bound von Willebrand Factor (vWF) is reduced by protamine, leading to disturbed platelet adhesion to the damaged vessel wall. [22]

A recent study showed that protamine reduced platelet aggregation by 50% in heparinized patients undergoing cardiac surgery with CPB. [23] This study was however limited by a 1:1 protamine-to-heparin dosing ratio that was calculated for the total heparin dose administered during CPB regardless of heparin consumption. [23] Similar findings were reported in patients undergoing pulmonary thromboendarterectomy with deep cooling, but this study also used

protamine-to-heparin ratios exceeding 1. [24] In vitro studies show that blood spiked with increasing doses of protamine reduces ADP-induced platelet aggregation. However, all studies used relatively high protamine-to-heparin ratios ranging from 1:1 to 10:1. [23-25] Using a protamine titration method, Shigeta and colleagues showed in a small patient study that the platelet inhibitory effects of protamine are indeed dose-dependent, and can be minimized by adaptation of the protamine dose to the residual heparin concentration at the end of CPB. [21]

Interference with coagulation factors

In vitro experiments previously elaborated that protamine mediates down-regulation of thrombin generation leading to a reduced conversion of fibrinogen to fibrin, [26,27] reduces the rate of factor V activation by thrombin and factor Xa,28 and decreases factor VII activation by tissue factor thrombin activity. [29] In haemophilia patients, a direct drop in factor VII clotting activity was observed upon protamine dose ranges between 30 and 100 µg ml^{-1}, irrespective of the presence of heparin. [30]

In a study in patients receiving a high or low protamine-to-heparin ratio, the recovery of thrombin generation following heparin neutralisation was impaired in the high protamine dosing group. [31] Others showed that part of the inhibitory effects of protamine on thrombin generation is neutralised by the addition of platelets or increased factor VIII/vWF concentrations. [32,33] The aforementioned observations could also be confirmed during functional viscoelastic testing, showing that incremental doses of protamine or protamine-to-heparin dosing ratios > 1 resulted in a prolonged clotting time of the intrinsic coagulation test. [31,32,34,35] Rotational thromboelastometry experiments show that the administration of extra protamine in cardiosurgical patients with an abnormal activated clotting time after CPB results in a brief prolongation of clotting times. [36] In agreement with these findings, it was also shown that protamine prolongs the clot initiation time in thromboelastography, and impairs clot kinetics and platelet function. [37] From these observations, it can be concluded that protamine may interfere with coagulation factors involved in clot formation, although this association is mostly seen for higher protamine doses.

Stimulation of clot breakdown

The decreased thrombin levels associated with protamine administration may also negatively influence the fibrinolytic system. [38,39] In vitro experiments in human plasma showed that protamine reduces clot strength and reduces the time to clot lysis, and enhances tissue-type plasminogen activator mediated

fibrinolysis, thereby promoting clot breakdown. [38] These observations could be counteracted by the addition of tissue factor to the plasma samples. [38] Although protamine-related hyperfibrinolysis might be of relevance in cardiac surgery with CPB, the routine use of antifibrinolytic therapy in clinical practice may prevent this phenomenon.

SIDE-EFFECTS OF PROTAMINE

Protamine administration is associated with immunological and inflammatory alterations, and may induce an anaphylactic response with hypotension, bradycardia, pulmonary vasoconstriction and allergy as most frequent reported side effects.[40,41] The reported incidence of anaphylactic reactions varies from 0.06% to 10.6%.[40] Patient risk factors for an anaphylactic response include treatment of diabetes mellitus with protamine-containing insulin and allergies for fish proteins. [41]

2

Antibody formation and protamine-induced thrombocytopenia

Protamine reactions are a complication of protamine infusion, and caused by IgE and IgG antibody formation. [42-44] In addition, the heparin-protamine complexes that are generated following protamine infusion have been shown to be immunogenic in several species. Incubation of ^{125}I-labeled protamine with blood cells in the presence of heparin resulted in tight binding of protamine to platelets and granulocytes. [45] It was further demonstrated that 29% of the patients develop antibodies to the protamine-heparin complex within the first month following cardiopulmonary bypass. [43] These antibodies can trigger neutrophil activation[44] and activate platelets in vitro. [46-49] The latter results in a decrease in the platelet aggregation capacity as measured by multiple electrode aggregometry. [24] In vitro studies further showed an association between protamine-related antibodies and thrombocytopenia. [49,50] However, only a small percentage of patients with postoperative thrombocytopenia reveal protamine-heparin complex antibodies. [46] Moreover, there is no evidence that protamine-heparin antibodies are associated with unfavourable clinical outcome. [47,48]

Complement activation

Exposure to cardiopulmonary bypass activates the complement system through the alternative pathway, while protamine administration is associated with complement activation through the classic C4a pathway. [51-54] In particular, in vitro experiments demonstrate that protamine-induced complement activation is mediated by C-reactive protein. [52] The cumulative activation of both complement pathways by the extracorporeal circuit and protamine may contribute to a pro-inflammatory response following cardiac surgery.

Vasoplegia

Infusion of protamine is frequently associated with a transient drop in blood pressure and a rise in pulmonary arterial pressure. [55] In vitro studies revealed that vascular reactivity to protamine is most likely mediated by alterations in mechanical stretch-induced intracellular endothelial calcium release [56] and nitric oxide production. [57] Unwanted effects of protamine on perioperative blood pressure can be prevented by administration of a H1-H2 blocker or hydrocortisone and methylene blue. [41,58]

Pulmonary hypertension

Pulmonary hypertension is the most severe clinical complication following a protamine-induced anaphylactic response. Comunale and colleagues reported a protamine-induced pulmonary hypertensive response defined as in increase in mean pulmonary arterial pressure of > 7 mmHg in 0.6% of patients. [59] In a second report, 1.78% of patients developed pulmonary hypertension with right ventricular failure following protamine administration. [60] It is assumed that thromboxane release underlies the pathophysiological mechanism underlying this severe complication, [61-64] and can be treated by prostacyclin EI infusion. [60,65] Others suggested that protamine-induced pulmonary hypertension is an anaphylactic response, which can be prevented by continuation of acetylsalicylic acid intake before surgery. [59] Due the low incidence of pulmonary hypertension following protamine infusion, clinical data on this topic are mainly restricted to case reports.

Rate and location of protamine infusion

The protamine-related haemodynamic side effects are enlarged when protamine is rapidly infused over a 3-minute period when compared a 15-minute infusion period. [66] Moreover, slow infusion of protamine over a 30-minute period was associated with a favourable postoperative coagulation profile when compared to fast bolus administration. [14] Chaney and colleagues investigated whether the location of protamine infusion influenced the adverse event rate. [67] They showed that administration of protamine via the ascending aorta was the least associated with blood pressure an arterial oxygenation changes when compared to administration through the central venous line, albeit that the changes were not clinically relevant and transient. [67]

Point-of-care haemostatic testing for protamine effects

Point-of-care coagulation tests for perioperative anticoagulation monitoring in cardiac surgery are primarily used to detect heparin anticoagulation and residual heparin. However, due to the effects of protamine on the coagulation system, these tests may also be affected by protamine itself.

Activated clotting time

The celite or kaolin activated clotting time (ACT) is typically used to assess the heparin neutralising effects of protamine following CPB. Theoretically, the ACT should return to preoperative values after protamine administration. Yet, there is substantial evidence that the post-protamine ACT does not accurately reflect the levels of residual heparin. In particular, the ACT even increases with incremental protamine-to-heparin dosing ratios. [25] It was further shown that a prolonged ACT following heparin neutralisation by 1:1 protamine administration has no predictive value in detecting residual heparin. [68] Others also reported these observations, with a weak correlation between ACT values and heparin concentrations. [69-71] Interestingly, several reports show that, despite the use of different protamine-to-heparin dosing ratios in comparative study arms, post-protamine ACT values were comparable. [72-74] These findings suggest that the protamine dose and post-CPB ACT value are only marginally associated.

Heparinase-containing viscoelastic test

The worldwide reintroduction of viscoelastic testing for haemostasis monitoring in cardiac surgery allows specific evaluation of residual heparin following cardiopulmonary bypass. However, the heparinase-containing INTEM rotational thromboelastometry test for intrinsic coagulation monitoring (HEPTEM) has also shown to be sensitive for protamine overdosing. In particular, the INTEM is prolonged in case of higher protamine-to-heparin dosing ratios. [35-37,48,75] The reason for this effect of protamine is unrevealed, but the observation has now been included as warning in the device manual by the manufacturer. In addition, Levin et al. showed that thromboelastography is responsive to different doses of protamine and might be used as method for protamine titration. [76] In summary, these data suggest that users of viscoelastic test devices should be aware of the test-modulating effects of both heparin and protamine, and that these tests should be interpreted accordingly.

PROTAMINE DOSING STRATEGIES

The 2011 Update to The Society of Thoracic Surgeons and the Society of Cardiovascular Anesthesiologists Blood Conservation Clinical Practice Guidelines recommend protamine titration or empiric low dosing regimens in order to reduce postoperative bleeding and transfusion requirements. [77] In the management of severe perioperative bleeding guidelines from the European Society of Anaesthesiology it is suggested that heparin monitoring using heparin concentration-based approaches are associated with smaller protamine doses, but that a protamine-to-heparin ratio of 1.3:1 is still common. [78]

Ideally, protamine dosing should be guided by its effect on patient haemostasis by means of the ACT. However, because the ACT is hardly associated with post-CPB heparin levels, [69-71] and additional protamine administration based on a prolonged post-CPB ACT when compared to pre-CPB levels can be harmful instead of beneficial for patient haemostasis. The quality of evidence on this topic is moderate. Current protamine dosing practices are based on protamine-to-heparin dosing ratios, low anticoagulation strategies, model-based titration or measurement-based titration.

Ratio-based dosing

Protamine-to-heparin dosing ratio strategies vary among institutions, and are subjected to local conventions. The dosing ratio is either based on the initial heparin dose or the total heparin dose administered during surgery. Protamine dosing only based on the initial heparin dose takes heparin consumption during bypass into account. The number of clinical studies focusing on the impact of different protamine-to-heparin dosing ratios on postoperative bleeding and transfusion requirements is limited.

Despotis and colleagues used a protamine-to-heparin dosing ratio of 0.8 for the total heparin dose administered, with the possibility to infuse additional protamine in case of a post-CPB ACT that did not return to pre-CPB levels. [79] In a multivariate analysis, they showed that a larger total protamine dose was associated with a higher risk of postoperative transfusions. [79] Others reported prolonged activated clotting times, [25] abnormal viscoelastic clotting times [37] and impaired ADP-induced platelet aggregation [25] when the protamine-to-heparin dosing ratio was above 1.3. In a small prospective study, randomisation of patients to a 0.8 versus 2.0 protamine-to-heparin dosing ratio revealed that high protamine dosing was associated with changes in platelet count and platelet factor 4, while post-protamine ACT levels were similar among groups. [74]

Our group showed that a protamine-to-heparin dosing ratio of 1.3 based on the total dose of heparin administered throughout the procedure associates with coagulation abnormalities, lower post-protamine thrombin levels and postoperative bleeding when compared to a protamine-to-heparin dosing ratio of 0.8. [32] In contrast, one retrospective study and one randomised trial revealed that a higher protamine dose regime was associated with better clinical outcome when compared to a lower protamine dose, albeit that both studies were limited by excessive volumes of postoperative blood loss. [80,81] Except for the study by Meesters et al. [32] none of abovementioned studies were powered to detect a difference in postoperative blood loss or transfusion requirements.

Low systemic anticoagulation strategies

Full heparin anticoagulation to achieve a target ACT exceeding 480 seconds is considered gold standard in cardiac surgery. In contrast, low anticoagulation protocols in combination with a tip-to-tip biocompatible coated extracorporeal circuit, aim for a target ACT of 250-300 seconds based on the assumption that excessive heparin is unfavourable for postoperative patient haemostasis. [82-84] Consequently, the total dose of protamine administered in these studies is reduced by about 50% due to the lower anticoagulation approach. [83,84] A lower dose of protamine may decrease the risk for an anaphylactic reaction. While low systemic anticoagulation strategies seem to be associated with reduced postoperative bleeding and transfusion, [82-84] the wide adaptation of these protocols is limited due to the presumed safety aspects associated with a low target ACT.

Model-based titration of protamine

There is increasing interest in the application of mathematical or pharmacokinetic algorithms for heparin and protamine dosing to support optimization of perioperative systemic anticoagulation in cardiac surgery. In particular, the estimation of a post-CPB heparin level may improve the accuracy of protamine dosing. Jia and colleagues developed a two-compartment pharmacokinetic (PK) model based on anti-FIIa levels to predict heparin concentrations during hypothermic cardiac surgery. [85] They suggested that their approach would result in a lower protamine dose regime after termination of CPB. [85] Others developed a formula based on perioperative kaolin ACT measurements to calculate the post-CPB protamine dose, which resulted in a decrease in protamine dosing compared to a conventional group. [86] However, the study was biased by the excessive volumes of chest tube drainage in both groups. [86]

In a group of patients receiving heparin and protamine based on an empirically based algorithm protamine dosing was reduced without any significant difference in postoperative bleeding in the algorithm group. [87] Davidsson and colleagues developed a statistical model based on body surface area, the administered dose of heparin, heparin clearance and the preoperative platelet count, that predicted the required dose of protamine in accordance with the haemostasis management system as reference. [88] In a second study, the same investigators implemented this model in a randomised controlled fashion and compared protamine dosing and postoperative coagulation with patients exposed to a protamine-to-heparin dosing ratio of 1:1. [89] While the heparin dosing was similar in both groups, they showed a reduction in protamine dosage from 426 ± 43 to 251 ± 66 mg, which was paralleled by more favourable postoperative haemostatic test results. [89] Interestingly, post-protamine ACT values were similar among groups. Using a pharmacokinetic two-compartment model, we showed in a retrospective fashion that patient-tailored protamine dosing was associated with a 50% reduction in the protamine-to-heparin dosing ratio and more favourable postoperative haemostatic test results when compared to patients subjected to a conventional protamine dosing strategy. [90] The abovementioned studies suggest the need for a more accurate protamine dosing strategy in cardiac surgery, but all have methodological limitations, including insufficient statistical power. Further studies are required to evaluate the benefits of a calculated protamine-based algorithm for patient bleeding and transfusion requirements.

Protamine titration based on heparin measurements
Titration of protamine based on measured heparin levels following CPB may improve the accuracy of heparin neutralisation and contribute to preserved patient haemostasis. Protamine titration is usually based on the measured heparin concentration or anti-Xa levels. There are several publications available focusing on the effects of protamine titration on postoperative outcome in cardiac surgery, with conflicting results. In order to understand the impact of inadequate protamine dosing, a theoretical distinction should be made between the effects of protamine underdosing and overdosing (Figure 3).

The most frequent clinically applied method for protamine titration is based on the measurement of post-CPB heparin levels using a haemostasis management system (HepCon/HMS®) or Hemochron RxDx device. Despite several attempts to develop novel protamine titration assays, including ultracentrifugation or an automatic protein dose assay, these methods never gained widespread acceptance in routine clinical care. [91,92]
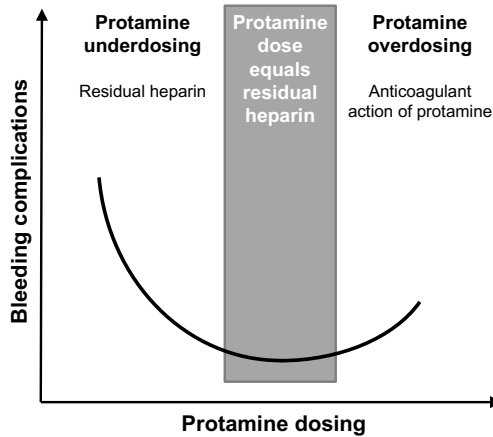
Figure 3. Hypothetical relation of protamine dosing with bleeding complications. While protamine underdosing is associated with residual heparin, protamine overdosing may lead to bleeding complications due to the anticoagulant properties of protamine in the absence of heparin.

Supplemental Table I provides an overview of comparative studies that appeared between 1990-2017 of conventional ACT-based heparin and protamine management with protamine titration based on blood heparin concentrations measured by Hepcon/HMS, RxDx or a protamine dose assay. [21,71,92-106] The majority of these studies reported non-clinical outcomes, including heparin or protamine dose, [71,92,94,100,103] thrombin antithrombin levels or thrombin potential, [93,99,102] haemostatic activation [95,96] or platelet function [21,96] as study endpoints. Only a few studies were primarily designed to study the association of protamine titration with postoperative blood loss and/or transfusion requirements, [98,101,104-106] but frequently without providing a sample size calculation. [98,104-106]

The protamine-to-heparin dosing ratios (P:H) are either based on the initial heparin dose [92,96,102,106] or total heparin dose, and ratios reported in these studies vary from 0.33 [21] to 1.3. [106] In half of the studies, a reduction in the protamine-to-heparin dosing ratio was associated with improved haemostatic [21,95,96,102] or clinical outcome. [71,97,98,105,106] One group showed no benefit of protamine titration on 12-hour blood loss, but this can be explained by almost similar protamine-to-heparin dosing ratios in the ACT group (0.84) and protamine titration group (0.86). [93] Others reported no difference in postoperative blood loss when an ACT and protamine titration strategy were compared, [99-101,104] albeit that two studies were not designed to show differences in postoperative bleeding. [99,100] Finally, two studies reported increased blood loss after

protamine titration. The first study found increased 6-hour blood loss in the protamine titration group, but this study was limited by the relatively high reported 6-hour bleeding volumes. [103] Albeit not the primary endpoint, the second study also reported higher 12-hour blood loss volumes in the protamine titration group. [94] It is however unknown whether the mixed inclusion of on-pump, OPCAB and miniaturized extracorporeal therapy cases and absent publication of concomitant ACT values may have influenced this outcome. [94]

In summary, despite the availability of several protamine titration studies, the quality of studies is limited and clinical outcomes were only rarely used as primary study endpoint. Moreover, the wide range in publication years (1994-2015) may introduce a bias, as cardiosurgical strategies have been largely improved over the last decade. In particular, modern cardiosurgical practices frequently implement a multifactorial and multidisciplinary patient blood management program to reduce transfusion rates. Larger randomised controlled trials in the modern cardiosurgical setting are warranted to understand the impact of protamine titration on postoperative blood loss and transfusion requirements.

Protamine-to-heparin dosing ratio and postoperative blood loss

In order to gain insight in the association of protamine-to-heparin dosing ratios and 12-hour blood loss an analysis was performed of studies comparing distinct protamine dosing strategies based on a fixed ratio, [32] model-based protamine titration [89,90] and protamine titration based on heparin measurements [72,93-95,98,99] and reported 12-hour blood loss (Figure 4). With a moderately good correlation, we here show that there might be a trend between protamine dosing and postoperative blood loss, albeit that these data have methodological limitations and are mostly retrieved from studies where postoperative outcome was not the primary endpoint, from mixed cardiosurgical populations with different perioperative strategies.

Additional protamine administration

When the post-protamine ACT exceeds the pre-CPB ACT, it is common in some hospitals to add additional protamine doses for neutralisation of residual heparin. However, in light of the weak association between post-protamine ACT levels and residual heparin, [25,68-71] it is unclear whether these additional protamine doses act as heparin neutralising agent or anticoagulant. Consequently, the administration of additional doses of protamine after compensating for the heparin dose may lead to protamine overdosing and enhanced bleeding.
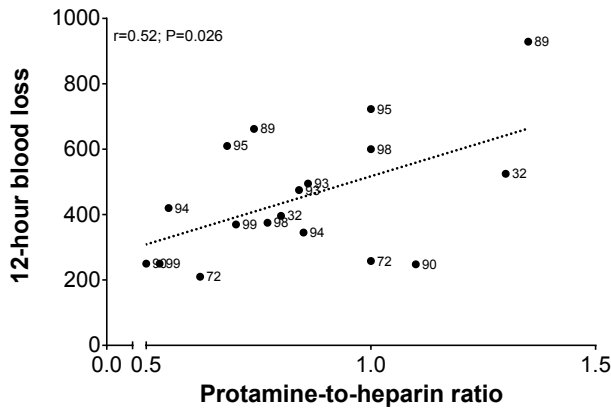
Figure 4. Association of the protamine-to-heparin dosing ratio and 12-hour postoperative blood loss. Numbers refer to citations in the reference list.

In 1968, Berger et al. already showed that administration of protamine in smaller aliquots resulted in sufficient restoration of clot formation after the first dose when compared to a full protamine dose, resulting in a protamine dose administration of 1.37 versus 0.79 mg for every 100 IU of heparin, respectively. [107] Others showed that the administration of additional protamine resulted in an increased clotting time. [37] In a small randomised controlled study, a substantial number of patients received additional protamine doses in the absence of residual heparin, and this approach was associated with increased postoperative blood loss. [108] Although the number of studies focusing on additional protamine dosing is low, there is substantial evidence that protamine dosing should not be adjusted based on the post-protamine ACT, as this coagulation test does not reflect the presence of residual heparin. Caution is warranted when additional protamine is administered in case of oozing, since there is no evidence that this approach is beneficial for patient haemostasis and postoperative bleeding.

AVOIDANCE OF HEPARIN REBOUND

Heparin rebound is the recurrence of anticoagulant activity after heparin neutralisation with protamine, and is frequently attributed to protamine underdosing. Heparin rebound is a feared phenomenon, as it might contribute to postoperative bleeding and increased transfusion requirements. The phenomenon is complicated by the diversity in definitions of heparin rebound, and the absent accuracy of most coagulation tests to detect heparin rebound. [109] In particular, in most studies the definition of heparin rebound is only limited to the presence of heparin in blood, without investigating its effect on postoperative bleeding complications.

It was recently shown that heparin rebound might be of less importance as cause for postoperative bleeding than previously assumed, showing only a weak association between post-protamine heparin levels and volume of blood loss. [110] Indeed, both low and high protamine dose approaches are associated with similar incidences of heparin rebound. [104] Additionally, we showed that post-protamine heparin concentrations at 3 and 30 minutes after protamine administration were similar in high and low protamine dosing ratio groups, and no heparin rebound in the late postoperative period was reported. [32] In particular, the high protamine-to-heparin dosing ratio was associated with more postoperative bleeding. [32]

A low dose protamine infusion (25 mg h^{-1} for 6 hours) following heparin neutralisation prevented heparin rebound as defined by the thrombin clotting time and anti-FXa levels. [111] They further showed that a protamine infusion aborts the presence of heparin following surgery, which was associated with a non-relevant reduction in postoperative bleeding when compared to control patients. [111]

ALTERNATIVES FOR PROTAMINE

A strategy to avoid the side effects and dosing complications of protamine is the use of alternatives for unfractionated heparin or protamine. The direct thrombin inhibitor bivalirudin is the most commonly used replacement for heparin anticoagulation. Bivalirudin has no antidote and is eliminated with a half-life of approximately 25 minutes, thereby avoiding the use of protamine. [112] In a randomised controlled trial, Dyke et al. showed that bivalirudin can be safely and effectively used for systemic anticoagulation during cardiac surgery, without differences in blood loss and clinical outcome compared to heparin anticoagulation. [113] However, as stasis should be avoided during bivalirudin, this anticoagulation therapy requires adjustments of perfusion approaches, and a widespread adaptation of this approach is slow. Moreover, the costs of bivalirudin outweigh the costs of heparin and protamine, and its use is therefore mainly restricted to patients with i.e. heparin-induced thrombocytopenia (HIT) and HIT antibodies. [114]

Due to the side effects of protamine, several experimental alternatives have been suggested, [115] including platelet factor [4,116] heparinase [20,117] and hexadimethrine. [118] Although their heparin-neutralising capacities could be proved, widespread introduction in the clinical setting has not yet been realized.

CONCLUSION

Protamine reversal of heparin anticoagulation is a necessary evil in cardiac surgery with or without cardiopulmonary bypass, and can be associated with haemostatic abnormalities when inadequately dosed. The available evidence suggests that protamine administration based on the initial heparin dose should target a ratio below 1:1 in order to prevent protamine-related coagulopathy and bleeding. However, large randomised controlled trials are warranted to improve the quality of evidence for this topic.

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Chapter 3

Effect of high or low protamine dosing on postoperative bleeding following heparin anticoagulation in cardiac surgery: a randomized clinical trial

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ABSTRACT

While experimental data state that protamine exerts intrinsic anticoagulation effects, protamine is still frequently overdosed for heparin neutralization during cardiac surgery with cardiopulmonary bypass (CPB). Since comparative studies are lacking, we assessed the influence of two protamine-to-heparin dosing ratios on perioperative haemostasis and bleeding, and hypothesized that protamine overdosing impairs the coagulation status following cardiac surgery.

In this open-label, multicentre, single-blinded, randomized controlled trial, patients undergoing on-pump coronary artery bypass graft surgery were assigned to a low (0.8; n=49) or high (1.3; n=47) protamine-to-heparin dosing group. The primary outcome was 24-hour blood loss. Patient haemostasis was monitored using rotational thromboelastometry and a thrombin generation assay.

The low protamine-to-heparin dosing ratio group received less protamine (329 ± 95 vs. 539 ± 117 mg; P<0.001), while post-protamine activated clotting times were similar among groups. The high dosing group revealed increased intrinsic clotting times (236 ± 74 vs. 196 ± 64 s; P=0.006) and the maximum post-protamine thrombin generation was less suppressed in the low dosing group (38 ± 40% vs. 6 ± 9%; P=0.001). Postoperative blood loss was increased in the high dosing ratio group (615 ml; 95% CI 500 – 830 ml vs. 470 ml; 95% CI 420 – 530 ml; P=0.021) when compared to the low dosing group, respectively. More patients in the high dosing group received fresh frozen plasma (11% vs. 0%; P=0.02) and platelet concentrate (21% vs. 6%; P=0.04) compared to the low dosing group.

Our study confirms in vitro data that abundant protamine dosing is associated with increased postoperative blood loss and higher transfusion rates in cardiac surgery.

INTRODUCTION

Protamine is used to neutralize the anticoagulant effects of heparin during cardiac surgery with cardiopulmonary bypass (CPB). [1-3] While under dosing of protamine may result in residual heparinisation and prolonged anticoagulation, protamine overdosing is associated with impaired clot formation exerted by the intrinsic anticoagulation properties of protamine itself. [4-6] Protamine inhibits the activation of coagulation factors, thrombin formation, platelet activation and enhances fibrinolysis. [7-13]

In 1968 Berger et al. already showed that reducing the protamine-to-heparin dosing ratio from 1.37 to 0.79 was associated with reduced postoperative blood loss and fewer rethoracotomies. [14] Moreover, individualized protamine dosing is associated with better coagulation parameters and less bleeding. [6,15] Despite these potential benefits, the optimal protamine dosing in cardiac surgery has only been scarcely investigated. Current guidelines advise empiric low protamine dose regimens, but these are only low class evidence recommendations due to scarce and inconsequent study results. [16-18] Moreover, as an artificial safety net, it is not uncommon that additional doses of protamine are administered in case of an oozing operation field after weaning from CPB, elevated post-pump activated clotting time (ACT) levels, or in case of a high postoperative thoracic drain production. [19]

The objective of this randomized controlled study was to investigate whether reducing protamine dosing based on a fixed protamine-to-heparin dosing ratio is associated with reduced postoperative bleeding and decreased allogeneic blood transfusions requirements. We therefore compared a dosing ratio of 1.3 with 0.8 in patients undergoing coronary artery bypass graft surgery with CPB, and hypothesized that the high protamine-to-heparin dosing ratio deteriorates haemostasis and is associated with more postoperative blood loss.

MATERIALS AND METHODS

Study population

This open-label, multicentre, single-blinded, randomized controlled parallel-group, superiority clinical trial was performed in the departments of cardiac anaesthesiology and cardio-thoracic surgery of the VU University Medical Centre (VUmc; Amsterdam, the Netherlands) and Medical Centre Leeuwarden (MCL; Leeuwarden, the Netherlands). Both hospitals had their own dedicated anaesthesia and surgical team for cardiothoracic surgery, and there was no exchange of anaesthesiologists or surgeons between centres. The protocol was approved by the Human Subjects Committee of both centres, and registered in the Netherlands Trial Register (NTR3528). All eligible patients were approached for informed consent by a dedicated investigator, and patients included in the study protocol provided written consent. Patient inclusion started January 2013 and ended January 2015.

Patients aging 18-85 years were eligible in case of elective first-time coronary artery bypass graft (CABG) surgery with CPB. Exclusion criteria were emergency surgery, a body mass index (BMI) < 18 kg m^{-2} or > 35 kg m^{-2}, haematology disorders, renal replacement therapy and the preoperative use of heparin or heparin-like medication. The primary study endpoint was 24-hour blood loss.

Study procedure

Patients were randomly assigned by a sealed envelope method through block randomization for both centres. Groups were based on the protamine-to-heparin dosing ratio after CPB, which was calculated based on the total amount of heparin administered to the patient. One group received a low protamine-to-heparin dosing ratio of 0.8 (0.8:1.0; total amount of heparin administrated (mg) * 0.8 = protamine dose (mg)), while the second group received a high ratio of 1.3 (1.3:1.0; total amount of heparin administrated (mg) * 1.3 = protamine dose (mg)). Both participating centres routinely used a protamine-to-heparin dosing ratio of 0.8 based on the total heparin dose, while the European perioperative bleeding guideline advises a protamine-to-heparin dosing ratio of 1.0-1.3. [17] We chose to compare our routine dosing ratio with the higher end of the spectrum, a protamine-to-heparin dosing ratio of 1.3. Patients were blinded for the allocated group.

Anaesthesia and cardiopulmonary bypass

According to clinical routine in both participating centres, acetylsalicylic acid was continued throughout the perioperative period, while clopidogrel was discontinued at least 5 days before surgery. Patients were anaesthetized according to local protocols based on a combination of sufentanil 3-7 µg kg^{-1}, rocuronium 0.6 mg kg^{-1} and midazolam 0.1 mg kg^{-1} for anaesthesia induction and tracheal intubation. Propofol (0.1–1.3 mcg kg^{-1} min^{-1}) with or without isoflurane/sevoflurane was used for maintenance of anaesthesia. In both centres, all patients received tranexamic acid. CPB was performed at a blood flow (2.2-2.4 l min^{-1} m^{-2}) during normo- or mild hypothermia (> 34°C) using either a C5 or S5 heart-lung machine (Stöckert Instrumente GMBH, Munich, Germany) or HL-30 heart-lung machine (Maquet, Cardiopulmonary AG, Hirrlingen, USA) with a centrifugal pump and biocompatible-coated circuit. Cell salvage was applied throughout the surgical procedure in both centres.

Heparin and protamine management

Heparinisation started with an initial bolus of 300 IU kg^{-1} heparin (LEO Pharma BV, Amsterdam, the Netherlands), to achieve an activated clotting time (ACT) test of 480 seconds. If necessary, additional doses of heparin (5000 IU) were administered to maintain this target ACT. The priming solution (1400 ml) contained 5000 IU (VUmc) or 7500 IU (MCL) heparin. After weaning from CPB, heparin was reversed by manual infusion of the calculated dose protamine hydrochloride (Meda Pharma BV, Amstelveen, the Netherlands) according to the subsequent study arm. The amount of protamine was in accordance with the study arm definitions based on the total amount of heparin that was given throughout the whole procedure, including the heparin administered during CPB. It was not allowed to administer an additional protamine after the study bolus was infused.

Local blood transfusion algorithm

The transfusion of packed red blood cells (PRBC) was based on the Dutch transfusion guidelines, recommending transfusion if haemoglobin levels below 9.7 g dL^{-1} (6 mmol L^{-1}) in case of ASA IV patients, patients with symptomatic cerebrovascular disease, sepsis, heart failure, or severe pulmonary disease. PRBC transfusion is advised in case of haemoglobin levels below 8.1 g dL^{-1} (5 mmol L^{-1}) in case of acute blood loss from one bleeding focus in a normovolemic ASA I patient aging > 60 years, in case of fever or following cardiac surgery. Fresh frozen plasma (FFP) or platelet transfusion was performed in case of clinical signs of persistent nonsurgical bleeding (oozing). When laboratory coagulation

test results were available, FFP was transfused when the international normalized ratio (INR) of the prothrombin time exceeded 1.5. Additional platelet concentrate administration was indicated in case of extended bypass times, clinical signs of bleeding and/or a platelet count below $75*10^9\,l^{-1}$.

Blood sampling

After anaesthesia induction, the first blood sample was collected for baseline haemostatic measurements (Pre-CPB). Blood was drawn in a citrated tube from the radial artery catheter. Three minutes (PROT-3) and thirty minutes (PROT-30) after protamine administration, the second and third blood samples were drawn. Blood for plasma determinations and thrombin generation measurements was only sampled in the VU University Medical Centre, Amsterdam, the Netherlands. At all three time points, blood was centrifuged and platelet-free plasma was extracted. Plasma was stored at -80 ^{o}C for further analysis.

Thromboelastometry

Rotational thromboelastometry™ (ROTEM delta; TEM International, Munich, Germany) was immediately started following blood withdrawal, and included the intrinsically activated coagulation test (INTEM), extrinsically activated coagulation test (EXTEM), fibrin polymerization test (FIBTEM) and the intrinsically activated coagulation test without heparin effect (HEPTEM). [20] Clot formation was assessed by the clotting time (CT) or the maximum clot firmness (MCF).

Coagulation parameters

Routine laboratory testing of the international normalized ratio (INR) of the prothrombin time (calcium thromboplastin) and the activated partial thromboplastin time (aPTT; cefaline) were determined in platelet-free plasma. Other haemostatic parameters included the activated coagulation time (ACT; Hemochron, ITC Medical, Edison, NY), haemoglobin levels and platelet count. Blood samples for laboratory coagulation testing were drawn directly upon arrival at the intensive care unit.

Plasma determinations
Antithrombin III

Plasma levels of antithrombin III (ATIII), heparin and platelet factor 4 (PF4) were not corrected for hemodilution during CPB. Antithrombin (ATIII) concentrations were determined in plasma diluted 40 times in 50 mM Tris-HCl buffer (pH 7.4). A calibration curve was made by diluting purified ATIII in eight steps of 1:1 in

Tris buffer starting at 12 µg ml^{-1}. Fifty µl heparin (1 IU ml^{-1}) / thrombin (5 IU ml^{-1}) mixture in Tris buffer was added to 50 µl of diluted plasma in a microtiter plate and prewarmed for 2 minutes in an incubator at 37 °C. Then 50 µl Substrate 2238 (2 mM) in Tris was added to each well and incubated for 2 minutes at 37°C. The color formation was stopped by addition of 2% citric acid. The yellow color was measured at 405 nm in a spectrophotometer (Power Wave 200, Bio Tek Instruments, Vermont, USA).

Heparin concentration by Xa inhibition

Fifty µl Xa solution (1.75 nkat) in Tris buffer pH 5.6 was added to 50 µl plasma and 25 µl Tris in a microtiter plate. A standard curve was made from serial diluted heparin in Tris buffer and normal human plasma. The baseline OD 405 nm was measured in a spectrophotometer and then 50 µl Substrate S2222 (2 mM) was added. The microplate was sealed and incubated for 30 minutes at 37°C, and the absorbance at 405 nm was again measured in the spectrophotometer. The baseline value was subtracted from the second reading and the amount of heparin in plasma was calculated by means of the standards.

Heparin concentration by thrombin inhibition

Plasma (12.5 µl) was added to 62.5 µl citrate/Tris buffer (pH 7.4) in a microtiter plate. A standard curve was made from serial heparin dilutions starting at 10 IU ml^{-1} in normal human platelet poor plasma. Then 25 µl thrombin solution (1.5 IU ml^{-1}) was added to all wells and the baseline optical density was read at 405 nm in a spectrophotometer. The 50 µl Substrate S2238 was added and the sealed plate was incubated for 20 minutes at 37°C. The OD 405 was read and corrected for baseline. The heparin concentration was calculated by means of the standard curve.

Platelet factor 4

Platelet factor 4 (PF4) was determined by ELISA based on antibodies and PF4 antigen standard from R&D. A Nunc Maxisorp microtiter plate was coated during the night with a carbonate coating buffer (pH 9.6) containing mouse-anti-human PF4 capture antibody. The plate was washed with PBS-Tween buffer, blocked with 1% BSA in PBS and washed again. Then samples (200 times diluted in 0.1% BSA/PBS) and serial diluted standards (starting at 1000 pg ml^{-1} in 0.1% BSA/PBS were incubated for 2 hours at room temperature. The plate was washed and then incubated for 2 hours with biotinylated goat-anti-human PF4. The plate was washed and then incubated with streptavidin-HRP solution in 1% BSA in PBS

and after washing the plate was incubated with OPD substrate solution. After 20 minutes the reaction was stopped with 0.3 M H_2SO_4. The color formation was read at 490 nm in a spectrophotometer and PF4 concentrations were calculated from the OD490 against the standard curve.

Thrombin generation assay

The thrombin generation assay was performed in a subgroup of 20 patients in the low dosing ratio group and 22 patients in the high dosing ratio group. Citrated platelet free plasma was analysed with calibrated automated thrombography (CAT) as previously described. [21,22] Thrombin generation was measured in blood samples obtained before surgery and 3 and 30 minutes following protamine administration.

Other study parameters

Patient demographics included age, gender and BMI. Surgical data were CPB time, aortic cross clamp time and the amount of heparin and protamine administration. Clinical outcome included 24-hour blood loss by chest drainage and postoperative allogeneic transfusion rates of packed red blood cells (PRBC; 275 ml), fresh frozen plasma (FFP; 310 ml) and platelet concentrates (340 ml). Assessment of drain production started immediately after placement of drains. Furthermore, time to extubation, length of postoperative ICU stay and mortality, rethoracotomy, myocardial infarction, transient ischemic attack (TIA), cerebral vascular incident (CVA), cardiac tamponade and the need for reintubation were assessed.

Statistical analysis

We hypothesized that the low protamine-to-heparin ratio would reduce 24-hour blood loss by 100 ml. With a power of 0.9 and alpha of 0.05, a sample size of 49 subjects per group was required to reach our primary study endpoint, with a total sample size of 98 patients. An intention-to-treat statistical analysis was performed using the SPSS statistical software package 22.0 (IBM, New York, USA) or Graphpad Prism 6 (La Jolla, USA). Continuous, normally distributed variables were expressed as mean ± standard deviation, while ordinal or skewed data were shown as median with interquartile range (IQR). Categorical data were expressed as frequencies with or without 95% confidence intervals and analysed by a Chi-square test or Mann-Whitney test, respectively. The primary endpoint (24-hours blood loss) was expressed as median with interquartile range and 95% confidence intervals. Normally distributed numeric variables were analysed

using an (un)paired T-test. In order to analyse between-group effects over time a two-way repeated measures ANOVA was performed for coagulation and plasma parameters at baseline and three (PROT-3) and 30 (PROT-30) minutes following protamine administration and upon ICU arrival (ICU). A P-value of 0.05 was considered as statistically significant.

3

RESULTS

Patient characteristics

Forty-seven (VUmc) and 51 (MCL) patients were included. VUmc and MCL included an equal number of males (83% vs. 73%; P=0.26) with similar ages (67 ± 9 vs. 68 ± 9 years; P=0.58). Two patients in the high protamine-to-heparin dosing ratio group were excluded due to inappropriate protamine dosing (Figure 1).

Patient and surgical characteristics were similar among groups (Table 1). There were differences in the use of anticoagulant medication before surgery between groups. The total administered protamine dose was higher in the high dosing ratio group (539 ± 117 mg) when compared to the low dosing ratio group (329 ± 95 mg; P < 0.001), resulting in an 0.8 and 1.3 dosing ratio per group, respectively as per protocol.
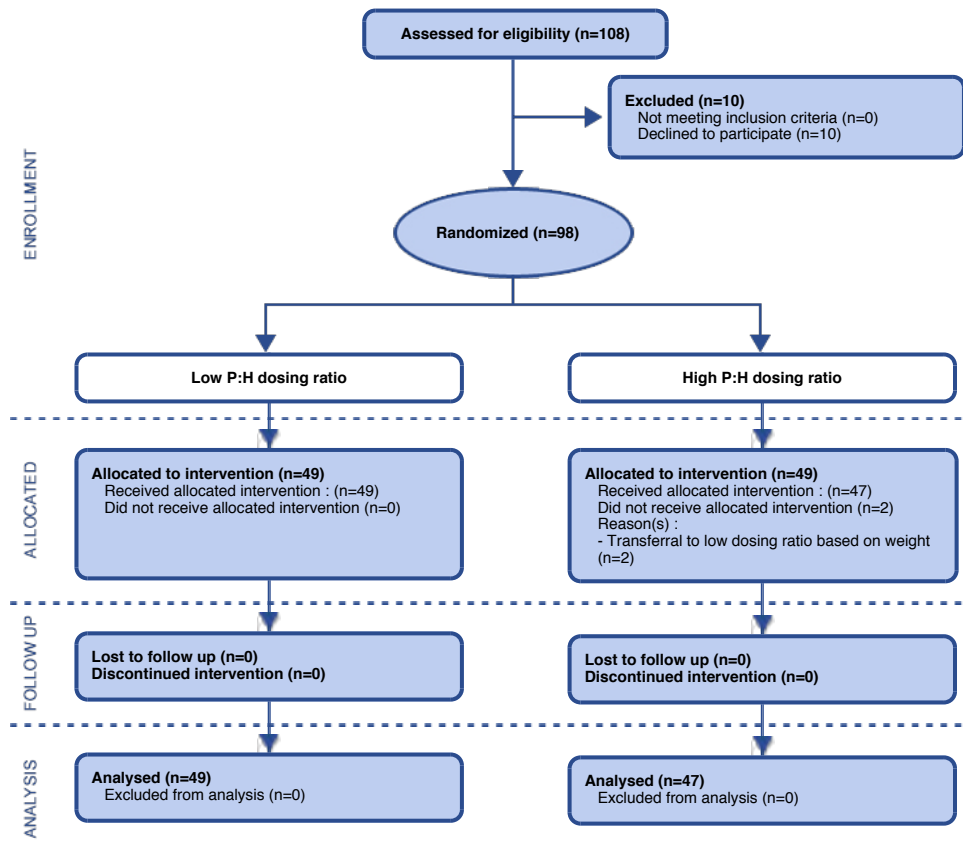

Figure 1.   CONSORT flow diagram of patient inclusion.

3

Perioperative haemostatic data

Repeated measures ANOVA revealed no differences between groups in the course of haemoglobin (panel A; F value 0.402; P=0.529), the aPTT (panel C; F value 0.360; P=0.550) or the ACT (panel D; F value 1.039; P=0.311; Figure 2). The prothrombin time was slightly prolonged in the high dosing group at three minutes following protamine administration (INR 1.6 ± 0.2 vs. 1.7 ± 0.2; P=0.001; see also Panel B), with a between-group subjects effect for protamine dosing (F value 10.571; P=0.002).

	Low P:H ratio	High P:H ratio	P-value
N	49	47	
Age (years)	67 ± 9	67 ± 9	0.79
BMI (kg m^{-2})	27 ± 3	27 ± 4	0.81
Males (n (%))	36 (74%)	39 (83%)	0.26
Preoperative anticoagulation			0.21
Acetylsalicylic acid (n (%))	41 (84%)	45 (96%)	
Clopidogrel (n (%))	2 (4%)	1 (2%)	
Acenocoumarol (n (%))	3 (6%)	0 (0%)	
ECC time (min)	91 ± 29	94 ± 35	0.57
Aorta clamp time (min)	61 ± 22	66 ± 27	0.37
Tranexamic acid (g)	2.8 ± 0.6	3.1 ± 1.7	0.34
Total heparin administration (IU)	41,000 ± 11,800	41,500 ± 9,000	0.81
Protamine administration (mg)	329 ± 95	539 ± 117	<0.001
Protamine-to-heparin dosing ratio	0.8 ± 0.0	1.3 ± 0.0	<0.001

Table 1. Patient demographics and surgical characteristics for the low and high protamine-to-heparin dosing ratio groups. BMI = body mass index; ECC = extracorporeal circulation. Data represent frequencies or mean ± standard deviation.

The course of the thromboelastometric test results are shown in figure 3. The course of the INTEM CT (F value 19.56; P<0.0001) and HEPTEM CT (F value 17.365; P<0.0001) over time was different between groups as analysed by repeated measures ANOVA. At three minutes following protamine administration, the INTEM CT (293 ± 72 vs. 243 ± 36 s; P<0.001) and HEPTEM CT (303 ± 68 vs. 241 ± 39 s; P<0.001) were both prolonged in the high dosing compared to the low dosing group, respectively. The course of the EXTEM CT (panel C; F value 2.23; P=0.137) and FIBTEM MCF (panel D; F value 0.188; P=0.665) over time did not differ among groups.
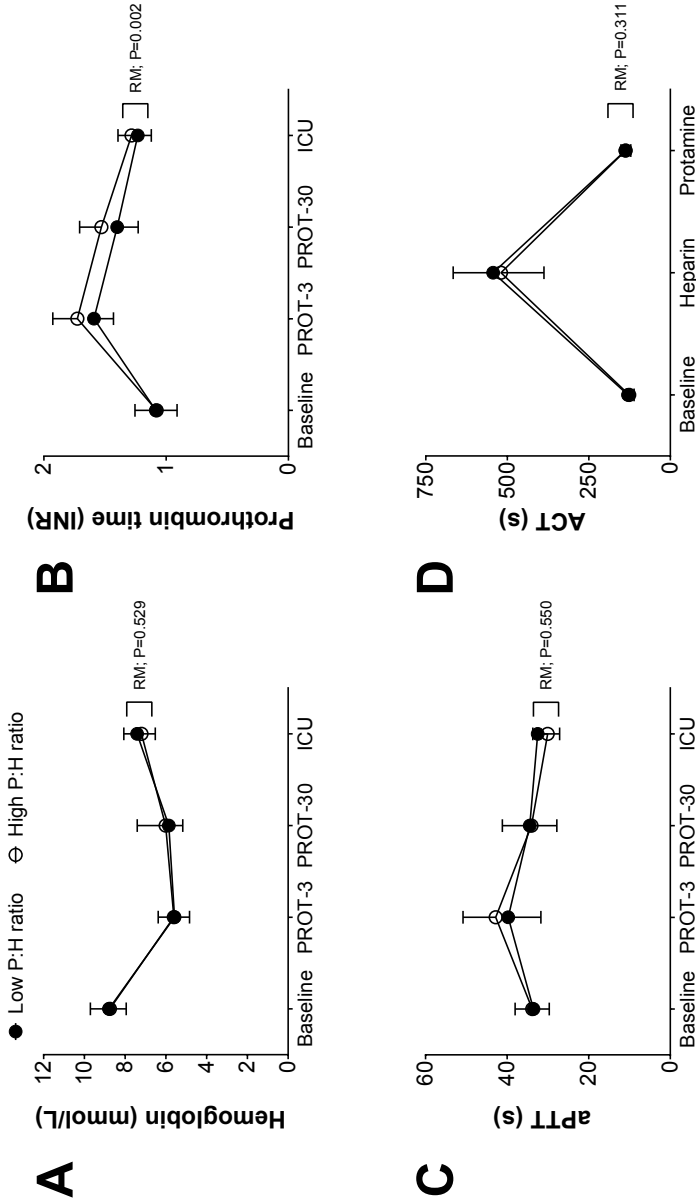
Figure 2. The course of haemoglobin values (panel A), the prothrombin time expressed as international normalized ratio (INR; panel B) and activated partial thromboplastin time (aPTT) at baseline, at 3 (PROT-3) and 30 (PROT-30) minutes after protamine administration and upon intensive care unit admission (ICU) in the low and high protamine-to-heparin dosing ratio groups. Panel D shows the activated clotting time (ACT) at baseline and following heparin and protamine administration. Data represent means ± standard deviation and are analysed by repeated measures (RM) ANOVA. The P-value indicates the between-group difference over time.
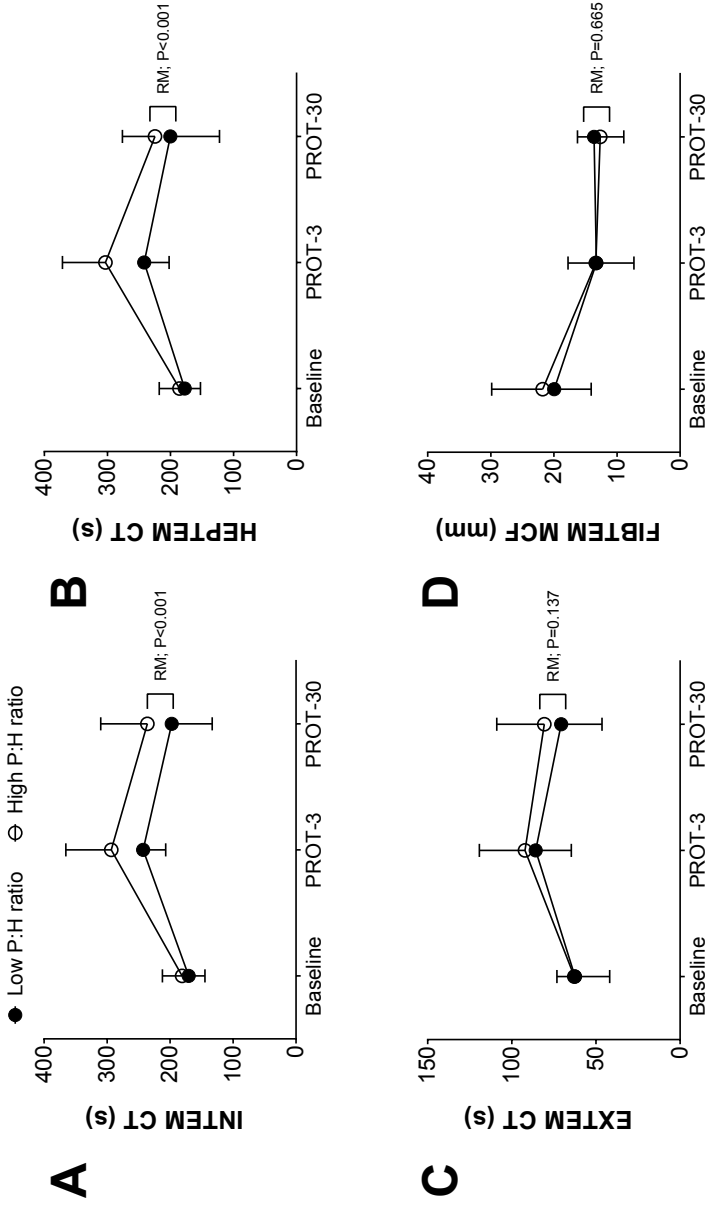
Figure 3. The course of rotational thromboelastometry values for the INTEM clotting time (panel A), the HEPTEM clotting time (panel B), the EXTEM clotting time (panel C) and the FIBTEM maximum clot firmness (MCF; panel D) at baseline and at 3 (PROT-3) and 30 (PROT-30) minutes after protamine administration in the low and high protamine-to-heparin dosing ratio groups. Data represent means ± standard deviation and are analysed by repeated measures (RM) ANOVA. The P-value indicates the between-group difference over time.
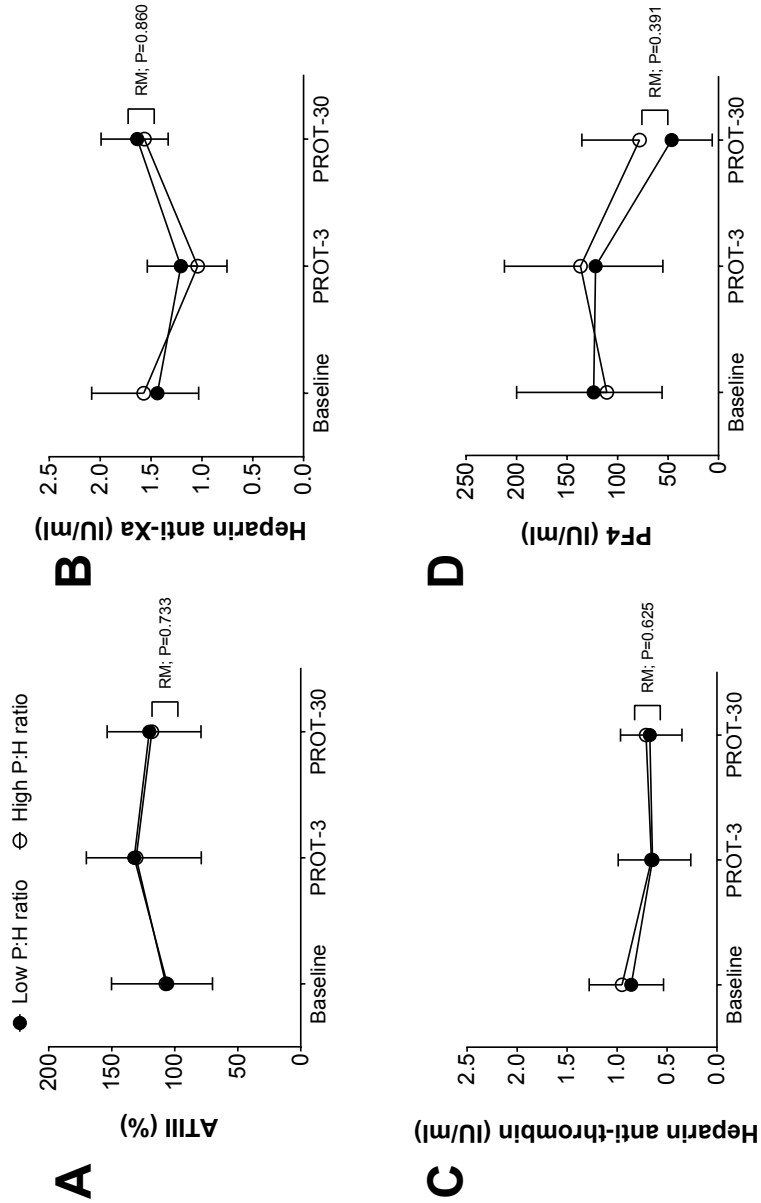
Figure 4. Plasma determinations of antithrombin III (ATIII; panel A), the heparin concentration by anti-Xa testing (panel B), the heparin concentration by anti-thrombin testing (panel C), and platelet factor 4 (PF4; panel D). Data represent means ± standard deviation and are analysed by repeated measures (RM) ANOVA. The P-value indicates the between-group difference over time.

The course of ATIII levels over time was comparable for both dosing ratio groups (Figure 4, panel A; F value 0.117; P=0.733). The available heparin concentration as revealed by factor Xa inhibition at 3 minutes following protamine administration was 1.21 ± 0.45 IU ml^{-1} and 1.04 ± 0.50 IU ml^{-1} in the low and high protamine dosing group (P=0.246) without a difference in the course of heparin levels over time between groups (panel B; F value 0.032; P=0.860). At 3 minutes following protamine administration, ATIII levels were comparable between the low and high protamine-to-heparin dosing groups (0.65 ± 0.38 IU ml^{-1} vs. 0.66 ± 0.33 IU ml^{-1}, respectively; P=0.92), without differences over time between groups (panel C; F value 0.243;P=0.625). A similar pattern was observed for PF4 levels, showing no different course over time among groups (panel D; F value 0.752; P=0.391).

Thrombin generation assay
The lag time, the endogenous thrombin potential (ETP), the peak height and the time to peak as derived from the thrombin generation assay are shown in figure 5. For every patient, data retrieved at 3 and 30 minutes following protamine administration were normalized to preoperative values. After stimulation with 1 pM of tissue factor the normalized lag time (Panel A; 404 ± 279 vs. 216 ± 144 %; P=0.01) and time to thrombin peak (Panel G; 240 ± 125 vs. 152 ± 64 %; P=0.009) were higher in the high dosing group at 30 minutes after protamine administration. Thrombogram parameters derived upon stimulation with 5 pM tissue factor, which activates the extrinsic coagulation system (panels B, D, F, H), did not differ between groups.

Blood transfusion and postoperative complications
There was no difference in the volume of retransfused cell saver blood between groups as shown in table 2. Postoperative blood loss was higher at 24 hours (615 ml; 95% CI 500-830 ml vs. 470 ml; 95% CI 420-530 ml; P=0.021) in the high protamine-to-heparin dosing ratio group compared to the low dosing group, respectively. More patients received fresh frozen plasma (11% versus 0%; P=0.02) and platelet concentrates (21% vs. 6%; P=0.04) in the high protamine-to-heparin dosing group when compared to the low dosing group (Table 2). There were no differences in serious adverse outcome between the groups.
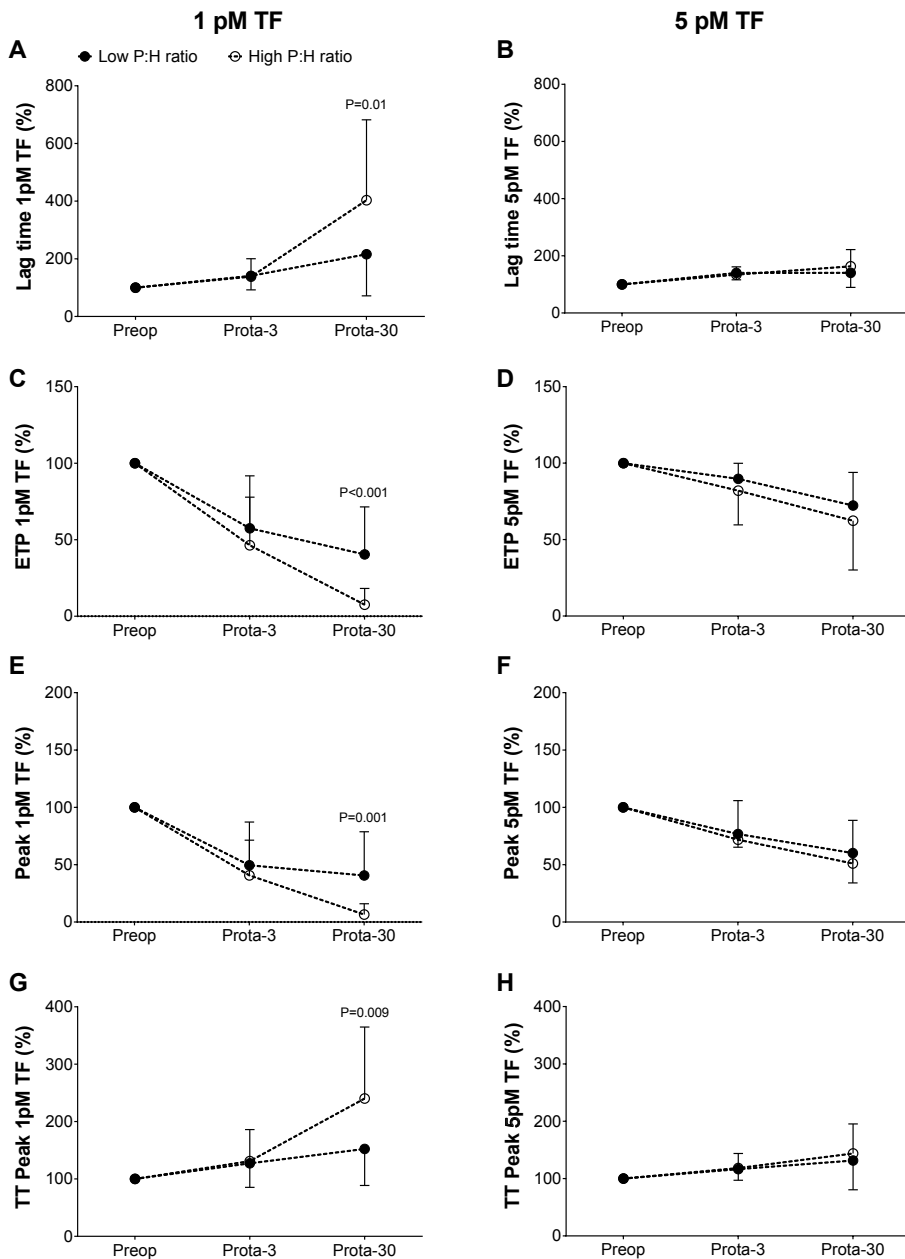
Figure 5. Thrombin generation assay. Data represent the lag time (Panels A and B), the endogenous thrombin generation potential (ETP; Panels C and D), the peak thrombin generation (Panels E and F) and the time to peak thrombin (Panels G and H) after stimulation with 1 or 5 pM tissue factor in the low dosing ratio group (n=20) and high dosing ratio group (n=22). Data are expressed as mean ± standard deviation.

	Low P:H ratio	High P:H ratio	P-value
Blood product transfusion			
Cellsaver blood (ml)	322 (113;450)	326 (223;465)	0.56
PRBC transfusion [% patients; 95% CI]	19% (8-30%)	32% (19-45%)	0.16
FFP transfusion [% patients; 95% CI]	0% (0-0%)	11% (2-21%)	0.02
Platelet transfusion [% patients; 95% CI]	6% (0-15%)	21% (11-34%)	0.04
Postoperative outcome			
ICU stay (hours)	26 ± 10	27 ± 15	0.81
Rethoracotomy (n (%))	1 (2%)	4 (9%)	0.17
Myocardial infarction (n (%))	1 (2%)	0 (0%)	0.32
TIA / CVA (n (%))	1 (2%)	0 (0%)	0.32
Cardiac tamponade (n (%))	0 (0%)	1 (2%)	0.32
Reintubation (n (%))	1 (2%)	0 (0%)	0.32
Died (n (%))	0 (0%)	1 (2%)	0.32

Table 2. Blood product transfusion and postoperative outcome parameters. PRBC = packed red blood cells; FFP = fresh frozen plasma; ICU = intensive care unit; TIA = transient ischemic attack; CVA = cerebral vascular accident. Data represent mean ± standard deviation, median with interquartile range (IQR) in brackets, or frequencies with 95% confidence intervals (95% CI).

3

DISCUSSION

This study shows that a protamine-to-heparin ratio of 1.3 is associated with disturbances in postoperative haemostasis, increased 24-hour blood loss and higher transfusion rates in patients undergoing cardiac surgery with CPB when compared to a low dosing group. The low dosing ratio of 0.8 did not result in residual heparin or prolonged activated clotting times following weaning from bypass. Excessive protamine was associated with reduced speed and level of thrombin generation irrespective of the total dose of administered heparin. These findings suggest that excessive protamine dosing is harmful for the restoration of perioperative haemostasis in cardiac surgery, and may contribute to increased bleeding and allogeneic blood transfusions.

Although several in vitro studies showed negative effects of high protamine dosing on haemostasis, [4,9-13] there are no randomized controlled trials available that confirmed these findings in the clinical setting. As a consequence, European guidelines still accept a protamine-to-heparin dosing ratio of 1.3:1.0. [17] Other guidelines suggest lowering protamine dosing, yet these are low class recommendations (IIb) as clinical data are scarce. [16]

Although the earliest publications on the anticoagulant effects of protamine originate from 1900 and 1927, the impact of a disturbed equilibrium between the heparin-neutralizing effects and anticoagulant actions of protamine has only been limitedly investigated. [23-27] Berger et al. were the first who suggested to adapt protamine dosing for heparin neutralization in light of the half-life of heparin and the intraoperative consumption of heparin. [14] When protamine dosing was administered in consecutive doses, the first dose was frequently sufficient to establish clot formation, while the second dose resulted in continuous bleeding. [5,14] In contrast to our findings, in two studies it was shown that a higher protamine dosing ratio (1.3), or the titration of protamine, was associated with better clinical outcome when compared to a lower protamine dose. [28,29]

A recent meta-analysis showed a minor reduction of perioperative blood loss when protamine titration was compared to conventional practice. [30] However, the four studies that were included in this meta-analysis were not primarily designed to compare different protamine dosing strategies in cardiac surgery [31,32] or based on a small sample size. [33,34]

A common denominator in the aforementioned studies is that protamine itself influences patient haemostasis and bleeding, and that protamine titration might support restricted allogeneic blood transfusion strategies in cardiac surgery. Despite the short half-life of protamine, [35] we still observed impaired coagulation at 30 minutes following protamine administration in the high protamine-to-heparin

dosing ratio group. This suggests that the detrimental effect of protamine on the coagulation system may persist even after protamine has disappeared from the circulation.

The most important reasons for protamine overdosing or postoperative protamine administration are fear for postoperative bleeding when post-CBP ACT levels are elevated, or a heparin rebound effect. Interestingly, we found no differences in the ACT between the two protamine-to-heparin dosing regimes, suggesting that even the lowest dose of protamine administered was an overdose in the light of sufficient normalization of heparin. The aPTT, thromboelastography and a heparin assay were more sensitive than the ACT to detect residual heparin anticoagulation. [36]

Heparin rebound is the reappearance of anticoagulant activity after adequate neutralization with protamine. While in the early days of cardiac surgery heparin rebound was a frequently observed phenomenon, a recent study suggested that this phenomenon might be of less importance as cause for postoperative bleeding. [37] Moreover, ACT values following protamine administration in the low and high dosing groups were similar in our study. Finally, we measured heparin concentrations after protamine administration and found no difference in the heparin concentration at 3 and 30 minutes after protamine administration in both groups, and therefore we concluded that residual heparin could be excluded.

The thromboelastometric intrinsic clotting time with and without the presence of heparinase was prolonged in the high protamine-to-heparin dosing ratio group when compared to the lower dosing ratio group, while the extrinsic clotting time was unaltered. These findings were supported by the thrombin generation measurements. During exposure to a low concentration of tissue factor, coagulation occurs predominantly through the intrinsic pathway, involving factor VIII, IX and XI. [21] The thrombin generation analyses as performed in the present study showed that high protamine dosing results in an increased lag time and reduced peak level of thrombin generation, [38] rather than recovering thrombin formation. This confirms previous statements that protamine interferes with the conversion of prothrombin to thrombin in whole blood, [39] or with the activation of factor V. [8] The thrombin assay measurements were performed in a small subgroup of patients, which restricts the generalizability of these findings to the whole population.

Platelet factor 4 (PF4) is a large polypeptide, which is released by activated platelets. It binds circulating heparins and thereby inhibits the action of antithrombin, promoting haemostasis. In this study (pleuro-)pericardial shed blood was washed by cell saver before returning to the patient's circulation. However,

as pericardial suction blood contains blood with activated haemostasis, including PF4, centres returning this blood directly to the patient's circulation might further reduce their protamine dosing as heparin captured by PF4.

The present study was limited by the one-sided blinding protocol, which might have influenced perioperative blood management strategies. A second limitation is the calculation of the protamine dose based on the total heparin dose, leading to relatively high doses when compared to others. [6,15] An additional limitation of this study is that there were small differences in CPB strategies between both centres with respect to the type of heart-lung machine used, the use of volatile anaesthesia and the amount of heparin in the extracorporeal circuit. However, these differences between centres have only limited influence on our study outcome. Due to logistic reasons the thrombin generation assay was performed in plasma, and future studies should investigate the effect of different protamine dosing regimens in whole blood to evaluate the role of platelets in the anticoagulation effect of protamine.

Worldwide, there is still a general belief that additional protamine administration in patients with microvascular bleeding might stop blood loss following heparin neutralization. Our study confirms that protamine might by itself enhance bleeding when inappropriately dosed. In light of patient blood management as multifactorial strategy that involves blood conservation techniques, our study contributes to improved awareness regarding the impact of common interventions in clinical routine.

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Chapter 4

A pharmacokinetic model for protamine dosing after cardiopulmonary bypass

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ABSTRACT

Objective: This study investigated postoperative hemostasis of patients subjected to conventional protamine dosing compared with protamine dosing based on a pharmacokinetic (PK) model following cardiopulmonary bypass.

Design: Retrospective case-control study.

Setting: Tertiary university hospital.

Participants: Patients undergoing elective cardiac surgery with cardiopulmonary bypass.

Interventions: In 56 patients, protamine was dosed in a fixed ratio (CD), while 62 patients received protamine based on the PK model.

Results: There was no difference in heparin administration (414 ± 107 mg (CD) vs 403 ± 90 mg (PK); p = 0.54), whereas protamine dosing was considerably different with a protamine-to-heparin dosing ratio of 1.1 ± 0.3 for the CD group and 0.5 ± 0.1 for the PK group (p<0.001). The changes in activated coagulation time (ΔACT) values (ACT after protamine minus preoperative ACT; +17 ± 77 s vs +6 ± 15 s; p = 0.31) were equal between groups. Yet, the thromboelastometric intrinsically activated coagulation test clotting time (CT; 250 ± 76 s vs 203 ± 44 s; p<0.001) and intrinsically activated coagulation test without the heparin effect CT (275 ± 105 vs 198 ± 32 s; p<0.001) were prolonged in the CD group. Median packed red blood cell transfusion (0 [0–2] v 0 [0–0]), fresh frozen plasma transfusion (1 [0–2] vs 0 [0–0]), and platelet concentrate transfusion (0 [0–1] v 0 [0–0]) were different between the fixed ratio and PK group, respectively (all p<0.001).

Conclusions: This study showed that patient-tailored protamine dosing based on a PK model was associated with a reduction in protamine dosing, with better hemostatic test results when compared with fixed-ratio protamine dosing.

INTRODUCTION

Protamine is used to reverse heparin after termination of cardiopulmonary bypass (CPB). The anticoagulant properties of heparin are antagonized by the formation of a neutral 1:1 protamine-heparin complex. Protamine is commonly administered in a fixed protamine-to-heparin ratio based on the intraoperative dose of heparin, irrespective of the patient's heparin consumption. Protamine dosing usually ranges from 0.5 to 1.3 mg of protamine per 100 IU of heparin administrated. [1,2] Yet, during CPB, heparin is broken down, with a half-life of approximately 4 hours in high dosage, and a small part of heparin is lost by blood loss. When these factors were taken into account, protamine dosing based on a fixed ratio was inadequate for the great majority of patients, and might lead to unantagonized heparin or protamine overdose, both causing coagulopathy. [3,4]

An alternative method for individualized protamine dosing is heparin and protamine administration using a point-of-care hemostasis management system. [5] The heparin concentration is estimated in vitro and multiplied by the calculated blood volume of the patient, leading to an advised protamine dosage. This method has been shown to contribute to individualized protamine dosing; however, the technique is costly. Therefore, its additive value and cost-benefit for its use in routine minor and moderate risk cardiac surgery is debatable.

In order to offer an alternative for the titration method the authors developed a two-compartment pharmacokinetic (PK) model that estimates the heparin blood concentration from the administered heparin doses and time of administration to calculate the loss of heparin over time. [6] By estimating the amount of heparin at the end of CPB, the required protamine dosage is calculated to neutralize heparin. In this study the authors investigated if implementation of this PK model improved clinical practice by evaluating postoperative bleeding, blood transfusion, and hemostasis by thromboelastometry.

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METHODS

Study Population

This retrospective observational study was performed in the Departments of Anesthesiology and Cardiothoracic Surgery of the VU University Medical Center (Amsterdam, The Netherlands). Two cohorts were compared, before and after the implementation of the PK protamine dosing. All measurements were part of routine clinical practice. The local Human Subjects Committee approved this retrospective analysis (METc 2012/438) and waived informed consent. During the study period no alterations were made in the perioperative cardiac surgery practice. Additionally, there were no changes in the dedicated anesthesia or cardiothoracic surgery team.

Patients aged 18 to 85 years were eligible if they were scheduled for elective cardiac surgery with CPB. Acetylsalicylic acid was not discontinued prior to surgery, whereas clopidogrel was stopped 5 days prior to the cardiac procedure. Exclusion criteria were emergency surgery, a body mass index below 18 kg m^{-2} or above 35 kg m^{-2}, a history of hematologic disorders, the necessity for renal replacement therapy, and the preoperative use of heparin or heparin-like medication.

Study Procedure

The first cohort of patients received protamine based on a fixed ratio at the discretion of the attending anesthesiologist. The second cohort received protamine based on a two-compartment model to estimate the heparin concentration in blood (Fig 1). The calculated residual heparin concentration was multiplied by the calculated blood volume, resulting in the residual amount of heparin. Protamine was dosed in a 1:1 ratio based on the calculated amount of residual heparin after termination of CPB.

Protamine Dosing Model

A two-compartment model was implemented using the following equation:

$$C_{t'} = C_0.A.e^{\alpha t} + C_0.B.e^{-\beta t}$$

C_t is the residual heparin in the patient at the moment of protamine dosing (t). A and B represent the distribution over the subsequent compartments of the initial dose (C_0), 0.1 and 0.9, respectively, [7] and e is the base of the natural logarithm (2.718) with α and β being the time constants of both compartments (α = 10 minutes and β = 250 minutes). The half-life of heparin increases by incremental

dosage. The highest dose investigated was 400 IU and showed a half-life of 150 minutes in humans. [8] Patients receive an average of 500 IU of heparin during a cardiac procedure in this center, leading to a half-life greater than 150 minutes. The half-life is expected to increase further with cooling (to approximately 34°C during CPB). As the detrimental effect of heparin overdosing is greater than protamine overdosing, the half-life of heparin was rounded up to avoid a residual heparin effect. In the absence of evidence about the half-life of high-dose heparin during CPB, an empirical half-life (β) of 250 minutes was chosen taking all the above-mentioned factors into consideration.

The final dose of protamine was calculated combining the remainder of the initial bolus (C_t) plus the remainder of the subsequent bolus ($C_{t'}$, $C_{t''}$,…). Protamine was dosed in a 1:1 ratio with the calculated remainder of heparin (mg).

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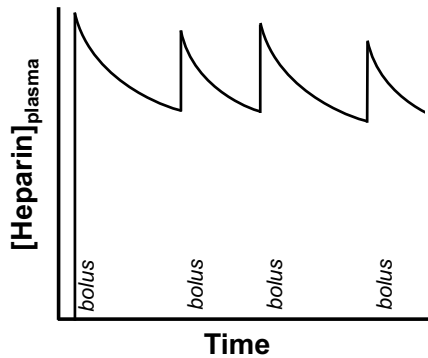
Figure 1.   Schematic representation of the pharmacokinetic heparin model. Prior to CPB a bolus of 300 IE kg^{-1} of heparin is administered to achieve an ACT > 480 s. During CPB, when the heparin concentration drops due to its half-life, the ACT will shorten. When the ACT falls below 480 seconds an additional bolus of heparin is administered, resulting in a second peak concentration of heparin in the blood. CPB, cardiopulmonary bypass; ACT, activated coagulation time.

Anesthesia and Cardiopulmonary Bypass

Patients were anesthetized according to local protocols based on a combination of sufentanil, 3-7 µg kg^{-1}, rocuronium, 0.6 mg kg^{-1}, and midazolam, 0.1 mg kg^{-1}, for anesthesia induction and tracheal intubation. Propofol (200-400 µg h^{-1}), with or without isoflurane/sevoflurane, was used for maintenance of anesthesia. All patients received tranexamic acid (1 gram prior to and 2 gram after CPB).

CPB was performed at a blood flow of (2.2-2.4 L min^{-1} m^{-2}) during normo- or mild hypothermia (> 34°C) using either a C5 or S5 heart–lung machine (Stöckert Instrumente GMBH, Munich, Germany) with a centrifugal pump and biocompatible

coated circuit. Cell saving was applied throughout the surgical procedure. Shed blood from the thoracic cavity and residual blood that remained in the extracorporeal system after bypass were sucked up into the cell-saver reservoir using a dual-lumen tube connected to a vacuum pump, and subsequently transferred to the centrifuge bowl of the cell saver (Autolog, Medtronic, Minneapolis, MN). After plasma removal, red blood cells were washed, and concentrated red blood cells were retransfused.

Heparin and Protamine Management

Heparinization started with an initial bolus of 300 IU kg^{-1} of heparin (LEO Pharma BV, Amsterdam, The Netherlands) to achieve an activated coagulation time (ACT) test of 480 seconds. If necessary, additional doses of heparin (5000 IU) were administered to maintain this target ACT. The priming solution (1200 mL) contained 5000 IU of heparin.

After weaning from CPB, heparin was reversed with protamine hydrochloride (MedaPharma BV, Amstelveen, The Netherlands). The amount of protamine in the first cohort was at the discretion of the attending anesthesiologist in a fixed ratio. After implementation of the PK-based protamine dosing model, the second cohort was evaluated.

Local Blood Transfusion Algorithm

The transfusion of packed red blood cells (PRBC) was based on the Dutch transfusion guidelines, recommending transfusion if hemoglobin levels are below 9.7 g dL^{-1} (6 mmol L^{-1}), in American Society of Anesthesiologists IV patients, and patients with symptomatic cerebrovascular disease, sepsis, heart failure, or severe pulmonary disease. Packed red blood cells transfusion is advised in cases of hemoglobin levels below 8.1 g dL^{-1} (5 mmol L^{-1}), in cases of acute blood loss from one bleeding focus in a normovolemic American Society of Anesthesiologists I patient aged > 60 years, and in cases of fever or following cardiac surgery. Fresh frozen plasma or platelet transfusion was performed in patients with clinical signs of persistent nonsurgical bleeding (oozing). When laboratory coagulation test results were available, fresh frozen plasma was transfused when the international normalized ratio of the prothrombin time exceeded 1.5. Additional platelet concentrate administration was indicated for extended bypass times, clinical signs of bleeding, and/or a platelet count below 75×10^9 L^{-1}.

Blood Sampling

After anesthesia induction, the first blood sample was collected for hemostatic baseline evaluation as part of routine practice. Blood was drawn in a citrated tube from the radial artery catheter. Three minutes after protamine administration another blood sample was drawn for routine hemostatic evaluation.

Thromboelastometry

Using rotational thromboelastometry (ROTEM delta; TEM International, Munich, Germany), the viscoelastic properties of a blood clot were measured in vitro using the intrinsically activated coagulation test (INTEM), extrinsically activated coagulation test (EXTEM), fibrin polymerization test, and the intrinsically activated coagulation test without the heparin effect (HEPTEM). Clot formation was assessed by the clotting time or the maximum clot firmness (MCF), which were represented in a thromboelastogram.

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Other Study Parameters

Patient demographics included age, gender, and body mass index. Collected surgical data were CPB time, aortic cross-clamp time, and the amount of heparin and protamine administered. Hemostatic parameters included pre- and postoperative prothrombin time (PT), activated partial thromboplastin time (aPTT), ACT, hemoglobin concentration, and platelet count. Clinical outcomes included blood loss assessed at 6, 12, and 24 hours by chest drainage and postoperative allogeneic blood transfusion.

Statistical Analysis

Statistical analysis was performed using the SPSS statistical software package 22.0 (SPSS Inc.®, IBM, New York). Descriptive statistics are presented as frequencies (nominal data), median with interquartile range (non-parametric data), or mean with standard deviation (numerical data). A Student's T-test, Mann–Whitney U test, or chi-square test was used for differences in numeric, ordinal, or nominal data, respectively, between the cohorts. Multiple linear regression was performed to evaluate the possible confounding effect of the type of surgery on thromboelastometric results, and postoperative blood loss for both groups, as this was different between groups. A p-value < 0.05 was considered as statistically different.

RESULTS

The study population included 118 patients undergoing cardiac surgery with CPB. The conventional dosing (CD) cohort consisted of 56 patients, and 62 patients received protamine dosing according to the PK model. Table 1 shows patient and surgical characteristics for both patient cohorts. There were no differences in patient demographics between groups, except for a higher proportion of male patients in the PK group.

	Conventional dosing (CD)	Pharmacokinetic dosing (PK)	P-value
N	56	62	
Age (years)	66 ± 12	67 ± 11	0.74
BMI (kg m^{-2})	28 ± 4	27 ± 4	0.61
Males (n)	30 (54%)	47 (76%)	0.02
Preoperative anticoagulation			
Acetylsalicylic acid (n)	20 (36%)	32 (52%)	0.07
Clopidogrel (n)	1 (2%)	8 (13%)	0.02
Heparin (n)	2 (4%)	4 (7%)	0.46
ECC time (min)	132 ± 53	117 ± 44	0.09
Aorta clamp time (min)	98 ± 41	85 ± 35	0.07
Lowest temperature (°C)	34 ± 1	34 ± 1	0.10
Lowest hemoglobin			0.79
mmol l^{-1}	5.3 ± 1.0	5.3 ± 0.8	
g dl^{-1}	8.5 ± 1.6	8.5 ± 1.3	
Type of surgery			<0.001
CABG (n)	6 (11%)	31 (51%)	
Valve surgery (n)	29 (51%)	16 (26%)	
CABG + valve (n)	18 (32%)	11 (18%)	
Bentall ± CABG (n)	3 (5%)	2 (3%)	

Table 1. Patient demographics and surgical characteristics. BMI = body mass index; ECC = extracorporeal circulation; CABG = coronary artery bypass graft surgery. Data represent frequencies or mean ± standard deviation.

Figure 2 shows heparin and protamine dosing in the CD and PK dosing groups. Implementation of the PK model did not alter heparin dosing, but significantly reduced protamine dosing from 416 ± 82 mg to 186 ± 42 mg (p = 0.001) compared with the CD group. This resulted in a major reduction in the protamine-to-heparin dosing ratio from 1.1 ± 0.3 for the CD to 0.5 ± 0.1 for the PK dosing (p<0.001). Table 2 shows whether reduced protamine dosing in the PK group was associated with alterations in patient hemostasis, differences for hemoglobin, aPTT, prothrombin time, and platelet count between groups before surgery and at 3 minutes following protamine administration.
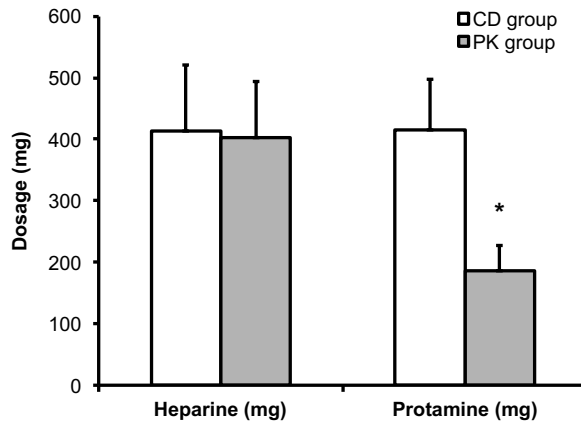
Figure 2. Protamine and heparin dosage. The protamine-to-heparin dosing ratio resulted in 1.1 ± 0.3 for the CD, and 0.5 ± 0.1 for the PK dosing (p<0.001). PK = pharmacokinetic dosing.

	Conventional dosing (CD)	Pharmacokinetic dosing (PK)	P-value
Preoperative			
Hemoglobin			<0.001
mmol l^{-1}	8.1 ± 0.9	8.8 ± 1.0	
g dl^{-1}	13.0 ± 1.4	14.1 ± 1.6	
aPTT (s)	37 ± 6	38 ± 8	0.50
PT (INR)	1.2 ± 0.4	1.1 ± 0.6	0.73
Platelet count (10^{*9} l^{-1})	245 ± 59	233 ± 73	0.38
3 minutes after protamine			
Hemoglobin			<0.01
mmol l^{-1}	6.1 ± 0.9	5.7 ± 0.7	
g dl^{-1}	9.8 ± 1.4	9.1 ± 1.1	
PT (INR)	1.9 ± 0.5	1.6 ± 0.2	<0.001
Platelet count (10^{*9} l^{-1})	109 ± 35	129 ± 39	<0.01
aPTT (s) after protamine			
3 minutes	42 ± 13	43 ± 5	0.73
1 hour	41 ± 8	42 ± 7	0.35
2 hours	39 ± 7	39 ± 9	0.99
Activated clotting time (ACT)			
ACT preoperative(s)	131 ± 15	124 ± 15	0.01
ACT after protamine dosing (s)	148 ± 74	130 ± 13	0.08
Delta ACT (preop – postop)	+ 17 ± 77	+ 6 ± 15	0.31

Table 2. Laboratory findings. PT = prothrombin time, INR = international normalized ratio; aPTT = activated partial thrombin time; ACT = activated clotting time. Data represent frequencies or mean ± standard deviation.

Figure 3 shows the thromboelastometric clotting times for the subsequent tests 3 minutes after protamine administration. Furthermore, INTEM MCF was 52 ± 8 in the CD group compared with 59 ± 9 in the PK dosing group, p<0.01. EXTEM MCF was higher in the PK group (60 ± 9) when compared with the CD group (54 ± 8, p = 0.02), respectively. The fibrin part of the clot (fibrin polymerization test MCF) was 11 ± 4 in the CD group and 14 ± 6 in the PK group, p = 0.01.
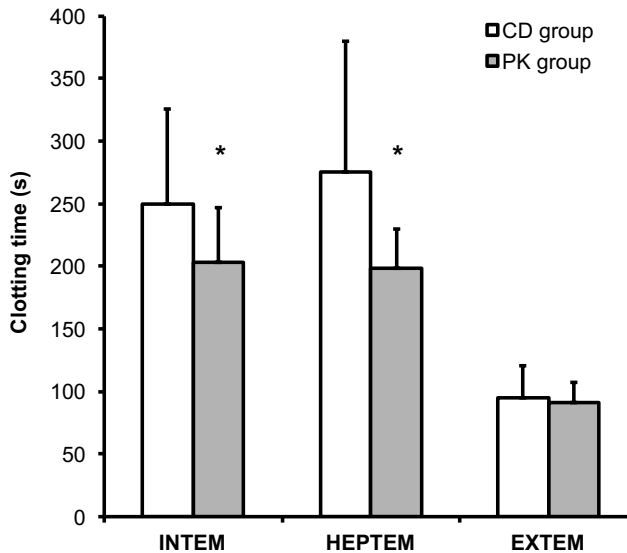
Figure 3. Thromboelastometric test results at three minutes following protamine administration. PK = pharmacokinetic dosing; INTEM = intrinsic coagulation cascade; HEPTEM = intrinsic coagulation cascade in the presence of heparinase; EXTEM = extrinsic coagulation cascade. *p < 0.05.

There was a difference in median postoperative blood loss 6 hours after surgery (110 [73;228)] vs. 180 [121,292] mL, p = 0.01), yet there was no difference at 12 hours (248 [141:375] vs. 250 [180,329] mL, p=0.34) nor 24 hours after surgery (340 [242,525] vs. 387 [293,548] mL, p=0.27) for the conventional versus the PK dosing group. Table 3 shows the postoperative transfusion outcome for both groups.

	Conventional dosing (CD)	Pharmacokinetic dosing (PK)	P-value
Packed red blood cells	0 (0-2)	0 (0-0)	<0.001
0 units (n (%))	27 (52%)	58 (93%)	
1-2 units (n (%))	13 (25%)	3 (5%)	
≥ 3 units (n (%))	12 (23%)	1 (2%)	
Fresh frozen plasma	1 (0-2)	0 (0-0)	<0.001
0 units (n (%))	26 (50%)	61 (98%)	
1-2 units (n (%))	17 (32%)	1 (2%)	
≥ 3 units (n (%))	9 (18%)	0 (0%)	
Platelet concentrate	0 (0-1)	0 (0-0)	<0.001
0 units (n (%))	27 (52%)	59 (95%)	
1-2 units (n (%))	23 (44%)	3 (5%)	
3 units (n (%))	2 (4%)	0 (0%)	
Fibrinogen concentrate	0 (0-0) n=3	0 (0-0) n=4	0.98
Prothrombin complex concentrate	0 (0-0) n=1	0 (0-0) n=8	0.48

Table 3. Postoperative transfusion. Data are represented as median with interquartile range.

Multiple Linear Regression

Multiple linear regression was performed to evaluate the possible confounding effect of the type of surgery on thromboelastometric results and postoperative blood loss for both groups (Tables 4 and 5). The effect of PK dosing remained significant after correcting for the type of surgery for the INTEM, and HEPTEM was improved by PK dosing after correcting for the type of surgery when compared with the CD group. There were no differences in EXTEM or postoperative blood loss 6 hours postoperative after correcting for the type of surgery.

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	INTEM			HEPTEM			EXTEM			6 h blood loss		
	Beta	t	P-value	Beta	t	P-value	Beta	t	P-value	Beta	t	P-value
PK-dosing	-0.386	-3.995	<0.001	-0.466	-5.083	<0.001	-0.063	-0.619	0.54	0.138	1.385	0.169
Valve	-0.141	-1.255	0.21	-0.130	-1.231	0.22	0.029	0.245	0.80	-0.231	-2.021	0.046
Valve+CABG	0.024	0.220	0.83	0.085	0.833	0.41	0.081	0.708	0.48	0.101	0.931	0.354
Bentall	0.108	1.165	0.25	0.114	1.296	0.20	0.241	2.465	0.02	0.111	1.155	0.251
R^2	0.17			0.25			0.06			0.136		
F	5.46			9.18			1.89			4.186		

Table 4. Multivariate linear regression to evaluate the possible confounding effect of type of surgery on the dosing group.

	INTEM CT (s)	HEPTEM CT (s)	EXTEM CT (s)	Protamine (mg)
CD group (n=56)	250 ± 76	275 ± 105	54 ± 8	416 ± 82
PK group (n=62)	203 ± 44	198 ± 32	60 ± 9	186 ± 42
Alfa (%)	5	5	5	5
Power (%)	98	100	97	100

Table 5. Two tailed power analysis for INTEM, HEPTEM, EXTEM clotting time and protamine dosing.

DISCUSSION

Protamine dosing based on a PK model for residual heparin after CPB was associated with a reduction of more than 50% of the protamine dose when compared with a conventional fixed protamine-to-heparin dosing strategy with full neutralization of the postoperative ACT. This reduction in protamine administration was paralleled by better postoperative hemostasis, less allogeneic blood transfusion, and might reduce postoperative blood loss when compared with the control cohort. This study suggested that patient-tailored protamine administration based on the concentration of residual heparin was beneficial for patient hemostasis following cardiac surgery with CPB.

Several studies have shown that unbound protamine has intrinsic anticoagulant properties that may impair hemostasis. Nielsen and Malayaman previously gave an overview of the negative effects of protamine on hemostasis, showing that protamine decreases thrombin (factor IIa) activity, reduces factor VII and V activation, enhances fibrinolysis, and impairs platelet function by inhibition of GPIb-vWF activity. [4] The complex balance between protamine underdosing, which may result in residual heparin, and protamine overdosing, which may result in impaired hemostasis, warrants better tools for protamine dosing.

More than half a century ago the first attempts to optimize protamine dosing were made. [9] Since then there have been studies showing that a protamine dose reduction led to better outcomes. [10,11] A more recent development was the introduction of protamine titration, which also showed a (minor) reduction of postoperative blood loss. [12] Yet, so far it is common practice that protamine is dosed based on a fixed ratio to heparin, with adjustments based on the clinical experience of the anesthesiologist. Current guidelines advise a protamine-to-heparin dosing ratio of 1:1 to 1:1.3, with a level IIb recommendation for lower protamine dosing. [1,2] As heparin is lost during CPB, a ratio of 1 mg of protamine for every milligram of heparin administered could result in protamine overdosing and impairment of hemostasis.

An alternative to a fixed protamine-dosing regimen is protamine titration, a method that titrates protamine ex vivo to find the optimal protamine-to-heparin dosing ratio. A meta-analysis by Wang et al showed that protamine titration might improve protamine dosing in cardiac surgery. [12] However, the meta-analysis was limited to the inclusion of studies not designed for comparison of protamine titration, and studies with a small sample size. Further attempts to improve protamine dosing were based on the perioperative anti-Xa or heparin concentration, the ACT or thrombin assay. [13-15] Unfortunately, none of these attempts resulted in major improvement of perioperative protamine management.

Recently, several groups have developed mathematical models for protamine dosing. Jia et al established a PK model to predict heparin concentrations during cardiac surgery, suggesting a lower protamine dosing regimen after termination of CPB. [16] Davidsson and colleagues developed another statistical model for protamine dosing and showed a good agreement with point-of-care protamine dosing. [17] Finally, Kjellberg et al built a protamine dosing algorithm and reduced protamine dosing without any significant difference in postoperative bleeding. [18] Yet, these authors were the first to develop a PK model for protamine dosing leading to reduced protamine dosing, improved postoperative hemostasis, and less blood loss 6 hours after surgery when compared with conventional protamine dosing.

Protamine generally is overdosed in cases of elevated ACT levels to neutralize residual circulating heparin, neglecting the possible detrimental effect of protamine overdosing. Interestingly, the PK dosing group showed a trend towards lower postoperative ACT values and no difference in aPTT, which is more sensitive for residual heparin, [19] when compared with the CD group. Another concern is postoperative "heparin rebound", the reappearance of circulating heparin after neutralization with protamine. Yet, a recent study suggested that this phenomenon might be a minor contributing factor to postoperative bleeding. [20] Additionally, the authors did not find any evidence of heparin rebound in the PK dosing group, as there was no difference in sequential postoperative aPTT values after surgery.

This study was limited by its retrospective design and the relatively small sample size per patient cohort. Because the two cohorts were sequential, the possibility of a chronology bias cannot be excluded. Furthermore, the absence of heparin measurements limits insight in the accuracy of the PK model, and the postoperative abundance of heparin or protamine. Moreover, the lack of repetitive heparin measurements following surgery limited the conclusions about the effect of the protamine dosing method on the occurrence of heparin rebound. Despite the limitations of this study, the benefits of PK-based protamine administration for postoperative hemostasis and allogeneic blood transfusion requirements warrant further exploration of patient-tailored heparin and protamine administration in cardiac surgery with CPB.

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Prediction of postoperative bleeding

part II

Chapter 5

Prediction of postoperative blood loss by thromboelastometry in adult cardiac surgery: cohort study & systematic review

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ABSTRACT

Objective: The aim was to evaluate the predictive value of thromboelastometry for postoperative blood loss in adult cardiac surgery with cardiopulmonary bypass.

Design: Retrospective cohort study & systematic review of the literature

Setting: A tertiary university hospital

Participants: 202 patients undergoing elective cardiac surgery

Interventions: Thromboelastometry was performed before cardiopulmonary bypass and 3 minutes after protamine administration.

Measurements and Main Results: The cohort study showed that the pre- and postoperative thromboelastometric positive predicting value was poor (0-22%), however the negative predicting value was high (89-94%).
The systematic review of the literature to evaluate the predictive value of thromboelastometry for major postoperative bleeding in cardiac surgery resulted in 1311 papers of which 11 were eligible, n=1765 (PUBMED and EMBASE, until June 2016). Two studies found a good predictive value whereas all other 9 studies showed a poor predictability for major postoperative bleeding after cardiac surgery. The overall negative predicting value was high.

Conclusions: Thromboelastometry does not predict which patients are at risk for major postoperative bleeding.

INTRODUCTION

While the incidence of major blood loss after cardiac surgery has been reduced in the last decades, massive bleeding after cardiopulmonary bypass is still common. [1,2] These bleeding patients frequently require blood product transfusion and reexploration for bleeding, which are both independently associated with poor outcome. [3-7] Therefore, many attempts have been made to predict, prevent and treat postoperative blood loss. [2, 8-10]

Several risk scores for blood transfusion in patients undergoing cardiac surgery have been validated, showing that post-surgical blood loss can be predicted by a set of demographic and surgical characteristics and medication use. [11-15] However, haemostatic tests, such as thromboelastometry, were only scarcely evaluated in these risk prediction models although thromboelastometry is increasingly used in cardiac surgery.

Thromboelastometry is mainly used to guide haemostatic therapy. Still, in the cardiosurgical setting thromboelastometry-guided therapy has not proven to reduce mortality, although blood product transfusion and thromboembolic events are reduced. [16,17] It remains difficult to distinguish whether the improvement in haemostatic management is a result of the application of thromboelastometry itself, the thromboelastometry-related increase in factor concentrates transfusion, or the implementation of an extensive transfusion algorithm creating more awareness and knowledge about hemostasis. [16,18]

Although, several studies showed improved outcome after implementation of thromboelastometry, there is increasing evidence that thromboelastometry test results after cardiac surgery do not predict postoperative blood loss. Yet, in the trauma setting thromboelastometry has shown to predict outcome19, Therefore, we evaluated the predictive value of thromboelastometry in our centre and conducted a systematic review to evaluate the predictive value of thromboelastometry for major postoperative bleeding in cardiac surgery.

5

METHODS

I. Cohort study
Study population
This retrospective study investigated haemostatic data of adult patients undergoing elective cardiac surgery with cardiopulmonary bypass (CPB) in a University hospital between 2008 and 2011. Data were retrieved from databases of previous observational studies performed in our centre, in which the patient characteristics and thromboelastometric data were prospectively collected. Data retrieval for the present investigation was approved by local Human Subjects Committee (Amsterdam, the Netherlands; METc 2012/337) and informed consent was waived. According to local protocols, acetylsalicylic acid was continued throughout the perioperative period, while clopidogrel was discontinued at least 5 days before surgery if deemed medically safe.

Anaesthesia and cardiopulmonary bypass
Patients were anesthetized according to the local protocol. All patients received 1 mg kg^{-1} dexamethasone before cardiopulmonary bypass and 2 grams of tranexamic acid after CPB. Heparinization started with an initial bolus of 300 IU kg^{-1} heparin (LEO Pharma BV, Amsterdam, the Netherlands), which was replenished until the activated clotting time (ACT) test exceeded 480 seconds. Cardiopulmonary bypass was performed during normothermia with a centrifugal pump and heparin-coated circuit. The priming solution (1400 ml) contained 5000 IU porcine heparin. After cardiopulmonary bypass, heparin was reversed with protamine (MedaPharma BV, Amstelveen, the Netherlands).

Haemostatic testing
Blood samples for haemostatic testing (haemoglobin, activated partial thromboplastin time (aPTT), prothrombin time (PT), platelet count, activated clotting time (ACT) and thromboelastometry) were drawn after anaesthesia induction and three minutes after protamine administration. Thromboelastometry (ROTEM delta; TEM International, Munich, Germany) was used to perform the extrinsically activated coagulation test (EXTEM), fibrin polymerization test (FIBTEM), intrinsically activated coagulation test with (HEPTEM) and without (INTEM) heparinise. Clot formation was assessed by the clotting time (CT) and the maximum clot firmness (MCF).

Local blood transfusion algorithm

The transfusion of packed red blood cells (PRBC) was based on the Dutch transfusion guidelines, recommending transfusion if haemoglobin levels below 9.7 g dL^{-1} (6 mmol L^{-1}) in case of ASA IV patients, patients with symptomatic cerebrovascular disease, sepsis, heart failure, or severe pulmonary disease. PRBC transfusion is advised in case of haemoglobin levels below 8.1 g dL^{-1} (5 mmol L^{-1}) in case of acute blood loss from one bleeding focus in a normovolaemic ASA I patient aging > 60 years, in case of fever or following cardiac surgery. Fresh frozen plasma (FFP) or platelet transfusion was performed in case of clinical signs of persistent nonsurgical bleeding (oozing). If laboratory coagulation test results were available, FFP was transfused when the international normalized ratio (INR) of the prothrombin time exceeded 1.5. Platelet concentrate administration was indicated in case of extended bypass times, clinical signs of bleeding and/or a platelet count below 75*10^9 L^{-1}.

5

Definition of major postoperative blood loss

Major blood loss was defined as more than 500 ml chest tube drainage at 6 hours after surgery (the 90th percentile of the total study population).

Other study variables

Patient demographics collected were age, gender and body mass index (BMI). Surgical characteristics were type of procedure, CPB time, aorta clamp time and lowest body temperature.

Data analysis

Statistical analysis was performed using the SPSS statistical software package 17.0 (SPSS Inc.®, Chicago USA) except for comparison of Area under the ROC curves which was done in STATA 14 (STATA corp. LP, College Station USA). Descriptive statistics were represented as frequencies (nominal data), median with interquartile range (ordinal data) or mean with standard deviation (numerical data). The Student's T-test was used to compare means of normally distributed variables between groups of patients with and without major blood loss. Mann-Whitney U test was used to compare distribution of ordinal variables and chi-square test was used for categorical variables. Univariate logistic regression was used to test for the association between known risk-factors and major postoperative blood loss. Multivariate logistic regression was used to investigate if thromboelastometric values were of added value to the known risk factors for prediction of major postoperative blood loss. Only models with two predictors

were considered as the limited number of patients with major postoperative blood loss made estimation of larger models infeasible. Area under the ROC curves were used to quantify the discriminative ability of thromboelastometric values and multivariate models for major postoperative blood loss. P<0.05 was considered as statistically different.

II. Systematic review

the systematic review was performed in accordance with the recommendations for systematic reviews. [20] The literature search was performed according to the PRISMA guideline. The following search was the fundament for the literature search: Does [(rotational) thromboelastometry] predict blood loss or blood product transfusion in [cardiac surgery patients]?

Inclusion criteria were papers written in English in non-paediatric cardiac surgery using cardiopulmonary bypass with rotational thromboelastometry analysis (not thromboelastography (TEG)) and predicting data. Pubmed/Medline and Embase were searched with the enclosed search queries in June 2016 (Appendix 1). Mesh terms and free text were used.

Embase search query
('thromboelastography'/exp OR (thrombelast*:ab,ti OR thromboelast*:ab,ti OR rotem:ab,ti OR roteg:ab,ti OR INTEM:ab,ti OR EXTEM:ab,ti OR HEPTEM:ab,ti OR FIBTEM:ab,ti OR TEM:ab,ti)) AND (('thorax surgery'/de OR 'heart surgery'/exp OR 'heart lung machine'/exp OR 'extracorporeal circulation'/de OR 'cardiopulmonary bypass'/exp OR 'heart left ventricle bypass'/exp) OR ((thoracic NEXT/1 surg*):ab,ti OR (thorax NEXT/1 surg*):ab,ti OR (cardiac NEXT/1 surg*):ab,ti OR (heart NEXT/1 surg*):ab,ti OR ('heart lung' NEXT/1 machine*):ab,ti OR (extracorporeal NEXT/1 circulation*):ab,ti OR (cardiopulmonary NEXT/1 bypass*):ab,ti OR ('heart lung' NEXT/1 bypass*):ab,ti OR cpb:ab,ti OR (heart NEXT/1 bypass*):ab,ti))

Pubmed search query
((Thrombelastography[Mesh]) OR (Thrombelast*[tiab] OR Thromboelast*[tiab] OR ROTEM[tiab] OR ROTEG[tiab] OR INTEM[tiab] OR EXTEM[tiab] OR HEPTEM[tiab] OR FIBTEM[tiab] OR TEM[tiab])) AND (("Thoracic Surgery"[Mesh] OR "Cardiac Surgical Procedures"[Mesh] OR "Heart-lung Machine"[Mesh] OR "Extracorporeal Circulation"[Mesh:noexp] OR "Cardiopulmonary Bypass"[Mesh] OR "Heart Bypass, Left"[Mesh]) OR ((thoracic[tiab] AND surg*[tiab]) OR (thorax[tiab] AND surg*[tiab]) OR (cardiac[tiab] AND surg*[tiab]) OR (heart[tiab] AND surg*[tiab]) OR Heart-Lung Machine*[tiab] OR Extracorporeal Circulation*[tiab] OR Cardiopulmonary Bypass*[tiab] OR Heart-Lung Bypass*[tiab] OR CPB[tiab] OR Heart Bypass*[tiab]))

Appendix 1. Literature search queries

Both searches were merged, duplicates removed and independently screened by two authors (MIM & DB). After each selection round, the selection of both screening authors was merged and further screened. First, all titles were screened for possible eligibility. When the title suggested that the study could investigate the predictive value of bleeding in cardiac surgery the title was selected. The abstracts of the selected titles were screened and when these could possibly contain predicting data in combination with viscoelastometric testing, the paper was selected. These selected papers were read and, when eligible, included in the final selection. The final selection was screened for possible bias and possible influencing funding sources.

5

RESULTS

I. Cohort study

Patients demographics and perioperative variables (n=202) for patients with more or less than 500 ml blood loss after 6 hours following surgery are shown in table 1.

	< 500 cc	≥ 500 cc	P=
N	181	21	
Age (yr)	67 ± 12	71 ± 10	0.23
BMI (m kg^{-2})	27 ± 4	25 ± 3	0.04
Type of surgery (n)			0.04
CABG	21 (12%)	5 (24%)	
Valve	96 (54%)	5 (24%)	
Valve + CABG	58 (32%)	11 (52%)	
Bentall ± CABG	4 (2%)	0 (0%)	
Extracorporeal circulation (min)	136 ± 57	149 ± 59	0.33
Aorta clamp time (min)	102 ± 44	108 ± 49	0.55
Heparin (mg)	493 ± 169	452 ± 99	0.28
Protamine (mg)	510 ± 165	564 ± 118	0.16
Protamine:Heparin ratio	1.1 ± 0.3	1.3 ± 0.3	<0.01
Lowest temperature (°C)	34 ± 1	34 ± 1	0.94
Lowest haemoglobin (mmol l^{-1})	5.1 ± 0.7	5.0 ± 0.6	0.65

Table 1. Demographic and surgical data of cohort study. Demographic and surgical data of cohort study. BMI = body mass index. Data represent frequencies or mean ± standard deviation.

In the 181 patients (90%) bleeding less than 500 ml the average amount of blood loss was 168 ml (100:260) 6 hours and 410 ml (281;600) 24 hours after surgery. In the 21 patients (10%) with major blood loss (> 500 ml) this was 890 ml (648;1270) after 6 hours and 1530 ml (1040;1865) after 24 hours, respectively. Body mass index, type of surgery and the protamine:heparin dosing ratio were different between groups. The minor bleeding group received less red blood cell transfusion in the operation room (2 (0;4) vs. 4 (2;6), p<0.01) and ICU (0 (0;0) vs. 1 (0;3.5), p<0.01) compared to the patients bleeding > 500 ml. Also, the amount of FFP transfusion was lower in patients bleeding less than 500 ml in the in the operation room (0 (0;2) vs 2 (1;4), p<0.01) and ICU (0 (0;2) vs 2 (1;3.5), p<0.01). The same goes for the numer op platelet concentrate transfusions in the operation room (1 (0;1) vs (1 (1;1.5), p=0.02) and ICU (0 (0;0) vs 2 (0;3.5) p<0.01).
Pre- and postoperative haemostatic results show that postoperative platelet count, the FIBTEM MCF and INTEM MCF were lower in the group with more than 500 ml blood loss (appendix 2).

	Preoperative			Postoperative		
	< 500 cc	≥ 500 cc	P=	< 500 cc	≥ 500 cc	P=
Laboratory hemostasis						
Haemoglobin (mmol l⁻¹)	8.1 ± 1.0	8.3 ± 1.0	0.35	6.0 ± 0.8	5.8 ± 0.8	0.22
aPTT (s)	37 ± 8	36 ± 3	0.65	41 ± 8	42 ± 5	0.35
PT (INR)	1.2 ± 0.3	1.1 ± 0.3	0.33	1.8 ± 0.4	1.8 ± 0.3	0.75
Platelets (*10⁹ l⁻¹)	246 ± 71	234 ± 50	0.46	116 ± 42	102 ± 17	<0.01
ACT (s)	137 ± 18	137 ± 18	0.98	137 ± 16	135 ± 11	0.66
Thromboelastometry						
INTEM CT (s)	173 ± 37	178 ± 101	0.83	248 ± 100	280 ± 70	0.08
INTEM MCF (mm)	64 ± 5	61 ± 6	0.06	53 ± 7	49 ± 6	0.01
EXTEM CT (s)	70 ± 28	70 ± 20	0.95	104 ± 65	106 ± 27	0.92
EXTEM MCF (mm)	64 ± 9	62 ± 5	0.21	55 ± 6	52 ± 6	0.05
FIBTEM MCF (mm)	19 ± 7	16 ± 5	0.01	12 ± 6	8 ± 3	<0.001
HEPTEM CT (s)	177 ± 55	166 ± 37	0.37	286 ± 165	341 ± 157	0.15
HEPTEM MCF (mm)	64 ± 5	63 ± 5	0.46	53 ± 7	49 ± 6	0.05

Appendix 2. Pre- and postoperative haemostatic data of cohort study

None of the pre- or postoperative thromboelastometric tests showed a good predictive value (PPV) for postoperative major blood loss (table 2).

	Preoperative				Postoperative			
	Spec	Sens	NPV	PPV	Spec	Sens	NPV	PPV
INTEM CT	94	10	90	17	63	65	94	17
INTEM MCF	100	5	90	100*	73	45	92	16
HEPTEM CT	92	5	89	7	55	65	93	14
HEPTEM MCF	99	0	89	0	73	45	92	16
EXTEM CT	83	30	91	17	26	80	92	11
EXTEM MCF	99	0	89	0	85	30	91	18
FIBTEM MCF	99	0	89	0	78	55	94	22

Table 2. Predictive value of thromboelastometry values for bleeding. CT = Clotting time, MCF = maximum clot firmness (MCF), INTEM = intrinsic pathway, HEPTEM = intrinsic pathway with heparinise, EXTEM = extrinsic pathway FIBTEM = fibrinogen, Spec = specificity, Sens = sensitivity, NPV = negative predicting value, PPV = positive predicting value, *n=1.

ROC analysis showed that only the INTEM CT and HEPTEM CT showed a good area under the curve (AUC) except for post-operative. AUCs of other thromboelastometric values were not significantly better (and some even worse) than an AUC of 0.5. Univariate logistic regression only showed a significant association between major postoperative blood loss and a protamine:heparin dosing ratio exceeding 1 (table 3).

	Preoperative ROC		Postoperative ROC	
	AUC	95% CI	AUC	95% CI
INTEM CT	0.374	0.240 – 0.508	0.678	0.573 – 0.784
INTEM MCF	0.382	0.247 – 0.516	0.313	0.197 – 0.429
HEPTEM CT	0.419	0.285 – 0.554	0.662	0.549 – 0.775
HEPTEM MCF	0.428	0.301 – 0.555	0.337	0.226 – 0.448
EXTEM CT	0.524	0.390 – 0.658	0.595	0.470 – 0.720
EXTEM MCF	0.386	0.264 – 0.509	0.358	0.233 – 0.483
FIBTEM MCF	0.324	0.194 – 0.454	0.219	0.128 – 0.309

Table 3. Receiver operating curve of TEM values for bleeding. CT = Clotting time, MCF = maximum clot firmness (MCF), INTEM = intrinsic pathway, HEPTEM = intrinsic pathway with heparinase, EXTEM = extrinsic pathway, FIBTEM = fibrinogen, AUC = area under the curve CI = confidence interval

The AUC for this risk-factor was found to be 0.69 (95% CI: 0.57 – 0.80) which was similar to AUCs found for postoperative INTEM CT and HEPTEM CT. In univariate logistic regression, postoperative INTEM CT and HEPTEM CT were not found to be significantly associated with major blood loss (table 4).

Risk factors	Odds ratio (OR)	95% CI for OR	p-value
Complex surgery	2.08	0.84 - 5.16	0.12
Age (per 1 year increase)	1.03	0.98 - 1.07	0.24
Male gender	1.55	0.58 - 4.20	0.38
BMI (per 1 unit increase)	0.91	0.81 - 1.02	0.11
Duration of CPB (per 1 hour increase)	1.26	0.80 - 1.99	0.33
Preoperative PT (INR)	0.45	0.09 - 2.29	0.33
Preoperative aPTT (s)	0.96	0.89 - 1.04	0.30
Preoperative Hb (mmol l^{-1})	1.25	0.78 - 2.00	0.35
Postoperative Hb (mmol l^{-1})	0.67	0.35 - 1.28	0.67
Protamine:Heparin ratio > 1.0	4.89	1.70 - 14.1	0.003
TEM values postoperative			
INTEM CT (per 10 s increase)	1.03	0.99 - 1.06	0.19
HEPTEM CT (per 10 s increase)	1.02	0.99 - 1.04	0.16

Table 4. Results from univariate logistic regression analyses of risk factors and most promising TEM values for prediction of major bleeding. BMI = body mass index, CPB = cardiopulmonary bypass, PT = prothrombin time, INR = international normalized ratio, aPTT = activated partial thromboplastin time, Hb = haemoglobin concentration, CT = Clotting time, INTEM = intrinsic pathway, HEPTEM = intrinsic pathway with heparinase

When separately added to protamine:heparin dosing ratio exceeding 1 in a multivariate logistic regression the odds ratios of both thromboelastometric values

became smaller (OR = 1.01, 95% CI: 0.97 – 1.06, p = 0.59 for INTEM CT and OR = 1.00, 95% CI: 0.98 – 1.03, p = 0.70). AUCs increased to 0.76 (95%: 0.61- 0.81) and 0.75 (95%: 0.64 – 0.85) when INTEM CT and HEPTEM CT were added to a model with protamine:heparin dosing ratio exceeding 1, however the increases in AUC were not statistically significant (p = 0.07 and p = 0.16).

II. Systematic review

Figure 1 shows the flowchart of the literature search. The literature search resulted in 1311 studies, of which 10 studies plus our cohort study were included in the systematic review. [21-30] This resulted in the inclusion of 1617 patients (table 5). Three studies had a possible conflict of interest; the thromboelastometry device and reagents were provided by the manufacturer to Coakly et al. and Ranucci et al.; the senior author of Lee et al. received a payment for consulting for the manufacturer of TEM. [21-23]
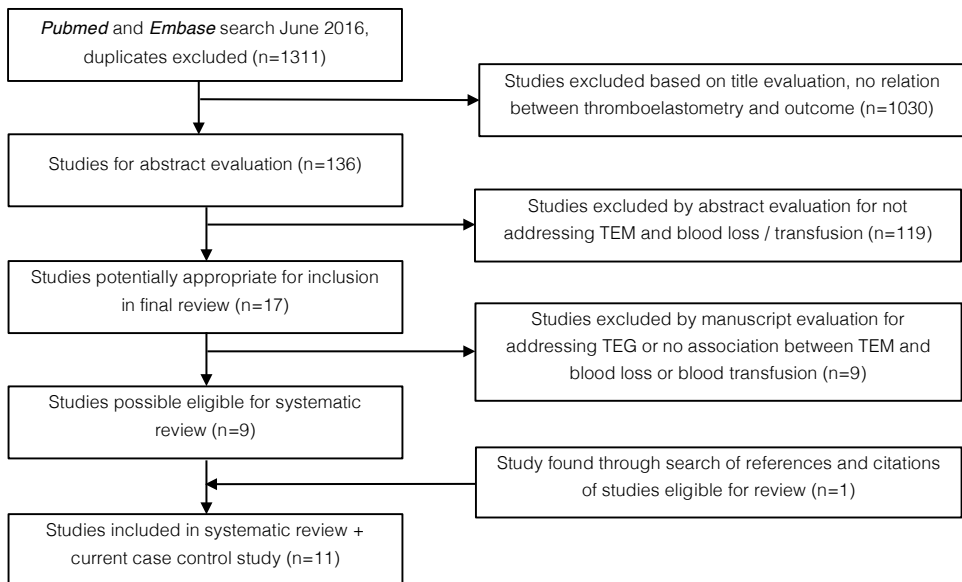

Figure 1. Flow chart of systematic review

1st Author (journal, year of publication)	Study design	n	Clinical Characteristics	Timing TEM		Outcome		TEM test				Main findings	Strengths (+) and Limitations (-)
				Before CPB	Timing after surgery	Major blood loss	Time-point blood loss measurement	INTEM	EXTEM	FIBTEM	HEPTEM		
Reinhofer[24] *Blood Coagulation Fibr, 2008*	PC	150	Elective on-pump surgery, expected CPB time > 45 min	Yes	immediately after surgery	> 600 ml OR PRBC ± (platelets OR FFP)	not stated	X	X	X	-	Postop EXTEM CFT and FIBTEM MCF showed moderate PPV (71%, 73%) with good specificity, 94%, 95%.	(+) Clinicians were blinded for TEM results, strict transfusion protocol (-) Concise analysis for the investigation of the predictive value of TEM
Davidson[25] *J Cardiothor Vasc Anesth, 2008*	PC	58	Elective on-pump CABG	Yes	1, 2 & 3 hours after surgery	> 200 m h^{-1}	'a single postoperative hour'	X	X	X	-	All pre- and postop TEM test results showed a low PPV (0-67%) and high NPV (85-100%). Highest PPV was INTEM CT in first postoperative hour.	(+) Clinicians were blinded for TEM results, multiple postoperative TEM testing time-points (-) Small sample size, antifibrinolytics were not structurally administrated. Abundant statistical analysis.
Coakley[21] *J of Thromb and Haem, 2011*	PC	44	Elective on-pump surgery	Yes	30 min	> 1000 ml	24 hours	X	-	X	X	Postoperative ROC analysis showed a poor predictability of TEM for postop bleeding, ROC AUC ranging from 0.49 – 0.63, all not significant.	(-) Small sample size, also including reoperations and ascending aortic aneurysm repair. Antifibrinolytics were only administrated when required. Hemostatic interventions might bias postop results.
Lee[23] *Anaesthesia & Analgesia, 2012*	PC	321	Elective on-pump surgery & high risk on bleeding patients	No	5 min & arrival at ICU	> 600 ml (75th %) > 910 ml (90th %)	8 hours	X	X	X	-	TEM does not add predictive value to a clinical predictive bleeding model.	(+) Clinicians were blinded for TEM results (-) Inclusion criteria changed during study. High transfusion rate of approximately 20-30%.
Petricevic[26] *J Thromb Thrombolysis, 2013*	PC	148	Elective on-pump surgery	Yes	15 min	> 12.46 ml kg^{-1} (75th %)	24 hours	-	X	X	X	Poor predictability of TEM for postoperative bleeding. Best predictor was postop FIBTEM A30 ROC AUC 0.70.	(+) Clinicians were blinded for TEM results (-) Two publications from same database with different TEM variables and different bleeding definitions. Abundant statistical analysis.
Petricevic[27] *J of Cardiothoracic surg, 2014*	RC	148	Elective on-pump surgery	Yes	15 min	> 1507 ml (75th %)	24 hours	X	-	-	-	Poor predictability of INTEM for postop bleeding. Strongest predictor was INTEM A10 ROC AUC 0.64.	

1st Author (journal, year of publication)	Study design	n	Clinical Characteristics	Timing TEM		Outcome		TEM test				Main findings	Strengths (+) and Limitations (-)
				Before CPB	Timing after surgery	Major blood loss	Time-point blood loss measurement	INTEM	EXTEM	FIBTEM	HEPTEM		
Ghavidel[28] *Asian Cardiovasc Thorac Ann, 2015*	PC	400	Elective on-pump CABG	Yes	30 min	> 200 ml h-1 OR > 1000 ml	'a single hour'	X	X	-	-	≥1 abnormal TEM value showed a high PPV (100%) and specificity (100%).	(+) Large cohort. (-) Arbitrary definition of surgical and non-surgical bleeding. High reexploration rate (≈10%). Unclear how the authors defined abnormal thromboelastometry results.
Bosch[29] *Cardiovasc and Thoracic Open, 2015*	PC	78	Elective on-pump surgery	Yes	arrival at ICU	> 1000 ml (75th %)	24 hours	X	X	X	-	ROC analysis showed a low AUC for all TEM values, ranging from 0.50 to 0.76. Best predictor was EXTEM α before surgery (ROC AUC 0.76, p=0.001).	(+) Patients receiving haemostatic therapy were excluded. (-) Small sample size. Patients with (multiple) reexploration for bleeding were excluded.
Tarzia[30] *World J of Card, 2015*	PC	100	ACS for CABG ± preoperative clopidogrel	Yes	Not performed	≥ 3 units blood transfusions	12 hours	X	X	-	-	EXTEM MCF and AUC showed good predictability of postoperative bleeding (cut-off MCF 60 mm and AUC 6000).	(+) Analysis for optimal cut off for prediction (-) Concise presentation of prediction data.
Ranucci[22] *BJA, 2016*	RC	116	Elective on pump cardiac surgery with a high bleeding risk	No	20 min before removal of aortic clamp	> 1000 ml	12 hours	-	-	X	-	ROC analysis for FIBTEM MCF showed a AUC of 0.721. Best cut off FIBTEM MCF = 14 mm, with poor PPV of 12%.	(+) Analysis for optimal cut off for prediction (-) Retrospective analysis of RCT. Exclusion of patients with excessive hemodilution.
Meesters *Current study, 2016*	RC	202	Elective on-pump surgery	Yes	3 min	> 500 ml (80th %)	6 hours	X	X	X	X	Pre- and postoperative ROC analysis showed a low AUC 0.22 – 0.68. Postoperative INTEM CT had the best predictive value.	(-) Retrospective cohort. Low blood loss. Relative long inclusion period.

Table 5. Details of studies included in systematic review. ACS = acute coronary syndrome, AUC= area under the curve, CABG = coronary artery bypass surgery, FFP = fresh frozen plasma, ICU = intensive care unit, PC = prospective cohort, PPV = positive predicting value, PRBC = paced red blood cells, RC = retrospective cohort, RCT = randomized controlled trial, ROC = receiver operating curve

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DISCUSSION

Our cohort study showed that thromboelastometric tests are poor predictors for major postoperative bleeding after cardiac surgery with cardiopulmonary bypass. These findings are in line with our systematic review of the available literature, which added 8 new studies to the previous literature review. [31]

Findings from our cohort study confirm previous reports that a lower BMI, longer CPB duration, more complex surgery and protamine excess are associated with postoperative bleeding. [32,33] The pre- and postoperative thromboelastometric positive predicting value was low with a high negative predicting value. The addition of heparinise to the intrinsic coagulation test (HEPTEM) did not improve the predictive value, further questioning the additive value of HEPTEM. [34]

The general conclusion from the systematic review is that the predictive value of thromboelastometry is insufficient to select patients at risk for major postoperative bleeding. This is in contrast with the trauma care where thromboelastometry are predict blood transfusion and mortality. [19] Our review showed insufficient predictive value in diverse study populations, i.e. with and without antiplatelet therapy, elective and emergency surgery, pre- and postoperative thromboelastometry values, with and without antifibrinolytics and all sorts of cardiac surgery procedures with a wide range of extracorporeal circulation duration.

Limitations of the review are that some studies did not state if the attending team was blinded for the thromboelastometry results. Furthermore, the definition of major blood loss and timing of thromboelastometric testing was very heterogeneous, ranging from measurement during CPB up to one hour after termination of cardiopulmonary bypass. Another limitation is the limited number of studies and lack of large trials. Also, several studies excluded a surgical cause for bleeding while in the clinical situation thromboelastometry might be used to investigate the origin of bleeding. Additionally, different statistical methods were used to investigate the predictive value of thromboelastometry. Three studies received support from the manufacturer of thromboelastometry. Yet, their results are in line with the non-supported studies suggesting there was no funding bias.

The definition of significant blood loss varied among studies, not only the cut off values and timing of blood loss measurement, also the inclusion of intraoperative chest tube drainage and blood product transfusion differed. A universal classification of postoperative blood loss remains difficult despite attempts to come to a general definition for clinically significant postoperative bleeding. [35] This difficulty is due to the heterogeneous practice among cardiothoracic centres leading to a diverse distribution of postoperative blood loss and transfusion.

[1,36,37] Additionally, there is a great influence of treatment (transfusion and reexploration for bleeding) on the amount of chest tube drainage. Also, the rapid physiological restoration of coagulation factors might influence the predictive value of thromboelastometry. In addition, the thromboelastometry reference values are wide impacting the predictive value of abnormal test results. The timing of chest tube drainage evaluation plays a supplementary role as late chest tube drainage might contain a greater part plasma and interstitial fluid than early more concentrated blood loss.

As stated, the treatment of coagulopathy might be a major confounder for the lack of predictability of bleeding by thromboelastometry. The natural course of a patient with postoperative coagulopathy would lead to a higher risk on bleeding. However, when coagulopathy is treated haemostasis will improve, weakening the predictive value of pre-intervention thromboelastometric test results. A study without haemostatic interventions would be unethical and clinically irrelevant as this does not reflect daily clinical practice.

The inability to predict postoperative bleeding might additionally be due to the discrepancy between the in vitro evaluation of coagulation and the local haemostatic conditions. Previous studies have shown that there is a high local fibrinolytic activity in the traumatized mediastinum which is not represented in a general blood sample. [38] Another bias might be that the centres investigating the predictive value of thromboelastometry have a more state of the art haemostatic management practice due to their interest in haemostasis. This would be in line with the hypothesis that not thromboelastometry but the implementation of thromboelastometry increases awareness and more knowledge about the haemostatic system leading to better outcome instead of the device itself.

Moreover, haemostasis is a stepwise process and requires functioning thrombocytes, sufficient clotting factors and fibrinogen. Thromboelastometry is able to accurately represent the latter two, however platelet function testing is suboptimal by thromboelastometry as the clot formation time (CFT), the main parameter for platelet functioning is affected by several other factors. This influence might explain the discrepancy between normal thromboelastometry values in a patient with impaired platelet functioning causing bleeding. This theory is supported by the inability of solely platelet function testing to predict postoperative bleeding (i.e. multiple electrode aggregometry, VerifyNow and PFA-100). [31]

Although postoperative fibrinogen was lower in the bleeding group the predictive value of the FIBTEM MCF was low. In the other studies fibrinogen had relatively the highest predictive value although the absolute values were poor to fair. A meta-

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analysis on the predictive value of fibrinogen levels on bleeding found a weak to moderate association between fibrinogen levels and postoperative bleeding.39 As fibrinogen is a key factor in haemostasis and easy to replenish with fibrinogen concentrate an increasing number of studies investigated the additive value of fibrinogen administration on postoperative bleeding after cardiac surgery. Preoperative fibrinogen supplementation showed controversial results although postoperative fibrinogen replenishment might reduce postoperative bleeding. [40-42] Still, the trigger for fibrinogen supplementation remains controversial. [43] Interestingly the findings of our systematic review contrast with the findings from the paediatric cardiac surgery literature where thromboelastometry has been found to predict major bleeding. [44,45] This might be explained by the smaller blood volume of children leading to more hemodilution and coagulopathy by CPB. These haemostatic disturbances as main cause of major blood loss in children would explain the discrepancy in the predictive value of thromboelastometry compared to adults. This hypothesis is supported by the fact that the body weight and duration of CPB are the best predictors for massive haemorrhage in paediatric cardiac surgery. [44-46]

The current cohort study is limited by the small sample size, retrospective data-analysis, the relative low blood loss and transfusion requirements and the inclusion of both patients at low and high risk for bleeding. Furthermore, the earliest data from the cohort study dates back to 2011. The number of patients with excessive bleeding was too small to perform multivariate logistic regression analyses with more than two predictors. Therefore, we used univariate analysis to identify the most important risk-factor (protamine:heparin dosing ratio exceeding 1) and checked to what extent thromboelastometric values were of added value to this risk factor. Furthermore, as patients demographics and thromboelastometry test results in cardiac surgery are not structurally stored in our centre only data from previous prospective observational studies in our centre was included, limiting the results.

As described above many factors complicate the predictive value of thromboelastometry. Nevertheless, the additive value of thromboelastometry might not be in predicting blood loss, thromboelastometry can be used to guide haemostatic management in a bleeding patient. [16,17] Several studies investigated if its implementation reduces bleeding, blood transfusion and improved outcome. However, the most recent systematic review shows that thromboelastometry can reduce blood transfusion and addresses the poor quality of the studies on thromboelastometry guided hemostatic treatment in cardiac surgery. [47] Most of the thromboelastometry algorithm studies have

a retrospective sequential cohort design. The implementation of a treatment algorithm with or without thromboelastometry creates awareness and might bias the results. This hypothesis is substantiated by the finding that studies implementing a general (non-thromboelastometry) blood management protocol showed improved outcome after implementation. [48]

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Monitoring coagulopathy

part III

Chapter 6

The value of the thromboelastometry heparinase assay (HEPTEM) in cardiac surgery

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ABSTRACT

Introduction: The thromboelastometry INTEM clotting time (CT) with heparinase (HEPTEM) is frequently used to detect residual heparin after cardiopulmonary bypass (CPB) in cardiac surgery. This study investigated whether the HEPTEM CT reflects the presence of residual heparin and the association of the protamine-to-heparin ratio to the INTEM and HEPTEM CT.

Methods: We retrospectively evaluated thromboelastometry data that were obtained before CPB and after protamine infusion following CPB in two tertiary hospitals. The number of patients with an INTEM:HEPTEM ratio (IH-ratio) > 1, suggesting residual heparin, were quantified. Moreover, the influence of different protamine-to-heparin-dosing-ratios (P:H) on the INTEM and HEPTEM CT was evaluated in the clinical setting and in blood drawn from healthy volunteers.

Results: An INTEM:HEPTEM CT ratio > 1.1 was observed in 16% of the patients prior to CPB, and in 15% after protamine administration. Interestingly, 23% and 36% of the patients had an HEPTEM CT exceeding the INTEM CT before CPB and following protamine administration. The HEPTEM CT was longer than the INTEM CT in patients with a P:H-ratio of 1:1 (265 ± 132 vs. 260 ± 246 s; P=0.002) or P:H-ratio of 1.3:1 (357 ± 174 vs. 292 ± 95 s; P=0.001). Increasing P:H-ratios induced a prolonged HEPTEM CT in fresh blood.

Conclusion: Limited agreement was observed between INTEM and HEPTEM clotting time in the absence of heparin. INTEM comparison to HEPTEM may not always reliably reflect the presence of residual heparin, while protamine may additionally affect the latter test. These observations complicate HEPTEM results interpretation in clinical situations with suspected residual heparin effect after protamine.

INTRODUCTION

The heparinase thromboelastometric test (HEPTEM) enables the detection of heparin in whole blood, and is increasingly used to identify residual heparin after protamine reversal following cardiopulmonary bypass (CPB) in cardiac surgery. [1] The HEPTEM test consists of the INTEM test in the presence of heparinase, a heparin-cleaving enzyme. Heparin prolongs the INTEM assay clotting time (CT) compared to the CT of the heparinase assay. In the absence of heparin, it is assumed that the INTEM and HEPTEM test produce a similar CT. Controversially, in several studies the HEPTEM CT appears to be longer than the INTEM CT following CPB. [2-4]

The antagonist of heparin, protamine, is administrated at the end of CPB and reverses the anticoagulant effects of heparin by the formation of a neutral protamine-heparin complex. [5] Yet, in vitro studies show that protamine itself has dose-dependent anticoagulant properties, with inhibitory effects on the intrinsic coagulation pathway through suppression of thrombin and factor V activation, enhancement of fibrinolysis and inhibition of platelet function. [1,6-13] Besides one small study, it is not known whether protamine by itself affects the HEPTEM CT in the clinical situation. [14]

As several clinical studies showed that the HEPTEM CT is frequently longer than the INTEM CT following CPB, we assessed the occurrence of a prolonged HEPTEM CT following CPB. In particular, if the HEPTEM CT does not only reflect the presence of heparin in whole blood, its value for coagulation monitoring during CPB might need further exploration. Furthermore, we determined the effect of different protamine-to-heparin ratios on the HEPTEM CT in blood from healthy volunteers in order to gain insight in the influence of protamine dosing on the HEPTEM test results.

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METHODS

I. Patient study

Patients

The retrospective study investigated thromboelastometric data of patients undergoing cardiothoracic surgery with cardiopulmonary bypass in VU University Medical Centre (Amsterdam, the Netherlands) and Maastricht University Medical Centre (Maastricht, the Netherlands). Thromboelastometry was part of routine clinical monitoring. The local Human Subjects Committee of both centres approved this retrospective analysis (METc 2012/438) and waived informed consent.

Surgical procedures included valve procedures, coronary artery bypass graft (CABG) surgery, and a combination of valve with CABG surgery. Exclusion criteria were missing data with regard to post-protamine thromboelastometry, aorta surgery, and emergency operations. Preoperative anticoagulant medication data was only available in the VUmc Medical Centre.

Anaesthesia and cardiopulmonary bypass

Patients were anesthetized according to local protocols based on a combination of sufentanil (3-7 µg kg^{-1}), pancuronium bromide (0.1 mg kg^{-1}) or rocuronium (0.5 mg kg^{-1}) and midazolam (0.1 mg kg^{-1}) for anaesthesia induction, and propofol (200-400 mcg h^{-1}) for anaesthesia maintenance. All patients received tranexamic acid.

Cardiopulmonary bypass was performed at a continuous blood flow (2.2-3.0 l min^{-1} m^{-2}) during mild hypothermia (> 34°C) using an S5 heart-lung machine (Stöckert Instrumente GMBH, Munich, Germany) with a centrifugal pump (Delphin, Terumo Europe NV, Leuven, Belgium) and heparin-coated circuit (Medtronic, Minneapolis, MN, USA) as described before. [15]

Heparinisation started with an initial bolus of 300 IU kg^{-1} heparin (LEO Pharma BV, Amsterdam, the Netherlands), which was replenished until the Celite activated clotting time (ACT) test exceeded 480 seconds (Hemochron® Response, Edison, NJ, USA). The priming solution (1400 ml) contained 5000 IU porcine heparin (LEO Pharma BV, Amsterdam, the Netherlands). After cardiopulmonary bypass, heparin was reversed with protamine hydrochloride (Meda Pharma BV, Amstelveen, the Netherlands). The primary dosing of protamine was based on local guidelines, which advises approximately 1 mg protamine for every 100 IU of heparin administered throughout the procedure.

Perioperative haemostasis monitoring

Haemostasis was routinely monitored after anaesthesia induction and 3-10 minutes after protamine administration using standard haemostatic testing including the activated partial thromboplastin time (aPTT), prothrombin time (PT), platelet count, haemoglobin concentration (Hb), and activated clotting time (ACT). The aPTT and PT were determined using calcium thromboplastin and cefaline/microcrystalline assays, respectively, in a STA-R instrument (Roche Diagnostics GmbH, Basel, Switzerland). PT was reported as the international normalized ratio (INR). Haemoglobin and platelet count were determined using a Cell-Dyn Sapphire® (Abbott Laboratories, Illinois, USA).

Thromboelastometry

Post-protamine thromboelastometry was routinely performed in both centres. In the VU University Medical Centre additional thromboelastometry was performed after induction of anaesthesia. Citrated blood samples were analysed using thromboelastometry (ROTEM®, TEM International, Munich, Germany).

Whole blood samples were recalcified with 20 microliter of calcium chloride. Subsequently, the following tests were performed in the patient study: INTEM (intrinsic coagulation pathway, activation by ellagic acid), HEPTEM (INTEM test in the presence of heparinase), EXTEM (extrinsic coagulation pathway, activation by rabbit brain tissue factor, the reagent supplied by the manufacturer at the time of the study period) and FIBTEM (fibrin part of clot formation, EXTEM assay plus cytochalasin D for platelet inhibition). Thromboelastometry parameters included the clotting time in seconds (CT) and maximum clot firmness in mm (MCF). In the in vitro study solely HEPTEM testing was performed. Reference values for clotting times for INTEM/HEPTEM and EXTEM are 100-240 s and 38-79 s, respectively. Reference values for the maximum clot firmness are 50-72 mm for both INTEM/HEPTEM and EXTEM and 9-25 mm for FIBTEM. [16]

Other study variables

Study parameters included patient demographics, surgical characteristics, standard coagulation and the preoperative and postoperative thromboelastometry values (INTEM, HEPTEM, EXTEM and FIBTEM). Demographic variables included age, gender, body mass index (BMI). Surgical characteristics were type of surgery, aorta cross clamp time, CPB time and lowest temperature. The INTEM:HEPTEM-ratio (IH-ratio) was calculated by dividing the INTEM by the HEPTEM clotting time. According to the manufacturer an IH-ratio > 1 indicates a heparin effect. The protamine-to-heparin ratio (PH-ratio) was calculated by dividing the initial

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protamine sulphate (IU) administration by the total heparin administration (IU). Patients were divided into low protamine dosing (PH-ratio < 0.8), normal dosing (PH-ratio of 0.8-1.0) and high protamine dosing group (PH-ratio > 1.0). A deviation of ± 0.1 was chosen to accept 10% inaccuracy in thromboelastometric tests.

II. In vitro study
Influence of protamine-to-heparin ratio on HEPTEM CT

The study in blood of healthy volunteers was approved by the Human Subjects Committee of VU University Medical Centre, Amsterdam, the Netherlands (NL43303.029.13). Written informed consent was obtained from each participant before blood sampling. Blood samples were collected from 13 adult subjects aging between 18 and 40 years and analysed using thromboelastometry. Exclusion criteria were the use of anticoagulant medication, known haemostatic deficiencies and pregnancy. Blood was sampled from a single venous puncture through a vacutainer system using 0.105 M sodium citrate-containing tubes, resulting in a final concentration of 3.2% of citrate. Heparin was added to the untreated blood samples to retrieve 4.0 IU of heparin per millilitre, which was the highest concentration used in the study of Mittermayr that was associated with clot formation in the INTEM test. [1] Higher concentrations of heparin completely blocked clot formation. [1] Protamine was added to obtain the following concentrations: 3.2, 4.0 and 5.6 IU ml^{-1}. A control sample was equally diluted with saline without heparin or protamine. Overall, the HEPTEM CT and MCF was determined for the following P:H ratios: 0:0 (control), 0.8:1.0, 1.0:1.0, 1.3:1.0.

Data analysis

Statistical analyses were performed using the SPSS statistical software package 17.0 (SPSS Inc.®, Chicago USA). All data were tested for normal distribution prior to statistical analysis. Demographic or coagulation measurement values are represented by mean ± standard deviation (SD), median with interquartile range or frequencies. Figures represent boxplots of ordinal variables or mean ± standard error of the mean. The difference between the distinct P:H ratios was tested using a two-way ANOVA (mean ± SD) and a Friedman test for related samples (median with interquartile range). Bland-Altman analysis was performed using GraphPad Prism 5.0 (La Jolla, CA, USA) and provided the bias, standard deviation of the bias, limits of agreement (LoA) and percentage error. Pearson correlation analysis was performed to assess the association between the postoperative INTEM:HEPTEM ratio and protamine-to-heparin dosing ratio. $P<0.05$ was considered to be statistical significant.

RESULTS

I. Patient study

Patient demographics

The study included 159 males (66%) and 81 females (34%). Eight patients received preoperative heparin, resulting in an aPTT of 46 ± 8 s in this subgroup prior to surgery, and excluding these patients did not alter the study results. Furthermore, 71 patients used preoperative acetylsalicylic acid, 1 clopidogrel, 2 acenocoumerol and 9 low weight molecular heparin. Table 1 shows the patient and surgical characteristics. Forty-four patients underwent CABG surgery (18%), 97 patients valve surgery (41%), 73 patients a combination of CABG and valve surgery (31%), 20 patients Bentall surgery (8%) and 4 patients a combination of Bentall and CABG surgery (2%). The mean preoperative ACT was 138 ± 28 s, and 607 ± 225 s after heparinisation. In table 2 the standard coagulation, blood and thromboelastometry test results before and after cardiopulmonary bypass are shown.

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	N	Value
Age (years)	240	66 ± 12
BMI (kg m^{-2})	240	27 ± 4
ECC time (min)	240	144 ± 64
Aortic clamping time (min)	240	103 ± 47
Lowest temperature during ECC ($^{\circ}$C)	233	34 ± 2
Cell saver blood transfusion (ml)	189	495 ± 408
Total heparin administered (IU)	239	436 ± 164
Protamine before thromboelastometry (mg)	201	448 ± 122
Total protamine administered (mg)	239	469 ± 172

Table 1. Patient and surgical characteristics BMI = body mass index; ECC = extracorporeal circulation time. Data represent mean ± standard deviation.

Distribution of the INTEM-HEPTEM clotting time ratio

Figure 1 shows the distribution of the INTEM:HEPTEM (IH) clotting time (CT) ratio before CPB (upper panel) and 3-10 minutes after protamine (lower panel). We observed an IH-ratio exceeding 1.1, which is suggestive for remaining heparin activity, in 16% of the patients following anaesthesia induction and in 15% of the patients after protamine administration. Interestingly, 23% and 36% of the patients had a HEPTEM CT exceeding the INTEM CT before CPB and following protamine administration, respectively. Bland-Altman analysis for the level of agreement between the preoperative INTEM and HEPEM clotting time revealed a bias of -4 ± 37 seconds, with limits of agreement from -76 to 67 seconds and a percentage error of 52.2% (Figure 2).
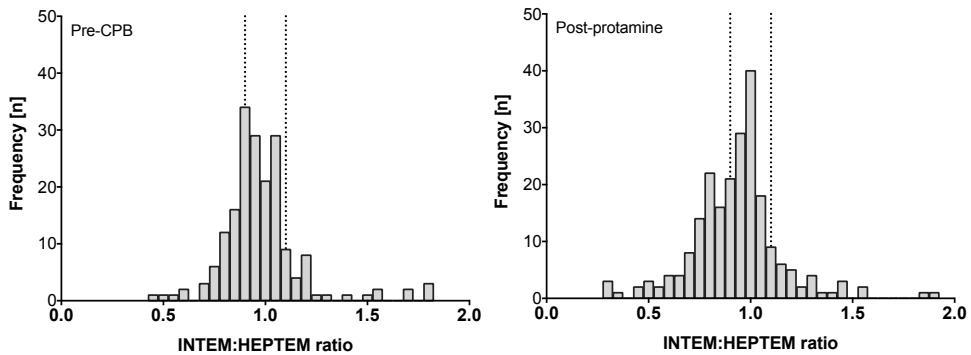
Figure 1. Histograms of the INTEM to HEPTEM clotting time ratio after anaesthesia induction (Pre-CPB, upper panel) and 3-10 minutes after protamine infusion (Post-protamine, lower panel). The dotted lines show 10% deviation around an INTEM:HEPTEM ratio of 1.0 (0.9-1.1).

	N	Before CPB	N	After protamine
Standard coagulation tests				
PT (INR)	202	1.16 ± 0.39	222	1.76 ± 0.43
aPTT (s)	204	37 ± 8	204	37 ± 8
Platelets (*10^9 L^{-1})	236	240 ± 62	237	114 ± 38
Haemoglobin (mmol L^{-1})	239	8.2 ± 1.0	237	6.0 ± 1.0
Thromboelastometry				
HEPTEM CT (s)	195	175 ± 42	228	282 ± 143
HEPTEM MCF (mm)	196	63 ± 5	225	51 ± 7
INTEM CT (s)	199	170 ± 31	229	262 ± 196
INTEM MCF (mm)	200	63 ± 5	229	51 ± 8
EXTEM CT (s)	197	69 ± 26	223	97 ± 31
EXTEM MCF (mm)	199	63 ± 5	214	54 ± 6
FIBTEM MCF (mm)	197	18 ± 5	231	10 ± 4

Table 2. Haemostatic parameters before cardiopulmonary bypass (CPB) and after protamine administration. PT = prothrombin time (PT); INR = International Normal Ratio (INR); aPTT = activated Partial Prothrombin Time; CT = clotting time; MCF = maximum clot firmness. Data represent mean ± standard deviation.

Association of the protamine-to-heparin ratio with the clotting time in patients

Figure 3 shows the relationship between different protamine-to-heparin ratios and the INTEM and HEPTEM clotting time. The distribution of distinct protamine-to-heparin dosing ratios in our population was 16.9% for a ratio < 0.8, 59.5% for a ratio between 0.8 and 1.0 and 23.6% for a ratio exceeding 1.0.

While an increased protamine-to-heparin ratio is suggestive for a reduction in residual heparin, the INTEM and HEPTEM clotting time increased. In particular, the HEPTEM clotting time was higher than the INTEM clotting time at protamine-to-heparin ratios of 0.81-1.00 (P=0.002) and a ratio exceeding 1.0 (P=0.001). The correlation between the post-CPB INTEM:HEPTEM ratio and the protamine-to-heparin dosing ratio was weak (r=-0.18; P=0.007).
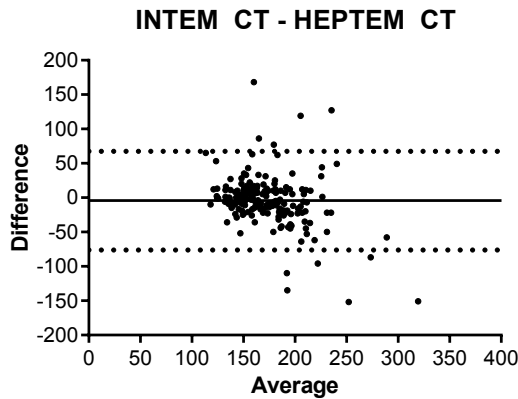

INTEM CT - HEPTEM CT

Figure 2. Level of agreement between the preoperative INTEM and HEPTEM clotting times.
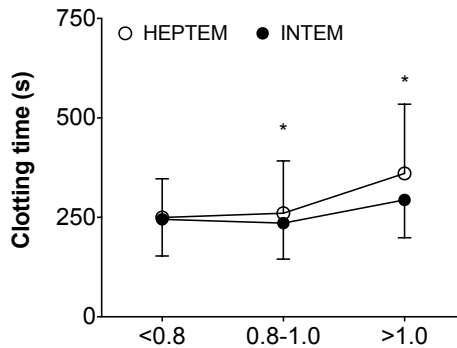
Figure 3. Mean HEPTEM and INTEM clotting time for a protamine-to-heparin dosing ratio < 0.8, 0.8 – 1.0 and > 1.0. Data represent mean ± standard deviation. * HEPTEM clotting time (CT) versus INTEM CT (P<0.05).

II. In vitro study

Influence of protamine-to-heparin ratio on HEPTEM CT

The study in blood from healthy volunteers included 6 males and 7 females with a mean age of 27 ± 4 years. Table 3 shows the relationship between subsequent protamine-to-heparin dosing ratios and HEPTEM test results. The HEPTEM clotting time was affected by an increase in the protamine-to-heparin dosing ratio, whereas the maximum clot firmness (MCF) was unaffected by a relative increase in protamine dosing. Interestingly, HEPTEM clotting time was 201 ± 17 for the control sample (0:0 ratio) whereas 273 ± 36 seconds for the 1:1 ratio (P<0.001).

	N	0:0	0.8:1.0	1.0:1.0	1.3:1.0	P-value
HEPTEM CT (s)	13	201 ± 17	244 ± 29	273 ± 36	374 ± 96	<0.001
HEPTEM MCF (mm)	13	54 ± 4	55 ± 4	54 ± 4	52 ± 5	n.s.

Table 3. In vitro relation between the protamine-to-heparin dosing ratio and the HEPTEM test. The protamine-to-heparin dosing ratio is calculated by dividing the amount of protamine (mg) by heparin (*100 IU). Clotting time (CT) and maximum clot firmness (MCF) for the intrinsic pathway with heparinase (HEPTEM). Data represent mean ± standard deviation (SD) or median with interquartile range (IQR).

DISCUSSION

Heparin anticoagulation during cardiac surgery is neutralised by protamine to normalise coagulation. The thromboelastometry HEPTEM test is frequently used to assess whether there is remaining heparin activity following protamine neutralisation following cardiopulmonary bypass (CPB). In particular, the INTEM and HEPTEM clotting time (CT) assess the intrinsic coagulation in the absence or presence of heparinase, respectively. Theoretically, the INTEM and HEPTEM CT should have a high level of agreement in the absence of heparin, while the INTEM CT will exceed the HEPTEM CT in the presence of remaining heparin activity.

The present study however shows that there is a wide diversion between the INTEM and HEPTEM CT before initiation of CPB, with a percentage error exceeding 30%. Moreover, in one quarter of the population we observed a HEPTEM CT that was longer than the INTEM CT before surgery. After heparin neutralisation by protamine, the HEPTEM CT also frequently exceeded the INTEM CT. Finally, in our patient data and *in vitro* study, we show that in case of higher protamine-to-heparin dosing ratios, both INTEM and HEPTEM CT values increase, with the most prevalent increase in the HEPTEM CT in our patient cohort. These findings suggest that protamine dosing might additionally influence the HEPTEM CT.

We are the first to describe these findings, but they are in line with several studies in cardiac surgery, showing that the mean INTEM CT is shorter than the mean HEPTEM CT. [2-4] Although one may assume that the observed diversion between INTEM and HEPTEM clotting times can be caused by local inadequate thromboelastometric testing, we could reproduce our findings in two tertiary hospitals.

Two points should be kept in mind with regard to our results. First, our findings need to be considered in light of a natural intrinsic variation between the test results due to the method of thromboelastometry. Bland-Altman analysis is not designed to investigate the level of agreement between tests with different purpose, but the large percentage error underlines the wide variation in preoperative INTEM and HEPTEM CT values. A recent study by Nagler et al. also showed large differences in the INTEM CT when assessed in parallel by two distinct ROTEM devices or channels, [17] while Theusinger et al. reported a coefficient of variation of 15% when the INTEM CT was repeated. [18] This intrinsic test variation might in part explain the differences in the preoperative INTEM and HEPTEM CT, although the observed variation between the HEPTEM and INTEM tests exceeds the intrinsic test variation of the ROTEM device. Secondly, there is a difference between measured residual heparin activity and the observation of a bleeding tendency that might be caused by heparin. The aberrant HEPTEM

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results might have no clinical impact, as this was beyond the scope of our study. The use of thromboelastometric test results should therefore always be placed in a clinical perspective, and isolated deviated test results should not lead to clinical decisions in a non-bleeding patient as therapy guidance that is solely based on test results might lead to inaccurate clinical decision-making. When bleeding is suspected to be caused by residual heparin it is advised to only administrate protamine in case of an activated clotting time (ACT) > 130 seconds *and* INTEM CT > 240 seconds *and* HEPTEM CT / INTEM CT < 0.8 as proposed by Weber et al. [19] Further studies should evaluate if the aberrant HEPTEM results lead to clinical consequences.

The association between heparin dosing and the HEPTEM CT has been clearly addressed by others. Mittmayer and colleagues showed *in vitro* that heparin titration prolongs the INTEM CT, whereas the HEPTEM CT remained unaffected. [1] Ichikawa and colleagues found a good correlation between the heparin concentration and ratio between the INTEM and HEPTEM CT in a clinical cohort at two hours following cardiac surgery. [20] Moreover, a recent clinical investigation confirmed this finding and showed that the HEPTEM CT is useful for the evaluation of haemostasis prior to protamine administration in the setting of high heparin concentrations. [21]

In contrast, the influence of protamine (over)dosing on the INTEM and HEPTEM CT is still under debate. In a small clinical study, protamine was overdosed in 13 patients, and a post-hoc anti-Xa assay showed no heparin activity after the first protamine dosing. However, both the INTEM and HEPTEM CT prolonged after additional protamine, while the HEPTEM remained shorter than INTEM. [14] We also showed recently that protamine dosing following CPB was moderately associated with the HEPTEM CT. [22] This is in line with the *in vitro* findings presented in the current study, and observations by others, albeit they showed that both the INTEM and HEPTEM CT were prolonged by protamine overdosing without affecting the IH-ratio. [1,13]

The manufacturer of the thromboelastometry device (TEM International™) nowadays proposes that HEPTEM clotting time values might exceed INTEM clotting times due to protamine overdosing. [22] Due to the limited number of clinical studies focusing on the influence of protamine dosing on the INTEM and HEPTEM CT, no conclusion can be drawn regarding the usefulness of the HEPTEM CT in the patient setting in the context of protamine dosing strategies.

Theoretically, heparinase degrades heparin bound to the heparin-protamine complex, resulting in free protamine. As protamine has dose-dependent anticoagulant properties when it is unbound to heparin, free protamine might

cause a more prolonged HEPTEM CT when compared to INTEM clotting time. To investigate this hypothesis we conducted an *in vitro* experiment where the control sample without heparin or protamine was equally diluted as the 1:1 protamine-to-heparin dosing ratio sample. We expected to find that the control sample and the 1:1 protamine-to-heparin dosing sample would reveal comparable HEPTEM clotting times, as heparin was in theory completely neutralised by protamine. Yet, we found a prolongation of 72 seconds of the HEPTEM CT in the 1:1 protamine-to-heparin dosing ratio group when compared to the control sample. This could confirm our hypothesis that heparinase frees protamine, which in turn exerts an anticoagulant effect. Unfortunately, our *in vitro* data are limited by the omission of an INTEM CT test, which makes it impossible to show whether the INTEM:HEPTEM CT ratio is affected by high protamine dosing. [1] However, as our clinical findings suggest that the HEPTEM CT is more affected by a higher protamine-to-heparin dosing ratio, further clinical studies should be executed to further elaborate our hypothesis.

The present patient study has all limitations of a retrospective study. The study is observational by nature, and protamine dosing was based on the discretion of the attending anaesthesiologist based on a 1:1 protamine-to-heparin ratio protocol. However, the observations presented in the current study originate from two independent hospitals. Moreover, our experiments in blood of healthy volunteers suggest that heparin bound to protamine is degraded by heparinase (HEPTEM), and the available free protamine subsequently prolongs the clotting time. Protamine (over)dosing also prolonged the HEPTEM CT, resulting in aberrant results. Interestingly, studies evaluating HEPTEM in the clinical situation are scarce and we are the first to evaluate HEPTEM in a large patient cohort. As our observations are not in line with previous studies, further clinical exploration of the value of the thromboelastometry HEPTEM assay in the clinical setting where heparin and protamine are used is warranted.

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Chapter 7

Instability of the non-activated rotational thromboelastometry assay (NATEM) in citrate stored blood

Michael I. Meesters, Alexander Koch, Gerard Kuiper,
Kai Zacharowski, Christa Boer

ABSTRACT

Introduction: The non-activated rotational thromboelastometric assay (NATEM) is increasingly used as sensitive test for the evaluation of the endogenous activation of haemostasis. The reproducibility of the test results in citrate stored blood has never been investigated.

Methods: The NATEM assay was performed in citrated blood samples stored for 0, 45 and 90 minutes using ROTEM® (TEM International, Munich, Germany). Blood samples were drawn from healthy volunteers and a population of patients admitted to the intensive care (ICU). In 10 ICU patients, citrate concentrations were measured at baseline and after 90 minutes of storage.

Results: The NATEM clotting time shortened in stored citrated blood from healthy volunteers (t=0 1226±160; t=45 986±171; t=90 903±177; p<0.001) and ICU patients (t=0 986±318; t=90 750±187; p<0.001). A similar decrease in clot formation time (CFT) was seen whereas the MCF remained unaffected. Citrate concentration did not change over time, baseline 13.3 ± 0.5 mmol l^{-1}; after 90 minutes 13.2 ± 0.7 mmol l^{-1}; n.s..

Conclusions: The non-activated rotational thromboelastometric assay test results change over time in citrate stored blood. The NATEM test should be initiated at a standardised time point, in order to prevent bias by different test initiation times,

INTRODUCTION

The non-activated rotational thromboelastometric assay (NATEM) is increasingly used as sensitive test for the evaluation of the endogenous activation of haemostasis. [1-6] The test solely recalcifies citrated blood prior to analysis, without additional coagulation activators. The reproducibility of the test results in citrate stored blood has however never been investigated.

Previous studies showed a low reproducibility of thromboelastography (TEG) tests in recalcified citrate stored blood, in particular shortening of the reaction time (r) in longer preserved blood samples. [7-12] These studies suggested that the citrated blood sample is activated during storage, thereby influencing the TEG results.

In order to gain more insight in a possible bias due to a delayed start of the NATEM test in citrate stored blood we performed a study to evaluate the influence of storage time on the test results in blood samples drawn from healthy volunteers. Furthermore, a population of patients admitted to the intensive care (ICU) was investigated in order to see if the results were equal and relevant in the clinical setting.

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METHODS

Study population

Blood sample drawing from healthy volunteers was approved by the Human Subjects Committee of VU University Medical Centre, Amsterdam, the Netherlands (NL43303.029.13). Written informed consent was obtained from each participant prior to blood sampling. Exclusion criteria were the use of anticoagulant medication, known haemostatic deficiencies and pregnancy.

The clinical patient study included patients admitted to the surgical intensive care unit (ICU) with two or more SIRS criteria. Exclusion criteria were anticoagulant therapy in the week preceding ICU admission, haematological disease, chronic liver or kidney failure, coagulopathy, increased thrombotic risk, use of an Extracorporeal Membranous Oxygenator (ECMO), administration of argatroban or protein C. The study was approved by the institutional ethics committee of the Klinikum und Fachbereich Medizin Goethe-Universität, Frankfurt am Main, Germany (75/11). Informed consent was obtained from patients or their relatives.

Study procedures

Blood from healthy volunteers was sampled from a single venous puncture through a vacutainer system using a tourniquet in healthy volunteers, or a flushed non-heparinized intra-arterial line in ICU patients. These methods were chosen to fully reflect the clinical situation. Blood was collected in 0.105 M sodium citrate-containing tubes, resulting in a final citrate concentration of 3.2%. Within 10 minutes after blood withdrawal, a rotational thromboelastometry NATEM test was performed (ROTEM®; TEM International, Munich, Germany). Blood samples were recalcified with 20 µl 0.2 M $CaCl_2$ (Star-TEM reagent, ROTEM®; TEM International, Munich, Germany) prior to analysis. Several ICU patients received anticoagulation with heparin, therefore blood samples in the clinical population were recalcified with 20 µl 0.2 M $CaCl_2$ containing additional heparinase (Hep-TEM reagent, ROTEM®; TEM International, Munich, Germany) in order to exclude a possible heparin effect. The heparinase assay might affect fibrinolysis [13], yet no reports indicate an effect on clotting time (CT), clot formation time (CFT) or maximum clot formation (MCF). The NATEM variables clotting time, clot formation time and maximum clot firmness were measured in blood stored for 0, 45 and 90 minutes (healthy volunteers) or 0 and 90 minutes (ICU patients). Other study parameters included demographic data: age, gender and reason for admission to the ICU.

Statistical analysis

Statistical analysis was performed using SPSS 22.0 Software (IBM, New York, USA). Continuous data are represented as mean ± SD. Data were analysed by a one-way ANOVA (healthy volunteers) or a Student's t-test (ICU). Statistical significance was achieved if the P-value was <0.05.

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RESULTS

Blood samples were drawn from 14 volunteers and 30 ICU patients. Healthy volunteers aged 25 ± 3 years and 50% of the volunteers were male. In ICU patients, the mean age was 66 ± 15 years and 23% was male. The reasons for ICU admission were cardiovascular (n=16), abdominal (n=5), trauma (n=5), neuro (n=2), urology (n=1), lung (n=1), or ENT surgery (n=1).

Figure 1 shows the NATEM clotting time (CT), the clot formation time (CFT) and the maximum clot firmness (MCF) measured after 0, 45 and 90 minutes of storage for both groups. The CT and CFT in healthy volunteers and ICU patients decreased with storage time (panels A and B, respectively), while the MCF was unaffected by storage time (panel C).

In 10 ICU patients, citrate concentrations were measured at baseline and after 90 minutes of storage. There were no differences in free citrate concentrations at baseline (13.3 ± 0.5 mmol l^{-1}) or after 90 minutes of storage (13.2 ± 0.7 mmol l^{-1}; n.s.).
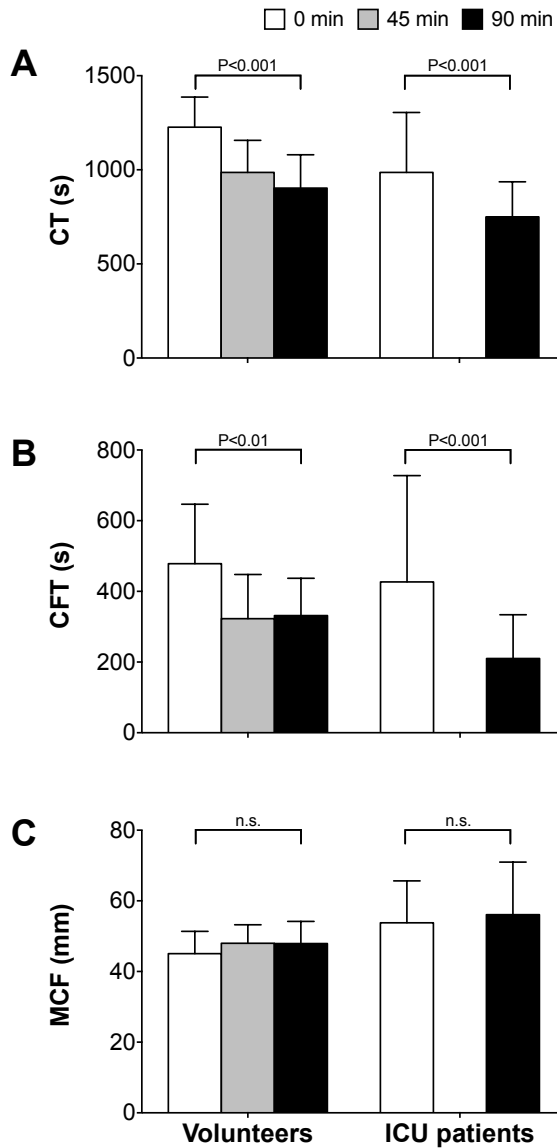
Figure 1. Rotational thromboelastometry clotting time (CT; panel A), clot formation time (CFT; panel B) and maximum clot firmness (MCF; panel C) for healthy volunteers and intensive care unit (ICU) patients in citrated blood (T=0 minutes) and after 45 and 90 minutes of blood sample storage. Data represent mean ± standard deviation. P-values are indicated in the figure. n.s. = not significant.

DISCUSSION

The NATEM clotting time and clot formation time shorten when citrated blood from healthy volunteers or patients is stored, while the MCF remains unaffected. These results are in line with findings observed using thromboelastography. It was primarily hypothesized that degradation of citrate by the Krebs-cycle in erythrocytes and thrombocytes increases the free calcium concentration, thereby reactivating the coagulation pathway. [14,15] However, in concordance with Camenzin et al. we found no changes in free citrate during blood sample storage. [7] Theoretically, the influence of storage time on NATEM parameters could also be explained by the initiation of the intrinsic coagulation cascade through contact of coagulation factor XII by kaolin or glass. Factor XIIa activates prekallikrein, which in turn is capable of activating factor XII, leading to a positive feedback loop. Factor XIIa activates factor XI that in turn will activate factor IX in the presence of calcium. [16] Over time, factor XIa and XIIa could accumulate, leading to activation of the downstream coagulation-cascade when the blood sample is recalcified, leading to shortening of the clotting time. Corn trypsin inhibitor (CTI) is a direct inhibitor of factor XIIa, and was added to blood samples of healthy volunteers to evaluate this hypothesis. [17,18] As expected CTI delayed clot formation. However there was no dependency on the time course seen so we could therefore conclude that FXIIa pathway activation seems not to play a role in the activation of the NATEM samples over time (data not shown). A third explanation may be a time-dependent accumulation of microparticles that are released from cells during activation. In particular, platelet derived vesicles are highly procoagulant, and it has previously shown that the concentration of microparticles increases during storage of blood. [19,20]

A limitation of the present study is the inclusion of two different subject cohorts that only limitedly reflect the clinical setting. We chose for a study in a healthy population of volunteers with normal thrombosis and haemostasis profile as first requirement to proof that the NATEM test results depend on the blood storage time. We further decided to choose a specific clinical population that is known for a procoagulant profile, in this case patients with SIRS. It is warranted that our findings should be further confirmed in a more heterogeneous clinical population. Thromboelastometric tests that require the addition of an activator to citrated blood samples, like the INTEM, EXTEM and FIBTEM tests, can be stored op to 2 hours. [21,22] It is thought that the strong activation of haemostasis by the addition of ellagic acid or tissue factor compensates for the endogenous change in coagulation potency in stored blood samples. Hence, our findings indicate that the NATEM test should be initiated at a standardised time point, preferably in

freshly drawn citrated blood in order to prevent bias by different test initiation times. In conclusion, we feel that initiation of the NATEM test directly after withdrawal of citrated blood will give the most realistic representation of the haemostatic status of the patients.

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Chapter 8

Level of agreement between laboratory and point-of-care prothrombin time before and after cardiopulmonary bypass in cardiac surgery

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ABSTRACT

Background: Hemostasis monitoring in cardiac surgery could benefit from an easy to use and fast point-of-care coagulation monitor, since routine laboratory tests have a delay of 30-45 minutes. This study investigated the level of agreement between the point-of-care prothrombin time (PT) with central laboratory PT before and after cardiopulmonary bypass.

Methods: Bland Altman and error grid analysis were used to analyze the agreement between the point-of-care Coaguchek XS Pro device (POC-PT) and the central laboratory prothrombin time (LAB-PT) before cardiopulmonary bypass (CPB) and 3 minutes after protamine administration. Prothrombin times were expressed in international normalized ratios (INR).

Results: The average POC-PT and LAB-PT values of 73 patients were 1.06 ± 0.14 and 1.09 ± 0.13 (P=0.10) before CPB. POC-PT measurements before CPB showed a good agreement with the LAB-PT, with a bias of -0.02 ± 0.07 INR and 94% of the values being represented in the clinical acceptable zone of error grid analysis. The mean POC-PT 3 minutes after protamine administration was significantly lower than the LAB-PT (1.35 ± 0.12 vs. 1.70 ± 0.18; P<0.001). The PT at 3 minutes after protamine administration showed a bias of 0.36 ± 0.14, and 82% of the values were located outside of the clinical acceptable zone in the error grid analysis.

Conclusions: Point-of-care prothrombin time testing was in concordance with conventional laboratory PT prior to cardiopulmonary bypass. At 3 minutes following protamine administration, PT values of the point-of-care device were structurally lower than the laboratory PT values, leading to a disagreement between both tests at that time point.

INTRODUCTION

Point-of-care prothrombin time (PT) monitoring is widely used in patients with vitamin-K-antagonist therapy. [1,2] Recently, point-of-care (POC) coagulation test devices have additionally been validated for intraoperative application, as their use may reduce the time between blood sampling and test results from 30-45 to 3 minutes. [3-5] This time reduction may be of particular value in the diagnostic support of perioperative plasma transfusion. [6] Most validation studies were however performed in non-cardiac surgery, while cardiac surgery is associated with the relatively highest number of blood product transfusion and subsequent risk of transfusion-induced-morbidity. [7-9] A complicating factor for coagulation monitoring in cardiac surgery is that patients are exposed to heparin, protamine and extracorporeal circulation, which may influence the coagulation test results.

Although the comparison of the point-of-care prothrombin time with laboratory testing in cardiac surgery has already described by others, [10,11] these studies did not evaluate the device shortly after cardiopulmonary bypass, whereas prompt evaluation could lead to improved hemostatic monitoring and intervention. Additionally, these studies did not specifically focus on the level of agreement or limited their evaluation to correlation analysis. Moreover, an advised evaluation of the clinical relevance of differences between point-of-care and central laboratory measurements was not included in these publications. [12]

In the present study we therefore investigated the level of agreement between the point-of-care Coaguchek XS Pro device (POC-PT) for whole blood analysis with the central laboratory prothrombin time (LAB-PT) in platelet free plasma before cardiopulmonary bypass and 3 minutes after protamine administration. Additionally, we evaluated the relevance of the observed differences between both methods of prothrombin time testing using Clarke's error grid analysis.

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METHODS

Patient characteristics

The present study investigated hemostatic laboratory test results of 73 patients undergoing cardiothoracic surgery with cardiopulmonary bypass in the VU University Medical Center (Amsterdam, the Netherlands). The local Human Subjects Committee approved the study (VU University Medical Center, Amsterdam, the Netherlands; METc 13/187), and informed consent was obtained according to local regulations. Included patients were 18 years or older and underwent elective cardiothoracic surgery with cardiopulmonary bypass (CPB). Surgical procedures included valve procedures, coronary artery bypass graft (CABG) surgery or a combination of valve with CABG surgery. Exclusion criteria were emergency operations and off-pump cardiac surgery.

Anesthesia and cardiopulmonary bypass

Patients were anesthetized according to local protocols based on a combination of sufentanil 3-7 µg kg^{-1}, rocuronium 0.5 mg kg^{-1} and midazolam 0.1 mg kg^{-1} for anesthesia induction and tracheal intubation, and propofol (200-400 mcg h^{-1}) for maintenance. All patients received dexamethasone and tranexamic acid.

Cardiopulmonary bypass was performed at a continuous blood flow (2.2-3.0 l min^{-1} m^{-2}) during normo- or mild hypothermia (>34 °C) using an S5 heart-lung machine (Stöckert Instrumente GMBH, Munich, Germany) with a centrifugal pump (Delphin, Terumo Europe NV, Leuven, Belgium) and heparin-coated circuit (Medtronic, Minneapolis, MN, USA). Heparinisation started with an initial bolus of 300 IU kg^{-1} heparin (LEO Pharma BV, Amsterdam, the Netherlands), which was replenished until the Celite activated clotting time (ACT) test exceeded 480 seconds (Hemochron® Response, Edison, NJ, USA). The priming solution (1400 ml) contained 5000 IU porcine heparin (LEO Pharma BV, Amsterdam, the Netherlands). After cardiopulmonary bypass, heparin was reversed with protamine (MedaPharma BV, Amstelveen, the Netherlands). The primary dosing of protamine was based on local guidelines, which advises approximately 1 mg protamine for every 100 IU of heparin administered throughout the procedure (1:1 ratio).

Prothrombin time measurements

According to local protocols, blood samples are routinely drawn directly after anesthesia induction and 3 minutes after protamine administration. The INR of the prothrombin time (PT) was measured by the POC-device and in the central hemostasis laboratory. The Coaguchek PRO XS Pro® (POC-PT; Roche Diagnostics, Almere, The Netherlands) was switched on and prepared prior to blood sampling.

A blood sample from a flushed arterial line was drawn into a 1 mL syringe and one drop of whole blood was immediately placed on the Coaguchek test strip containing rabbit brain thromboplastin (Roche PT test strip®, Roche Diagnostics FmbH, Basel, Switzerland). Simultaneously, a second blood sample was drawn in to a citrated tube and send to the central hemostasis laboratory. Laboratory testing included the international normalized ratio (INR) of the prothrombin time based on calcium thromboplastin (LAB-PT) and the activated partial thromboplastin time (aPTT) based on cefaline/microcrystaliline using a STA-R instrument® (Roche Diagnostics FmbH, Basel, Switzerland) in platelet-free plasma.

Other study parameters

Patient demographics included age, gender and body mass index (BMI). Surgical characteristics were type of surgery, CPB time, aorta clamp time, heparin and protamine dosing and the lowest body temperature. Hemostatic parameters included preoperative heparin use, activated clotting time (ACT), hemoglobin concentration (Hb), the activated partial thromboplastin time (aPTT), platelet count before CPB and 3 minutes after protamine administration.

Data analysis

Data are represented as mean ± standard deviation, median with interquartile range or frequencies. Data analysis was performed with the SPSS statistical software package 17.0 (SPSS Inc.®, IBM, New York, USA). A P-value <0.05 was considered as statistical significant.

Bland-Altman analysis was performed using GraphPad Prism 5.0 (La Jolla, CA, USA). Bland-Altman analysis provided the bias, standard deviation of the bias, and limits of agreement between both methods. Bland Altman analysis shows the mean of both prothrombin time measurements of one patient on the x-axis ((LAB PT + POC PT) / 2) and the difference between the to measurements on the y-axis (LAB PT - POC PT). The *bias* shows the general difference between the measurements, with the fluctuations around this difference as *standard deviation of the bias*. The *95%-limits of agreements* shows where the values will be for most patients (bias ± 1.96 SD), if these differences are not clinically relevant the laboratory and point-of-care method are interchangeable. The association between cardiopulmonary bypass and aortic clamp time, preoperative and post-CPB hemoglobin values and LAB-PT or POC-PT test results were analyzed using Pearson correlations.

Error grid analysis was used to evaluate the clinical relevance of the differences between point-of-care and central laboratory measurements. [12] A prothrombin

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time below 1.0 INR was indicated as normal, and a PT > 1.5 INR was defined as coagulopathy, potentially requiring treatment. The scatter plot was divided into three zones showing the clinical relevance of the (dis)agreement between the two methods. Zone A shows the clinical acceptable area with an acceptance of 10% deviation between the two methods. Zone B represents an error greater than 10% and in this area coagulopathy is over- or underestimated with possibly changing clinical decision-making. Ten percent was chosen since a deviation of 0.10 – 0.15 INR from the golden standard (i.e. laboratory prothrombin time) was considered clinically acceptable. Zone C reflects a clinically unsafe situation leading to inadequate or inappropriate treatment. More than 95% of the values should be in zone A and none in zone C for the POC-method to be clinical acceptable, reflecting a P-value < 0.05.

RESULTS

Patient characteristics

The study population included 73 patients undergoing cardiac surgery with cardiopulmonary bypass, with a mean age of 68 ± 10 years and body mass index of 27 ± 4 kg m^{-2}. Table 1 shows the surgical and hemostatic characteristics.

Surgical and hemostatic characteristics	Values
N	73
Preoperative hemoglobin (mmol l^{-1})	8.6 ± 0.8
Preoperative aPTT (s)	35 ± 4
Preoperative platelet count (10*^{9} l^{-1})	272 ± 84
Preoperative ACT (s)	131 ± 16
Pre-CPB ACT (s)	587 ± 187
CPB time (min)	104 ± 33
Aortic clamp time (min)	72 ± 25
Lowest intraoperative hemoglobin (mmol l^{-1})	5.2 ± 0.7
Lowest intraoperative temperature (°C)	34.5 ± 0.9
Heparin before CPB (*100 IU)	327 ± 78
Heparin during CPB (*100 IU)	173 ± 107
Total protamine dosing (mg)	371 ± 97
Postoperative hemoglobin (mmol l^{-1})	6.0 ± 1.0
Postoperative aPTT (s)	39 ± 4
Postoperative platelet count (10*^{9} l^{-1})	145 ± 51
Postoperative ACT (s)	133 ± 13
Intraoperative red blood cell transfusion [units]	0 (0;0)

Table 1. Surgical and hemostatic characteristics. Data represent mean ± standard deviation, median with interquartile range or frequencies. aPTT = activated partial thromboplastin time; CPB = cardiopulmonary bypass; ACT = activated clotting time.

The majority of patients were male (71%). Most patients underwent CABG (67%) or valve (22%) surgery. Patients did not receive red blood cell transfusion during surgery. Figure 1 shows the average values for the POC-PT and LAB-PT before and after cardiopulmonary bypass.

The whole blood POC-PT after protamine administration was lower when compared to the LAB-PT test in platelet-free plasma.
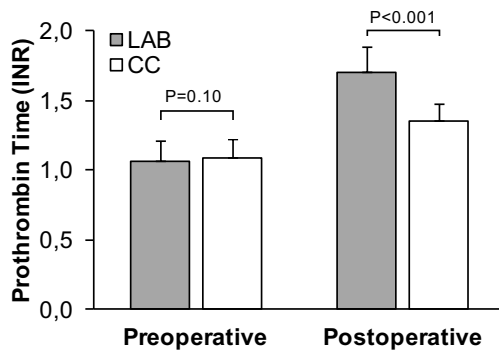
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Figure 1. Point of care (CC) and central laboratory (LAB) prothrombin time (I.N.R.) before and after cardiopulmonary bypass. Data represent mean ± standard deviation.

Level of agreement between POC-PT and LAB-PT

Figure 2 represents the Bland-Altman analyses for the POC-PT and LAB-PT after induction of anesthesia (panel A) and 3 minutes following administration of protamine (panel B).
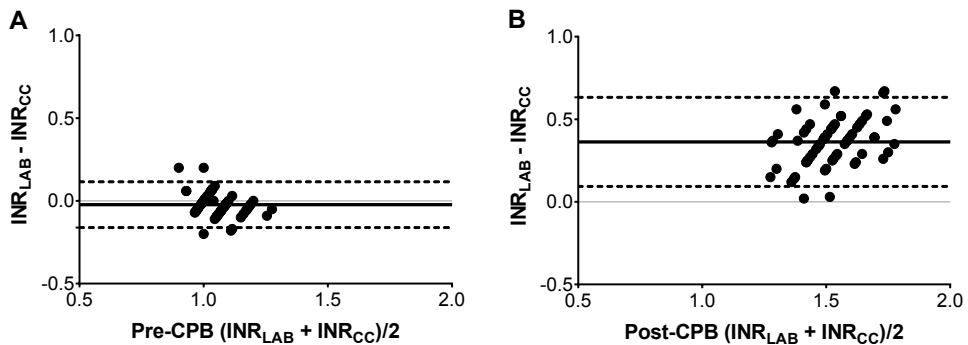

Figure 2. Bland Altman analysis of point of care (CC) vs. central laboratory (LAB) prothrombin time (INR) before (Panel A) and after (Panel B) cardiopulmonary bypass (CPB).

Before cardiopulmonary bypass, Bland-Altman analysis revealed a good level of agreement as shown by the bias with standard deviation of -0.02 ± 0.07 with limits of agreement ranging from -0.16 to -0.12. Three minutes after antagonizing heparin by protamine, the bias was severely altered and estimated 0.36 ± 0.14, with limits of agreement ranging from 0.09 to 0.63.

There were no differences in the POC-PT test (1.34 ± 0.13 vs. 1.34 ± 0.13; n.s.) and LAB-PT test (1.68 ± 0.16 vs. 1.73 ± 0.16; n.s.) following cardiopulmonary bypass between patients undergoing CABG or valve surgery, respectively.

Pearson correlation analysis showed no association between duration of cardiopulmonary bypass with the LAB-PT (r=0.069; P=0.56) or POC-PT (r=0.028; P=0.082) or the aortic clamp time with the LAB-PT (r=-0.02; P=0.87) or POC-PT (r=-0.13; P=0.26). There was no correlation between the cardiopulmonary bypass time (r=-0.09; P=0.44) and aortic clamp time with the difference between LAB-PT and POC-PT (r=-0.15; P=0.20). There was a correlation between postoperative hemoglobin concentration and LAB-PT (r=-0.30; P=0.01), this was not found for the POC-PT (r=-0.04; p=0.72). Postoperative hemoglobin concentration correlated with the difference between LAB-PT and POC-PT (r=-0.28; P=0.018).

Clarke's Error Grid Analysis

Figure 3 shows the error grid analysis for the deviation between POC-PT and LAB-PT before (panel A) and after (panel B) cardiopulmonary bypass. Before cardiopulmonary bypass, 94% of the values were located in zone A, 6% in zone B and 0% in zone C. After reversal of heparin by protamine, this distribution shifted, and 18% was located in zone A, 82% in zone B and 0% in zone C.
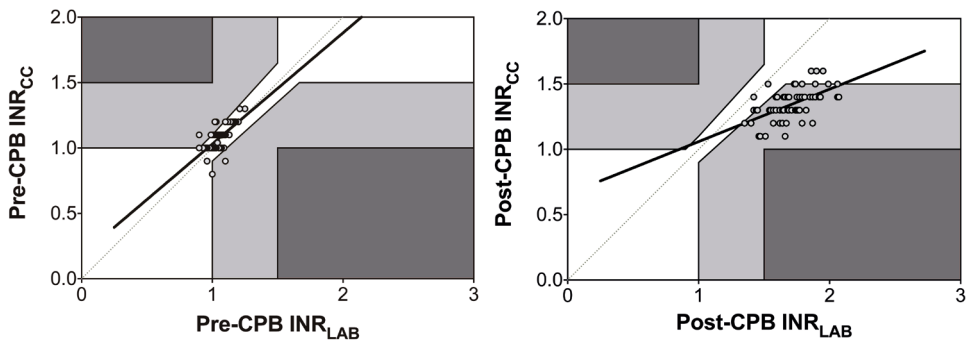

Figure 3. Error grid analysis of point of care (CC) vs. laboratory (LAB) prothrombin time (INR) before and after cardiopulmonary bypass (CPB). The implication of zones A (white), B (light grey), and C (dark grey) are described in detail in the method section.

DISCUSSION

This study shows that point-of-care whole blood prothrombin time testing showed a good level of agreement with a conventional laboratory plasma prothrombin time before initiation of cardiopulmonary bypass, but this agreement disappeared at three minutes after protamine administration. Point-of-care PT values were lower following cardiopulmonary bypass than laboratory PT values. The majority of data pairs as measured before cardiopulmonary bypass were located in zone A during Clarke's grid analysis. However, the error grid analysis showed that 82% of the values are represented in Zone B following heparin and protamine administration, while this should be <5% to be clinically acceptable. Our findings suggest that point-of-care testing may be of added value in hemostatic testing before heparin and protamine administration in cardiac surgery. However, the disagreement between POC-PT and LAB-PT three minutes following protamine administration warrants further evaluation of the optimal POC-PT monitoring timing after cardiopulmonary bypass before clinical implementation.

An early impression of the hemostatic state of a patient could differentiate between surgical and coagulopathic bleeding and influence subsequent therapy. Although POC-PT measurements have previously been evaluated in two other cardiosurgical studies, these studies evaluated the prothrombin time at least 10 minutes after protamine administration and found a good relation with laboratory PT values. [10,11] Yet, some patients require hemostatic evaluation at the earliest convenience following weaning from bypass, as an early goal directed approach of coagulopathy may improve outcome. We therefore performed post-CPB measurements shortly after protamine administration. However, 3 minutes after protamine administration shows to be too early for accurate PT monitoring by POC-PT. This prompt timing of prothrombin measurements might explain the disagreement of our findings with previous studies.

It might be suggested that the difference between POC and laboratory PT testing 3 minutes after protamine administration could be explained by the duration of protamine-heparin complex formation, as this takes up to 10 minutes. [13] As blood is drawn 3 minutes after protamine infusion not all heparin may have formed a complex with protamine. The manufacturer of the POC-PT device states that the device is insensitive to heparin concentrations up to 0.8 IU ml^{-1}. This insensitivity to heparin by the POC device could explain the shorter POC-PT, as the laboratory PT is more affected by the unbound heparin. Yet, the infusion of protamine itself takes several minutes in which protamine-heparin complexes can be formed, suggesting that nearly all complexes will have been formed at the time of the POC-PT measurement.

Interestingly, hemoglobin concentrations could rationalize the difference between the laboratory and POC prothrombin time. The found negative correlation between postoperative hemoglobin level and laboratory PT was absent for the POC-PT test suggesting that the POC-PT is less sensitive for the influence of erythrocytes on hemostasis. [14] This is in line with earlier findings by van den Besselaar et al. showing a distinct influence of hematocrit on the LAB-PT and POC-PT. [15]

The Clarke's grid analysis shows that, before cardiopulmonary bypass, 94% of the values were located in zone A and 6% in zone B. This means that in 6% of the cases, the level of coagulopathy as assessed by PT might be over- or underestimated with possible effect on the decision to transfuse hemostatic products. Three minutes after reversal of heparin by protamine, this distribution shifted to 18% in zone A and 82% in zone B. All values in zone B are located on the right side of zone A in the Clarke's grid plot, showing that PT values were structurally shorter than laboratory PT values after heparin reversal with protamine. As we compared the point-of-care PT with a gold standard, this might suggest that the use of the Coaguchek may lead to underestimation of the presence of coagulopathy. On the other hand, as the laboratory PT is also influenced by residual heparin or protamine overdosing, it is unclear whether the gold standard accurately reflects the hemostatic status of the patient. Further studies should reveal which test is the best representative for the post-heparin phase during cardiac surgery.

Our study is limited by the single measurement time point of the prothrombin time following cardiopulmonary bypass, as repeating measurements will give better insight in the optimal timing of PT monitoring and level of agreement between laboratory and point-of-care PT tests. Since point-of-care PT testing may contribute to fast insight in the hemostasis of our patients, additional studies should be performed to further determine the clinical value of point-of-care PT testing.

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Chapter 9

Validation of a point-of-care prothrombin time test after cardiopulmonary bypass in cardiac surgery

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ABSTRACT

Point-of-care coagulation monitoring can be used for the guidance of hemostasis management. However, the influence of time on point-of-care prothrombin time testing following protamine administration after cardiopulmonary bypass has not been investigated. Bland–Altman and error grid analysis were used to analyze the level of agreement between prothrombin time measurements from point-of-care and laboratory tests before cardiopulmonary bypass, and then 3 min, 6 min and 10 min after protamine administration. Prothrombin times were expressed as International Normalized Ratios. While the point-of-care and laboratory prothrombin time measurements showed a high level of agreement before bypass, this agreement deteriorated following protamine administration to a mean (SD) bias of - 0.22 (0.13) [limits of agreement 0.48 – 0.04]. Error grid analysis revealed that 35 (70%) of the paired values showed a clinically relevant discrepancy in international normalized ratio. At 3 min, 6 min and 10 min after cardiopulmonary bypass there is a clinical unacceptable discrepancy between the point-of-care and laboratory measurement of prothrombin time.

INTRODUCTION

Point-of-care coagulation monitoring in the peri-operative setting is used routinely for the guidance of hemostasis management. The point-of-care prothrombin time test was primarily developed for the rapid, outpatient monitoring of warfarin therapy. This test has recently been validated for use in non-cardiac surgery, reducing the time between blood sampling and test result by up to 60 min [1] and, therefore, may be useful for rapid guidance of transfusion practice. [2-5]

The value of prompt insight into patient hemostasis might even be greater in cardiac surgery, due to its association with postoperative coagulopathy and high rate of allogeneic blood transfusion. The coagulopathy is thought to be due to a combination of blood contact with the non-biological surface of the cardiopulmonary bypass circuit, hemodilution and acute blood loss. Residual heparin or abundant protamine administration may further impair hemostasis. These factors complicate peri-operative hemostatic monitoring in cardiac surgery, and might have distinct effects on the prothrombin time as measured by point-of-care and formal laboratory testing.

We recently showed a low level of agreement between point-of-care and laboratory prothrombin time results, when measured 3 min after protamine administration. [6] However, two previous studies demonstrated that point-of-care prothrombin time measurement was comparable with laboratory testing when blood was withdrawn more than 10 min after protamine administration. [7,8]

In order to clarify whether the timing of testing following protamine administration influences the results, we evaluated point-of-care and laboratory prothrombin time measures at 3 min, 6 min and 10 min after protamine administration in patients having cardiac surgery.

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METHODS

This prospective observational study was performed in the VU University Medical Center (Amsterdam, the Netherlands). Patients undergoing cardiothoracic surgery with cardiopulmonary bypass were included. The local Human Subjects Committee approved the study and informed consent was obtained from all patients. Patients were enrolled if they were undergoing elective cardiac surgery with bypass and aged $\geq$ 18 years. We did not study patients with renal or hepatic failure, those who had received pre-operative erythropoietin or warfarin less than 3 days before surgery and those with hemoglobin concentrations < 89 g l^{-1}.

Patients were anesthetized according to local protocols based on a combination of sufentanil (3–7 µg kg^{-1}), rocuronium (0.5 mg kg^{-1}) and midazolam (0.1 mg kg^{-1}) for induction of anesthesia, and propofol (200–400 µg h^{-1}) for anesthesia maintenance. All patients received tranexamic acid (1 g before and 2 g after bypass). Cardiopulmonary bypass was performed at a continuous flow of 2.2–3.0 l min^{-1} m^{-2} during mild hypothermia (> 34 °C) using an S5 heart-lung machine (Stöckert Instrumente GMBH, Munich, Germany) with a centrifugal pump (Delphin, Terumo Europe NV, Leuven, Belgium) and heparin-coated circuit (Medtronic, Minneapolis, MN, USA). Initial heparinization was achieved with 300 IU kg^{-1} heparin bolus (LEO Pharma BV, Amsterdam, the Netherlands), which was then repeated until the celite-activated clotting time test (Hemochron® Response, Edison, NJ, USA) exceeded 480 s. The priming solution (1400 ml) contained 5000 IU porcine heparin (LEO Pharma BV). After bypass, heparin was reversed with protamine hydrochloride (Meda Pharma BV, Amstelveen, the Netherlands). The primary dose of protamine was based on local guidelines, which advised approximately 0.8 mg protamine for every 100 IU of heparin administered throughout the procedure, with adjustments at the discretion of the attending anesthetist. Acetylsalicylic acid therapy was continued peri-operatively, while clopidogrel was stopped 5 days before surgery.

Four arterial blood samples were collected during the procedure from a non-heparinized arterial line; the first 5 ml of blood withdrawn was discarded before blood sampling. Samples were collected after induction of anesthesia and then 3 min, 6 min and 10 min after protamine administration. The international normalized ratio (INR) of the prothrombin time was measured using a point-of-care device (Coaguchek® Pro DM; Roche Diagnostics, Almere, the Netherlands) and in the central hemostasis laboratory. The Coaguchek Pro DM device was switched on and prepared before blood sampling. A blood sample from the flushed arterial line was drawn into a 1-ml syringe and one drop of whole-blood was immediately placed on the Coaguchek test strip containing rabbit brain thromboplastin (Roche

Diagnostics, FmbH, Basel, Switzerland). Simultaneously, a second blood sample was drawn into a citrated tube and sent to the central hemostasis laboratory. Laboratory testing included the INR of the prothrombin time based on calcium thromboplastin, and the activated partial thromboplastin time based on cefaline/ microcrystaliline using a STA-R instrument® (Roche Diagnostics) in platelet-free plasma. Other hematological data collected included activated clotting time, hemoglobin concentration, hematocrit, activated partial thromboplastin time and platelet count. All parameters were measured before cardiopulmonary bypass and 3 min after protamine administration.

To calculate sample size, based on a previous study we estimated that mean (SD) point-of-care and laboratory prothrombin time measurements at 10 min following weaning from bypass would be 1.40 (0.30) and 1.55 (0.30), respectively. [9] Based on the estimated difference, we accepted a bias of 0.15 between the point-of-care and laboratory prothrombin time measurements with estimated limits of agreement of –0.15 to 0.45. In order to achieve the desired 95% CI of 0.15 around the limits of agreement, we required 50 patients.

Data analysis was performed using the SPSS statistical software package 17.0 (IBM, New York, NY, USA). A p value < 0.05 was considered as statistically significant. Bland–Altman analysis was performed using GraphPad Prism 5.0 (La Jolla, CA, USA). Bland–Altman analysis provided the bias, standard deviation of the bias, and limits of agreement between both methods. [10] The percentage error was calculated as $1.96 \times$ SD of the bias/mean INR of the two methods. A percentage error below 20% was considered clinically acceptable. [11] Error grid analysis was used to evaluate the clinical relevance of the difference between point-of-care and laboratory measurements. [12] International normalized ratio < 1.0 was considered normal, and INR > 1.5 was defined as a coagulopathy which would potentially require treatment. The scatter plot was divided into three zones showing the clinical relevance of the (dis)agreement between the two methods. Zone A showed the clinical acceptable area with an acceptance of 10% deviation between the two methods. Zone B represented an error greater than 10%, and in this area coagulopathy is over- or underestimated; 10% was chosen since a deviation in INR 0.10–0.15 from the gold standard of laboratory prothrombin time measurement was considered clinically acceptable. Zone C reflected a clinically unsafe situation leading to inadequate or inappropriate treatment. More than 95% of the values should be in zone A and none in zone C for the point-of-care method to be clinical acceptable, reflecting a p value < 0.05. The difference between point-of-care and laboratory prothrombin time was correlated with fibrinogen level, thrombocyte count and hematocrit using Pearson correlation.

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RESULTS

We recruited 50 patients (31 males) with a mean age of 68 ± 10 years and BMI 29 ± 5 kg.m^{-2}. Overall, 38 patients underwent coronary artery bypass grafting or single valve surgery, whereas the remaining patients underwent a combination of coronary artery bypass grafting with valve surgery or a Bentall procedure. The surgical and hematological characteristics of the participants are shown in Tables 1 and 2, respectively. Six patients received packed red blood cell transfusion during surgery. Directly after cardiac surgery, six patients received platelet transfusions and two patients received packed red blood cells; no patient required transfusion of fresh frozen plasma.

	N = 50
CPB duration (min)	95 ± 37
Aortic clamp time (min)	70 ± 26
Minimum temperature (°C)	35 ± 1
Heparin dosing (mg)	412 ± 79
Protamine dosing (mg)	241 ± 54

Table 1. Cardiopulmonary bypass and anticoagulation characteristics for all included patients (n = 50). Values are mean (SD).

	Preoperative	3 min after protamine
Haemoglobin (g l^{-1})	139 ± 16	91 ± 13
Haematocrit (%)	41 ± 5	29 ± 4
Platelet count (×10^9 l^{-1})	243 ± 60	125 ± 35
Activated partial thromboplastin time (s)	35 ± 5	44 ± 13
Activated clotting time (s)	125 ± 11	130 ± 11

Table 2. Pre-operative and post-cardiopulmonary bypass haemoglobin, haematocrit, thrombocyte count values and haemostatic test results for included patients. Values are mean (SD). There were no missing values for haemoglobin, haematocrit, thrombocyte count and activated clotting time (n = 50). Activated partial thromboplastin time data from three patients were missing (n = 47).

The whole-blood prothrombin time measured using point-of-care testing was lower at all time points after bypass compared with those measured in the laboratory using platelet-free plasma (Figure 1).

Bland–Altman analyses for point-of-care and laboratory measurements of prothrombin time are shown in Figure 2. Before bypass, Bland–Altman analysis revealed a high level of agreement between the two measures with bias (SD
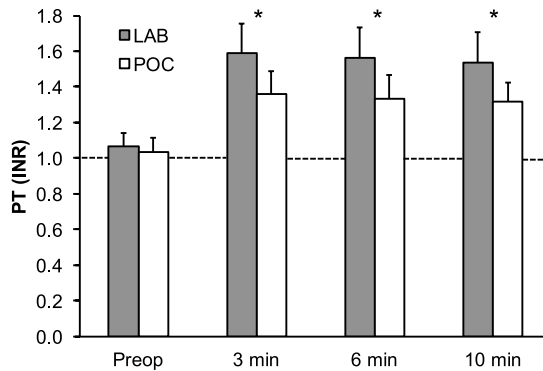
Figure 1. Laboratory (grey bars) and point-of-care (open bars) prothrombin times (PT) expressed as international normalised ratio (INR) before (Pre-op) and at 3 min, 6 min and 10 min following protamine administration. Values are mean (SD). Student's t-test was used to compare laboratory and point-of-care prothrombin times at the relevant time points. *p < 0.001.

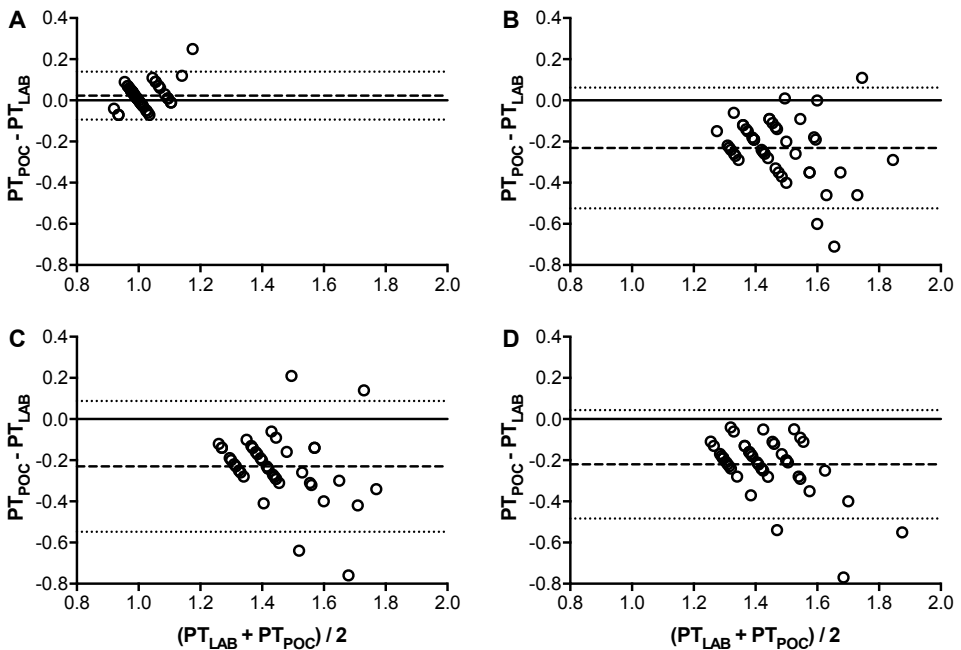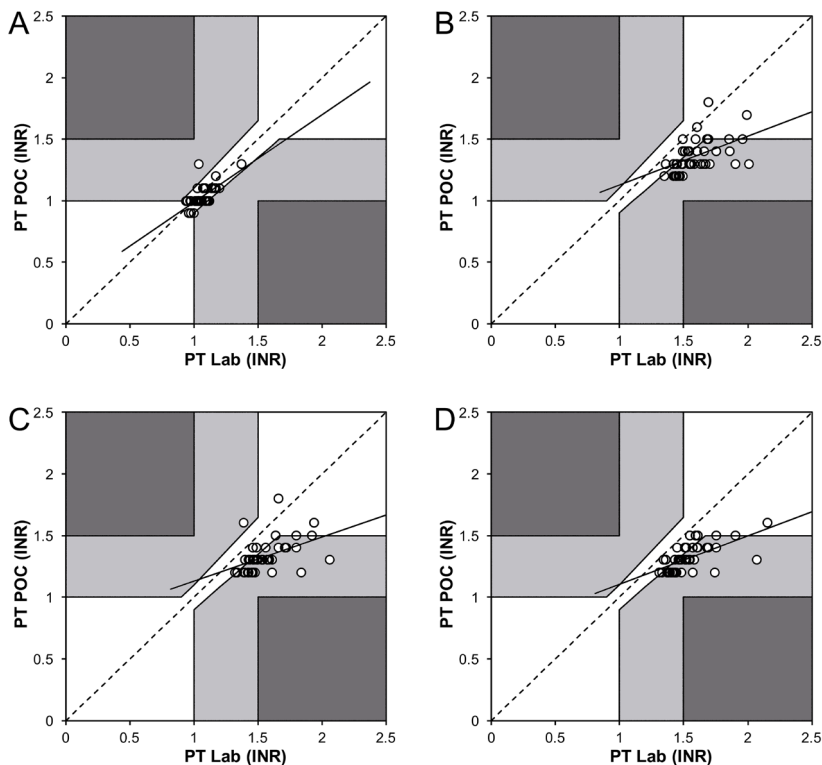**Figure 2.** Bland–Altman analysis of the level of agreement between the laboratory (LAB) and point-of-care (POC) prothrombin time (PT) expressed as international normalised ratio (INR) before (Pre-op; panel a) and at 3 min (panel b), 6 min (panel c) and 10 min (panel d) following protamine administration. The figures represent the bias (- - -) with limits of agreement (······).

[limits of agreement]) of 0.00 (0.04 [−0.09 to 0.14]). After reversal of heparinization with protamine, the bias (SD [limits of agreement]) was markedly altered at 3 min (−0.23 (0.15) [−0.53 to 0.06]), 6 min (−0.23 (0.16) [−0.55 to 0.09]) and 10 min (−0.22 (0.13) [−0.48 to 0.04]). The time of prothrombin time measurement after protamine administration did not influence the results. The precision error between both methods at baseline and 3 min, 6 min and 10 min following protamine administration was 11.4%, 20.0%, 21.7% and 17.9%, respectively.

Figure 3.   Clarke's Error Grid analysis of the level of agreement between the laboratory (LAB) and point-of-care (POC) prothrombin time (PT) expressed as international normalised ratio (INR) before (Pre-op; panel a) and at 3 min (panel b), 6 min (panel c) and 10 min (panel d) following protamine administration. The implication of zones A (white), B (light grey), and C (dark grey) are described in detail in the method section

The error grid analyses for the deviation between point-of-care and laboratory measures of prothrombin time are shown in Figure 3. Before bypass, 96% of the values were located in zone A and 4% in zone B. After reversal of heparin by

protamine, this distribution markedly altered: 32% zone A and 68% zone B after 3 min; 22% zone A and 78% zone B after 6 min; and 28% zone A and 72% in zone B after 10 min. There were no readings in zone C at any time point.

The difference between point-of-care and laboratory measurements of prothrombin time did not correlate with postoperative platelet count (R = 0.56, p = 0.714) or fibrinogen concentration (R = −0.45, p = 0.85), although there was a weak correlation with postoperative hematocrit (R = 0.31, p = 0.04).

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DISCUSSION

This study showed that, after cardiopulmonary bypass and administration of protamine, there is a great discrepancy between point-of-care whole-blood prothrombin time testing compared with conventional laboratory plasma prothrombin measurement. The average difference was approximately 0.3 INR at 3 min, 6 min and 10 min after protamine administration. Only the precision error at baseline and 10 min following protamine administration were below the Westgard criterion of 20%. [11] However, this seemingly minor difference is of great practical importance as around 70% of the values showed a clinically relevant discrepancy between the two tests in the error grid analysis, whereas this should be < 5% to be clinically acceptable. Bland–Altman analysis showed a similar excessive discrepancy between laboratory and point-of-care prothrombin time measures after protamine administration. Our results imply that the use of the Coaguchek leads to underestimation of the hemostatic disturbance, as the point-of-care test measured a shorter prothrombin time when compared with the laboratory value. The discrepancy might be explained by the nature of the tests, as the point-of-care test uses whole blood, whereas the laboratory test is performed with platelet-free plasma. As previously shown, pre-operative values showed a good level of agreement between laboratory and point-of-care prothrombin time measurements. [6]

Rapid insight in patient hemostasis after bypass can differentiate between bleeding due to coagulopathy from that of a surgical origin. Early goal-directed treatment of hemostatic abnormalities can prevent further blood loss and minimize exposure to allogeneic blood products with their inherent risks. However, our results show that the point-of-care prothrombin time test shows insufficient level of agreement to be used until at least 10 min after protamine administration in patients who have been on bypass. These results are in conflict with two earlier reported studies that showed a good correlation between point-of-care and laboratory prothrombin time measurements 10 min after protamine administration and on arrival in the ICU. [7, 8]

There may be factors that influence the point-of-care prothrombin time testing without affecting the laboratory prothrombin time measurement. Previously, it has been found that the discrepancy between laboratory and point-of-care prothrombin time measurements increases with increasing prothrombin times (> 4.0 INR). [13] However, in the case of a higher prothrombin time, the point-of-care prothrombin time was prolonged when compared with the laboratory results. Another study conflicts this finding showing a good level of agreement at higher prothrombin times (> 4.0 INR). [14] Prothrombin time values in our study were all < 2.0 INR.

Other studies have reported a confounding effect of lower hematocrit and fibrinogen levels on point-of-care prothrombin time test results. [15-17] Although our study was not powered for this endpoint, we did not find any relationship between the postoperative hematocrit, platelet count or fibrinogen level and the disagreement between the point-of-care and laboratory prothrombin time results. It should be noted, however, that the number of patients with very low fibrinogen levels in our study was small.

The strength of this study is the point-of-care measurement of prothrombin time at multiple time points after protamine administration. The time interval after protamine administration is critical for the management of bleeding and insight into the patient's hemostatic profile is of great value. However, point-of-care measurement of the prothrombin time is unreliable during a clinically relevant time period after protamine administration, and cannot be reliable upon to guide hemostatic therapy. The point-of-care prothrombin time test should not be used until at least 10 min after protamine administration in patients who have been on bypass.

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9

Treatment of coagulopathy

part IV

Chapter 10

Clinical decision versus thromboelastometry based fresh frozen plasma transfusion in cardiac surgery

Michael I. Meesters, Nick J. Koning,
Stephan A. Loer, Christa Boer

LETTER TO THE EDITOR

The prompt decision to transfuse fresh frozen plasma (FFP) after cardiopulmonary bypass (CPB) is frequently based on clinical signs of coagulopathy, patient characteristics, duration of cardiopulmonary bypass and experience of the surgical team, all united in the clinical view of the anaesthetist. [1,2] We investigated whether FFP transfusion based on this clinical judgment in the absence of haemostatic test results was appropriate when retrospectively validated by a thromboelastometry-based algorithm.

Data were retrieved from our observational study databases, in which thromboelastometry test results were not used by the attending anaesthetist to guide transfusion. Data retrieval for the present investigation was approved by local Human Subjects Committee (Amsterdam, the Netherlands; METc 2012/337) and informed consent was waived. Blood samples for haemostatic testing were routinely drawn after anaesthesia induction and three min after protamine administration. FFP transfusion based on clinical judgment of the anaesthetist was defined as administration of FFP after protamine administration without availability of classical coagulation and rotational thromboelastometry test results.

In most viscoelastic testing-based algorithms a HEPTEM clotting time (CT) > 240 s with or without EXTEM CT > 80 s is an indication for coagulopathy, requiring coagulation factor replenishment by prothrombin complex concentrate or FFP. [3] This definition was used to evaluate post hoc the accuracy of the clinical decision to transfuse FFP. Patients requiring protamine (HEPTEM: INTEM CT ratio < 0.8) were excluded from the analysis as protamine would be the first line treatment in these patients. [4,5] Patients who did not receive FFP showed no signs of clinical bleeding.

In our database, 159 patients were retrieved with available viscoelastic test results. Patients with a HEPTEM/INTEM ratio < 0.8 were excluded (n = 7). Table 1 shows patient and perioperative data. Patients in the non-FFP and FFP groups were divided according to thromboelastometry-based criteria for coagulopathy as defined above. In patients who received FFP, eight of 84 patients should not have received FFP according to the TEM algorithm (10%), whereas in the non-FFP group the decision to not transfuse FFP was retrospectively supported by the thromboelastometry algorithm in 68 out of 68 patients (100%).

Fresh frozen plasma transfusion based on the clinical judgment of the anaesthetist was appropriate in 95% of the patients when retrospectively validated by thromboelastometric transfusion algorithms. Our data imply that signs of clinical bleeding in light of patient and surgical characteristics are a major determinant in the decision to transfuse FFP. In agreement with others we found that patients who

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received FFP were older, had lower preoperative haemoglobin concentrations, underwent more complex surgery with longer extracorporeal circulation, and had more disturbed thromboelastometry and classical coagulation test results after cardiopulmonary bypass (thromboelastometric test results not shown). [6,7]
The clinical rationale for FFP transfusion is based on signs of clinical bleeding in combination with patient characteristics and surgical factors. This clinical judgment to transfuse FFP had a high accuracy when validated by a thromboelastometric transfusion algorithm. In light of the increasing availability of transfusion protocols, the presence of clinical bleeding and the judgment of the anaesthetist should not be neglected as part of the decision algorithm to transfuse FFP.

	No FFP group n=68	FFP group n=84	P-value
Age (yr)	63 ± 13	70 ± 10	<0.01
Type of surgery (n)			<0.01
CABG	14 (21%)	6 (7%)	
Valve	45 (67%)	28 (33%)	
Valve + CABG	8 (12%)	45 (54%)	
Bentall	0 (0%)	5 (6%)	
BMI (kg m^{-2})	27 ± 4	26 ± 4	0.24
Male (%)	44 (65%)	55 (66%)	0.92
CPB time (min)	106 ± 35	161 ± 50	<0.01
Aortic clamp time (min)	79 ± 28	121 ± 42	<0.01
Haemoglobin during CPB (mmol l^{-1})	6.0 ± 1.1	5.4 ± 0.8	<0.01
FFP transfusion (units)	0 (0-0)	2 (2-2)	<0.01
Platelet transfusion (units)	0 (0-0)	1 (1-1)	<0.01

Table 1.   Patient and perioperative data

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Chapter 11

Administration of fibrinogen concentrate during cardiac surgery

Michael I. Meesters, Christian von Heymann, Christa Boer

LETTER TO THE EDITOR

The randomized clinical trial by Dr Bilecen and colleagues showed no benefit of fibrinogen concentrate use, compared with placebo, on intraoperative blood loss in patients with bleeding following high-risk cardiac procedures. [1] We believe that the results may unfairly characterize fibrinogen concentrate use.

First, the authors chose intraoperative blood loss as their primary study endpoint. However, intraoperative blood loss is less relevant in cardiac surgery than blood loss that occurs after chest closure. [2] Unfortunately, 24-hour blood loss, which showed a significant reduction in the fibrinogen concentrate group, was a secondary endpoint.

Second, in their sample size calculation, the authors assumed a 40% reduction in intraoperative blood loss after fibrinogen supplementation based on findings from a small retrospective study, a small prospective study including patients with normal fibrinogen levels, and a porcine study. [1] Due to the low level of evidence of these studies, we doubt the likelihood of this effect size, leading to an underpowered outcome. Moreover, it is unclear whether the anticipated intraoperative blood loss of 2200 ml in the control group used for the sample size calculation was realized.

Third, most transfusion protocols in cardiac surgery recommend a fibrinogen measurement by the Clauss test or visco-elasting testing as a requirement for fibrinogen supplementation. [3] A limitation of this trial is that fibrinogen supplementation was not guided by fibrinogen measurements. The placebo and intervention groups both showed normal fibrinogen levels exceeding 1.5 g l^{-1} in most patients before trial drug administration, which usually does not require fibrinogen supplementation. Additionally, while the authors targeted fibrinogen levels of 2.5 g l^{-1} after fibrinogen supplementation, this endpoint was achieved in less than 50% of the patients. Thus, this trial only shows that supplementing clotting factors in patients with normal levels will not benefit the patient. [4]

We believe that the benefits of fibrinogen concentrate for perioperative hemostasis should be evaluated in a large and sufficiently powered trial with a clinically relevant primary endpoint in patients with hypofibrinogenaemia before it can be concluded that supplementation is of no benefit for bleeding patients undergoing high-risk cardiac surgery.

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Chapter 12

General discussion and conclusions

GENERAL DISCUSSION

The goal of patient blood management is to reduce bleeding and blood transfusion, with the subsequent aim to prevent unfavorable outcome. This is a multimodal approach comprising the *prevention*, *prediction, monitoring* and *treatment* of microvascular bleeding caused by coagulopathy. Figure 1 gives an overview of all individual major patient blood management modalities in cardiac surgery. As shown previously, each blood management intervention attributes to a small improvement in outcome, and when combined leads to a major reduction in blood loss and transfusion requirements. [1,2] All components are combined in a multidisciplinary approach involving the anesthesiologist, cardiac surgeon, perfusionist, intensivist and hematologist. This thesis focuses on several aspects of patient blood management in cardiac surgery, marked in grey in figure 1. First, optimal protamine dosing to prevent postoperative coagulopathy was investigated. Second, several aspects of different point-of-care monitors were assessed in search of optimal hemostasis monitoring to guide patient blood management. Finally, different coagulation treatment methods were evaluated.
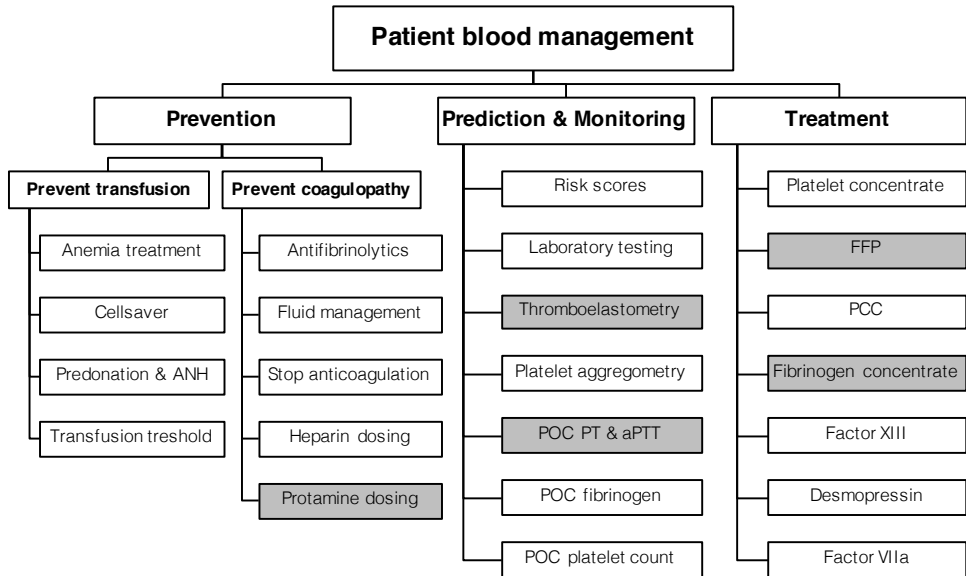
Figure 1. Multimodal patient blood management in cardiac surgery. Grey marked boxes = themes of this thesis. ANH = Acute normovolemic hemodilution, aPTT = activated partial thromboplastin time, FFP = Fresh frozen plasma, PCC = prothrombin complex concentrate, POC = point-of-care, PT = prothrombin time.

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PART I. PREVENTION OF COAGULOPATHY

Protamine dosing

An important step in patient blood management after cardiopulmonary bypass is the optimization of hemostasis by the reversal of heparin by protamine. In cardiac surgery, protamine is historically dosed in a fixed ratio and not much has changed since the first dosing studies which date back to 1968. [3] For many years, high protamine dosing was considered safe. [4] However, as described in **chapter 2,** an increasing number of studies show negative effects of protamine on hemostasis. Still, well-designed studies investigating the clinical effect of protamine overdosing were lacking.

In 2002, the first evaluation of different protamine-to-heparin dosing ratios investigated if reduced protamine dosing resulted in improved clinical outcome. [5] No outcome difference between a dosing ratio of [0.75] vs. [1.3] vs. [1 + 0.15 + 0.15] mg protamine for every milligram of heparin was found, except for more red blood cell transfusion in the low dosing ratio group without a difference in postoperative blood loss. This study had major limitations, as it was a retrospective sequential cohort study in which three groups were compared, no power analysis was performed, no transfusion algorithm was used, a single anesthesiologist and cardiac surgeon were involved and the effect on hemostasis was not evaluated. We were the first to investigate the effect of different fixed dosing regimens on outcome in a prospective randomized design (**chapter 3)**. We showed that protamine overdosing in the clinical setting impairs hemostasis. As in line with others we refute the misconception that a prolonged ACT after regular protamine dosing is caused by residual heparin. [6]

While optimization of clinical fixed dosing ratios is scarcely investigated, several studies did investigate the effects of heparin titration based protamine dosing. [7] The general conclusion is that postoperative blood loss is reduced when protamine titration is used. Still, these studies are limited by their small sample size, reporting of non-clinical outcome and lack of a sample size calculation. Furthermore, protamine dosing based on the titration method has several limitations and possible flaws. [8,9] The method bases the heparin concentration on ACT measurements, estimates patient blood volume and neglects the sequestration and plasma protein binding of heparin. Besides, only few centers use these heparin and protamine titration systems in routine clinical practice. [10] Therefore, optimization of a clinical fixed dosing ratio could lead to greater adaptation in clinical practice as implementation of a new dosing ratio is easy, resulting in improved outcome.

An alternative to protamine dosing based on a fixed ratio or the titration method is dosing based on a pharmacokinetic model of heparin. This method estimates the amount of heparin after bypass by a pharmacokinetic model, as proposed in **chapter 4**. Several studies, including ours, found lower protamine dosages and improved outcome when compared to a fixed protamine dosing ratio. [11-15] As pharmacokinetic dosing models only use clinical parameters, implementation is easy. A great advantage over the heparin titration technique is the lack of maintenance, training and costs of a heparin management system.

Fixed dosing [1.3:1.0]

Reduced dosing [0.8:1.0]

Protamine titration

Pharmacokinetic dosing

Anti-Xa measurement (POC)
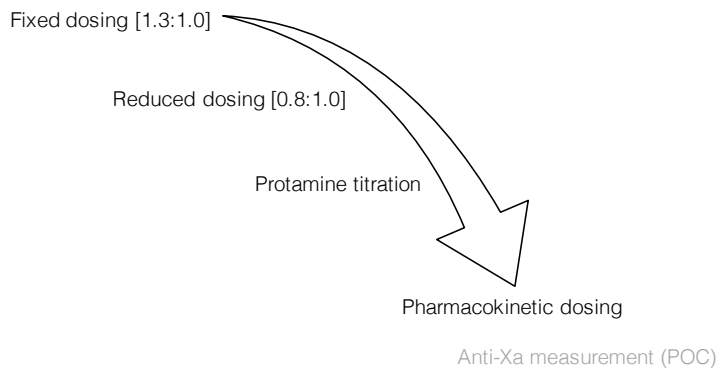
Figure 2. The evolution of protamine dosing.

Limitations & Future directions
The studies on protamine dosing in this thesis are limited by some factors. The 'Ratio PRO' study (**chapter 3**), comparing a fixed dosing ratio of 0.8 vs. 1.3, includes solely CABG patients. In this patient category blood loss may differ from other forms of cardiac surgery such as valve surgery. Another limitation is the calculation of the protamine dose based on the total amount of heparin administrated, including the heparin administrated during cardiopulmonary bypass. Still, the aim of this study was to investigate if a high protamine-to-heparin dosing ratio deteriorates hemostasis in the clinical setting. Interpretation for clinical dosing ratio should be seen in the light of this design. Future studies should compare a dosing ratio of 1.0 on the initial heparin dose to a protamine dosage of 0.7 – 0.8 to find the optimal fixed dosing ratio.

Our pharmacokinetic protamine dosing study, **chapter 4**, is limited by its retrospective design and the estimation of patients' blood volume. Heparin concentration measurements after protamine administration would add value to the evaluation of the adequacy of the model. Future studies should compare

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the pharmacokinetic model to the protamine titration method in a prospective randomized non-inferiority design to see if the heparin management system is redundant for protamine dosing. An easy to use web application or smartphone app could be developed to implement its use. However, the medicolegal status of such an application should be evaluated and disclaimed before distribution.

The future of protamine dosing might be the measurement of anti-Xa activity, the gold standard for heparin measurement. By relating the anti-Xa level to a known amount of heparin in a control sample the heparin concentration and protamine requirement can easily be calculated. [16] This could be more accurate than the prediction of the residual heparin concentration by a pharmacokinetic model and lead to more accurate protamine dosing. However, the dosing of protamine will still be based on an estimation of patients' blood volume and the sequestrated heparin will be neglected. Point-of-care anti-Xa measurements are currently under development. [17-18]

Another field for further exploration is the phenomena of heparin rebound, defined as reappearance of heparin hours after reversal with protamine. This is caused by release of heparin from the bound surface of the endothelium, proteins, circulating cells and platelet factor 4 (PF4; released by thrombocytes). Another hypothesis is the release of heparin from the heparin-protamine-complex before elimination. It was thought that the free heparin released during heparin rebound would lead to anticoagulation and postoperative bleeding. [19,20] Some studies even suggested administration of additional protamine to treat heparin rebound in order to reduce blood loss. [21,22] However, several studies refute clinical consequences of heparin rebound. [23-28] In order to reject the myth of heparin rebound a thorough large study should be performed to evaluate the existence and clinical consequences of heparin rebound.

PART II. PREDICTION OF POSTOPERATIVE BLEEDING

Identification of patients at risk for bleeding

Patients at high risk for bleeding should be identified before and during surgery in order to optimize patient blood mangement. Many attempts have been made to predict which patients are at risk for major blood loss after cardiac surgery. The major risk factors for postoperative bleeding and transfusion are female gender, renal impairment, cerebrovascular disease, preoperative anemia, continuation of antiplatelet drugs and/or anticoagulants, low body surface area, emergency - and complex surgery. [29] Clinical designed risk scores combine several risk factors and show a predictive value ranging from 15% to 91%. [30-37] Unfortunately, these risk scores are unpractical in clinical practice due to a discriminative rate that is too narrow or too broad, resulting in a high sensitivity and low specificity, or low sensitivity and high specificity respectively. In the clinical setting preoperative risk assessment for transfusion is scarcely used. [38]

Previous reports conclude that classical laboratory hemostatic test results do not predict which patients are at risk for bleeding. [39-41] In **chapter 5** we investigated if thromboelastometry, unlike classical coagulation tests, would be able to identify patients at risk for major hemorrhage. This was evaluated by a cohort study in our center in addition to a systematical review of the literature. Interestingly, we found that abnormal thromboelastometry test results do not risk the patient for bleeding. This is an important finding as it suggests that in the non-bleeding patient there is no need for prophylactic treatment of hemostasis abnormalities. Interestingly, classical laboratory testing is thought not useful to guide coagulation treatment, as the test results do not predict bleeding, and thromboelastometry is then suggested as an alternative. However, as we have shown, also thromboelastometry is not able to predict bleeding in cardiac surgery.

Limitations & Future directions

Our cohort study on the predictive value of thromboelastometry is limited by its relative small sample size and low rate of major blood loss (20%), limiting the possibility for extensive statistical analysis. However, the systematic review underlines the conclusion that thromboelastometry was unable to detect patients at risk for bleeding. A rationale for this finding might be that macrovascular bleeding is the main cause of excessive bleeding. Furthermore, rapid physiological restoration of the clotting factor level in the postoperative phase limits the predictive value of thromboelastometry. [42,43] Additionally, in the bleeding patient coagulation factors are replenished by the administration of fibrinogen concentrate, FFP, PCC and/or platelet concentrate in order to treat coagulopathy. Both phenomena

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lead to (rapid) improvement of the hemostatic status after thromboelastometric testing. This makes the test results directly after cardiopulmonary bypass used for the prediction of bleeding outdated and unlikely to predict bleeding. Therefore, thromboelastometry might better be used as a comparator to patients' baseline than using absolute values.

Another explanation for the inability to predict bleeding by thromboelastometry might be the discrepancy between reference values and critical (threshold) values. When a value falls outside the reference range the patient might not be at risk, as the clotting capacity might be lower but still sufficient. Additionally, thromboelastometry reference ranges are wide, caused by the multiple factors affecting the test results (i.e. red blood cells, leukocytes, platelets, fibrinogen and coagulation factors). The critical level of hemostatic components, i.e. thromboelastometry test results, might be more appropriate to predict bleeding. A critical level is the threshold sufficient for adequate clot formation and might differ from the reference range. The critical level can be different between patients and different clinical scenario's. Therefore, it is difficult to assess the individual critical value as it might depend on other parameters and clinical circumstances. [44,45] While thromboelastometry test results below the reference range do not predict bleeding, results below a critical threshold might. Future studies should look for the critical value as this might be a better predictor for bleeding and be of guidance for treatment and transfusion.

PART III. MONITORING COAGULOPATHY

The value of thromboelastometry & clinical decision-making

Thromboelastometry is used in cardiac surgery aiming to optimize patient blood management. Thromboelastometry was first described by dr. Hellmut Hartert, almost 70 years ago. [46] Only in the last decades, thromboelastometry has gained renewed interest for the rapid evaluation of patients clotting capacity and usability has improved leading to a more user friendly application. With this method, several aspects of the hemostatic system are evaluated, specific deficiencies can be identified and replenished aiming to improve outcome.

As mentioned before, thromboelastometry does not predict bleeding in cardiac surgery (chapter 5). However, several studies investigated if its implementation reduces bleeding, blood transfusion and improved outcome, shown below in table 1. Besides one study, all found a reduction in blood transfusion and improved outcome compared to the control group. [47] Interestingly, only two randomized controlled trials have investigated the impact of thromboelastometry implementation, of which one has a controversial design (Weber et al. 2012). [48,49] The most recent systematic review addresses the poor quality of the studies on thromboelastometry guided hemostatic treatment in cardiac surgery. [50]

The implementation of thromboelastometry resulted in an increased use of factor concentrates compared to conventional practice. As shown in table 1, there was a general increase in the use of fibrinogen in the thromboelastometry groups without an effect on the use of prothrombin complex concentrate. The use of recombinant activated factor VII ceased. As fibrinogen concentrate use has demonstrated to improve outcome this might bias the positive effect attributed to thromboelastometry implementation. [51-55] The hypothesis that factor concentrates are more effective in treatment of coagulopathy has recently been confirmed in a prospective trial. [56]

The main problem with these (retrospective) studies of thromboelastometry implementation is that it is unclear the implementation of a transfusion algorithm *or* the implementation of point-of-care hemostatic monitoring led to improved outcome. [57] Moreover, the increased use of fibrinogen concentrate can also have been the cause of improved outcome. It remains indistinguishable what the individual additive value of each variable is and which factor causes the improved outcome, the transfusion algorithm *and/or* the point-of-care testing *and/or* increased fibrinogen concentrate use.

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Year	1st author	N.	Design	RR concentrate use			Conclusion
				PCC	Fib	rVIIa	
2016	Karkouti	7402	R	n.a.	1.26	n.a.	Fewer blood transfusion
2016	Trevisan	768	R	2.00	3.00	n.a.	Fewer blood transfusion
2016	Pearse	1295	R	1.25	n.a.	0.78	Fewer blood transfusion
2015	Karkouti	2481	R	1.00	1.25	0.33	Fewer blood transfusion
2013	Fassl	62	R	1.00	0.90	n.a.	Fewer blood transfusion
2012	Weber	100	RCT	0.84	1.07	0.08	Fewer blood transfusion
2012	Hvas	1676	R	n.a.	3.00	0.50	No transfusion difference
2012	Hanke	10	R	0.33	0.66	n.a.	Fewer blood transfusion
2011	Görlinger	3865	R	2.02	2.70	0	Fewer blood transfusion
2010	Girdauskas	56	RCT	0.17	0.87	0.57	Fewer blood transfusion
2007	Spalding	1422	R	0.22	2.00	0	Fewer blood transfusion
2006	Anderson	990	R	n.a.	n.a.	n.a.	Fewer blood transfusion
			Average:	0.98	1.67	0.32	

Table 1.   All studies in cardiac - and/or proximal aortic surgery on rotational thromboelastometry based coagulation management. Fib = fibrinogen concentrate, PCC = prothrombin complex concentrate, R = retrospective study, RCT = randomized controlled trial, RR = relative risk compared to control group, rVIIa = recombinant activated factor VII.

A closer look at the transfusion algorithms shows that there is a large discrepancy in the indication for different treatment options (table 2). For instance, the indication for fibrinogen administration even ranges from a FIBTEM maximum clot firmness (MCF) of 6 to 16 mm. The indication for platelet transfusion varies from an INTEM with or without heparinase MCF of 35 to 50 mm. Besides, several studies use a stepwise approach with reappraisal after each intervention whereas others treat all abnormal values at the same time. The heterogeneity between studies makes it hard to compare the results. This highlights that the critical value, as mentioned earlier, is unknown. Still, the implementation of thromboelastometry based transfusion algorithm seems to reduce bleeding and transfusion. [47,50]

Year	1st author	Indication			
		PCC or FFP	Fibrinogen or Cryoprecipitate	Protamine	Platelet transfusion
2016	Karkouti	$CT_{EX}>90$	$A10_{FIB}<8$	n.a.	$A10_{EX}<35$ **and** $A10_{FIB}>8$
2016	Trevisan	$A10_{EX}<45$ **and** $A10_{FIB}>15$	$A10_{EX}<45$ **and** $A10_{FIB}<15$	$CT_{HEP}/CT_{IN}<0.8$	n.a.
2016	Pearse	$CT_{EX}>90$ **or** $CT_{HEP}>280$	$MCF_{EX}<40$ **and** $MCF_{FIB}<10$	n.a.	$A10_{EX}<40$ **and** $A10_{FIB}>10$
2015	Karkouti	$CT_{EX}>100$	$MCF_{FIB}<7$	Prolonged ACT	$A10_{EX}<35$ **and** $A10_{FIB}>7$
2013	Fassl	$CT_{IN}>240$ **and** $CT_{HEP}>240$	$A15_{EX}>48$ **and** $A15_{FIB}<10$	$CT_{IN}>240$ **and** $CT_{HEP}<240$	$A15_{EX}<48$ **and** $A15_{FIB}>10$
2012	Weber	$CT_{EX}>100$	$MCF_{FIB}<7$	Prolonged ACT	$A10_{EX}<35$ **and** $A10_{FIB}>7$
2012	Hvas	$CT_{HEP}>300$ **or** $CT_{EX}>100$	$MCF_{FIB}<9$	$CT_{IN}>+25\%\,CT_{HEP}$	$MCF_{HEP}<45$ **and** $MCF_{FIB}>9$
2012	Hanke	$CT_{HEP}>280$ **or** $CT_{EX}>90$	$MCF_{EX}<50$ **and** $MCF_{FIB}<16$	$CT_{IN}>240$ **and** $CT_{HEP}/CT_{IN}<1.0$	$MCF_{EX}<50$ **and** $MCF_{FIB}>16$
2011	Görlinger	$CT_{EX}>90$ **or** $CT_{HEP}>280$	$A10_{EX}<40$ **and** $A10_{FIB}<10$	$CT_{IN}>240$ **and** $CT_{HEP}/CT_{IN}<0.8$	$A10_{EX}<40$ **and** $A10_{FIB}>10$
2010	Girdauskas	$CT_{HEP}>260$	$MCF_{FIB}<8$	$CT_{IN}/CT_{HEP}>1.5$	$MCF_{HEP}<35$ **or** (MCF_{HEP} 35-45 **and** $MCF_{FIB}>8$)
2007	Spalding	n.a.	n.a.	n.a.	n.a.
2006	Anderson	$CT_{IN}>240$ **and** $CT_{HEP}>240$	$MCF_{IN}<50$ **and** $CFT_{IN}<30$ **and** $MCF_{FIB}<6$	$CT_{IN}>240$ **and** $CT_{HEP}<240$	$MCF_{IN}<50$ **and** $CFT_{IN}<30$ **and** $MCF_{FIB}>6$

Table 2. Indications for administration of hemostatic interventions in all studies in cardiac - and/or proximal aortic surgery on rotational thromboelastometry based coagulation management. CT = clotting time, EX = EXTEM, FFP = fresh frozen plasma, FIB = FIBTEM, HEP = HEPTEM, IN = INTEM, MCF = maximum clot firmness, PCC = prothrombin complex concentrate.

Most studies have a retrospective sequential cohort design, comparing outcome before and after implementation of a thromboelastometry guided treatment algorithm. This is a major limitation as the implementation of a treatment algorithm with or without thromboelastometry creates awareness for the coagulopathic component of bleeding. This might lead to earlier, more adequate and more aggressive treatment of coagulopathy resulting in better outcome, possibly biasing the study results. This hypothesis is substantiated by the finding that studies implementing a general (non-thromboelastometry) blood management protocol showed improved outcome after implementation, shown in table 3.

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Year	1st author	N.	Design	Intervention	Outcome
2015	Gross	2662	R	PBM protocol	Fewer transfusion, better outcome
2014	Bilecen	2534	R	Tailor made transfusion protocol	Fewer transfusion and myocardial infarction
2013	Silva	533	R	PBM protocol	Fewer blood transfusion
2013	Gutsche	495	R	Anemia guideline implementation	Fewer unnecessary transfusions
2012	Xydas	1032	R	PBM protocol	Fewer blood transfusion
2001	Capraro	58	RCT	Hemostasis monitoring vs. clinical judgement	No difference in outcome

Table 3. Studies investigating the effect of blood management (PBM) implementation on outcome. R = retrospective study, RCT = randomized controlled trial.

Still, several guidelines suggest that using thromboelastometry improves outcome. [58,59] However, the clinical judgement of the cardiac anesthesiologist should not be neglected. Together with the cardiac surgeon the anesthesiologist determines if there is excessive microvascular bleeding, the first decision in all blood management protocols.

In the era before point-of-care testing the anesthesiologist decided whether to treat possible coagulopathy after bypass. This was based on clinical features as the duration of extracorporeal circulation, the volume of blood loss, type of surgery and patients' characteristics. This advanced clinical assessment of hemostasis is evaluated in **chapter 10**. We showed that the interpretation of the clinical scenario reflects an accurate hemostatic appraisal of the need for fresh frozen plasma transfusion when assessed by thromboelastometry.

The study is limited by its retrospective design and was conducted in a center with focus for hemostasis as many previous studies on hemostasis were conducted in cardiac surgery in this hospital. This might influence the results as the attending team might have more attention for hemostatic abnormalities and gained a more 'advanced' view on a bleeding patient. Furthermore, during the study period the surgical and anesthesia teams included solely senior staff members with ample experience in cardiac surgery. Results might not be reproducible in a more junior team.

The importance of pre-analytic variables

The adequacy of hemostasis monitors to guide patient blood management strategies is of vital importance as wrong information leads to wrong decisions. Errors in blood analysis most commonly occur in the pre-analytic phase. [60,61] This phase resembles all procedures before the testing of the specimen. Here

we will focus on possible errors in hemostatic testing, with special notice for thromboelastometric testing. [62]

Starting with blood withdrawal, insufficient filling of the collecting tube causes increased dilution by the citrate solution leading to an abundance of citrate. When the sample is recalcified prior to the analysis, this can lead to insufficient calcium as it binds to the abundant citrate. The lack of sufficient calcium in addition to the increased dilution of the blood sample can impair clot formation and thereby influence the test results. [63,64] This effect also occurs in thromboelastometry testing, however the effect on the viscoelastometric test results is limited. [65]

During blood withdrawal, the intravascular line from where blood is drawn should be adequately flushed in order to prevent dilution of the blood sample. This is especially important in the case of an heparin or glucose line as these molecules affect the clotting time. [66] The site of blood withdrawal is unimportant as there is no difference between venous and arterial drawn blood, whereas intraosseous blood seems hypercoagulable in vitro. [67,68]

Inappropriate storage can affect clotting test results. Storing blood samples at high or low temperature affects the concentration of (activated) clotting factors. The influence of high temperature is relevant after several hours of storage, causing coagulation factor activation (f.VII) and degradation (protein S, f.VIII). [69,70] Low temperature also affect hemostasis in vivo and vitro. [71-73] As a low temperature slows the clotting process down the blood sample should be warmed to body temperature before (thromboelastometric) testing.

In the case of point-of-care assays, the setting of testing might influence the test results. Thromboelastometry performed at bedside does not change the test results compared to thromboelastometry testing in the laboratory. Still, the results are 11 minutes quicker available when performed at the point-of-care. [74] When decided to perform thromboelastometry in the laboratory, the pneumatic tube transport system affects the test results. [75-78] However, this effect is clinically irrelevant for most assays. The inter- and intra-rater variability does not significantly influence viscoelastometric testing. [79-81] The type of testing cups alters the results, therefore smaller cups should not be used. [82]

Patient and medication factors affect viscoelastometric testing. The hematocrit is inversely correlated with the maximum clot firmness, resulting in denser clot results for low red blood cell concentrations. [83-85] This appears to be an in vitro artefact and clinicians should therefore take care in interpretation of the thromboelastometry test results in anemic patients as this might mimic solid clot formation. [86] This is especially relevant in a bleeding patient. Furthermore, suggamadex seems to alter the test results towards a more thrombogenic profile,

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which is also attributed to an in vitro artefact. [87] The (pre)analytic factors influencing thromboelastometry are summarized in figure 3. The clinician working with thromboelastometry should be well aware of these influences in order to prevent inadequate decision making.
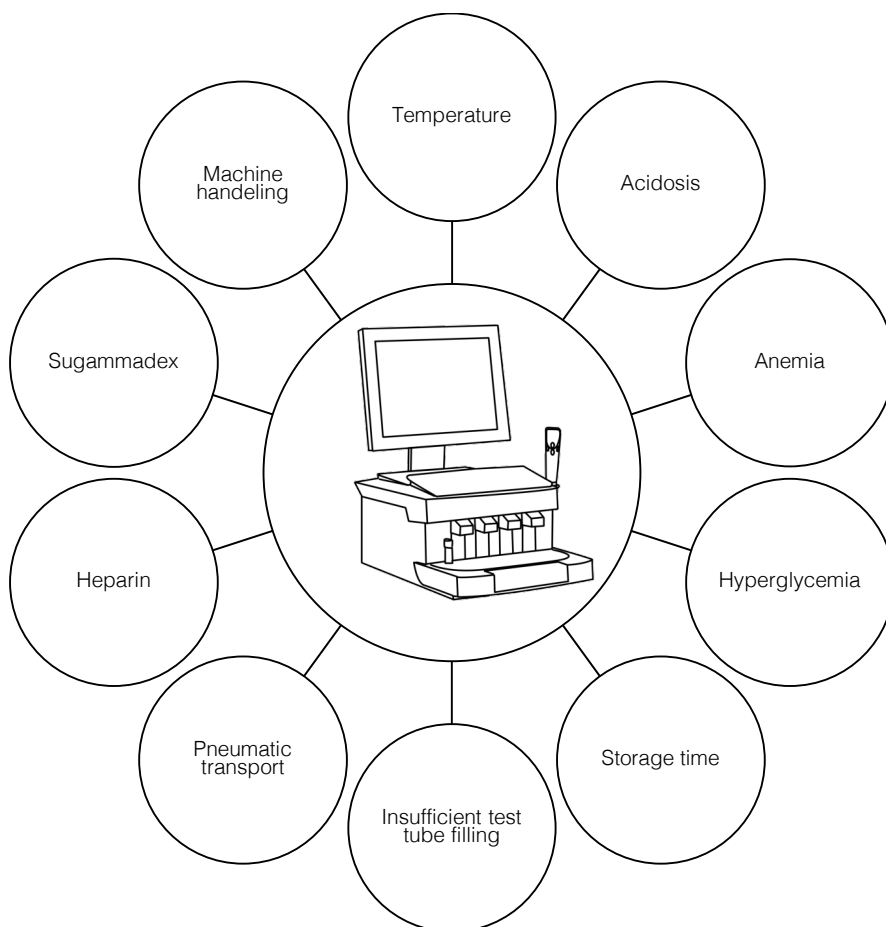

Figure 3. (Pre)analytic influences on thromboelastometry. (Copyright M.I. Meesters 2017)

I. Non-activated Thromboelastometry (NATEM)

In 2010, the stability of thromboelastometric testing was evaluated and showed constant results up to 120 minutes of storage. [88] This study only investigated the activated clotting tests, e.g. INTEM, EXTEM and FIBTEM. Still, the reproducibility

of the non-activated test (NATEM) has never been investigated. As the NATEM test requires much time until test results are available it is not used in routine clinical practice. However, an increasing number of studies use NATEM, while it is very sensitive to hemostatic changes. For example, fibrinolysis is rarely observed in routine viscoelastometric tests (INTEM and EXTEM), whereas NATEM is very sensitive to this phenomena. [89] Likewise, the effect of fractionated heparin is frequently not detected during routine thromboelastometry testing whereas the NATEM test is significantly affected by low molecular weight heparins. [90]

Since the NATEM test is very sensitive, also to pre-analytic variables, we investigated its stability for citrated blood samples over storage time in healthy volunteers and intensive care patients, **chapter 8**. We showed significant coagulation activation during storage. Endotoxin, released by gram negative bacteria during infection, might be responsible for this activation in the intensive care population. [91] However, our study in healthy volunteers showed similar results.

Future directions

The findings from our NATEM study are relevant since there will be a difference in test results between direct (near patient) testing and testing in the laboratory as transportation and handling of the specimen requires time. This phenomenon must be taken into consideration in addition to the influence by pneumatic transportation on the test results, which both lead to considerable activation of the blood sample. [78] Repeat sampling from the same citrated collecting tube has been shown to give a progressive hypercoagulable trace which might bias test results. [92] Future studies on the effect of citrate storage time on thromboelastometric test results should therefore warrant not to reuse the same collecting tube at different time-points.

Several studies found a difference between fresh and citrated whole blood. [92-94] This might be caused by the activation of non-citrated blood in the test tube, as the wall of the test tube or syringe may function as a coagulation activator. Therefore, citrated blood sampling and testing is preferred. When non-citrated whole blood is used, the time between blood withdrawal and testing should be minimized in order to prevent hemostatic activation of the blood sample.

II. Heparinase-modified thromboelastometry

Addition of heparinase to a blood sample is an old method to investigate the coagulation capacity without the effect of heparin, as it was first used in 1972 in laboratory coagulation testing. [95] This principle is also used in the

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thromboelastometry heparinase assay (HEPTEM). The HEPTEM test consists of the INTEM test with the addition of heparinase, which inactivates heparin by cleavage of the molecule. In the case of a heparin effect, the INTEM clotting time (CT) will be prolonged whereas the heparin effect will be omitted in the heparinase assay (HEPTEM), resulting in an $CT_{INTEM} > CT_{HEPTEM}$.

The first viscoelastometric testing with heparinase investigated the hemostatic potential during cardiopulmonary bypass in the setting of pre- and peroperative anticoagulation with heparin. [96] In 2001, the heparinase assay was used to guide transfusion prior to heparin reversal and indicate the need for additional protamine. [97,98]
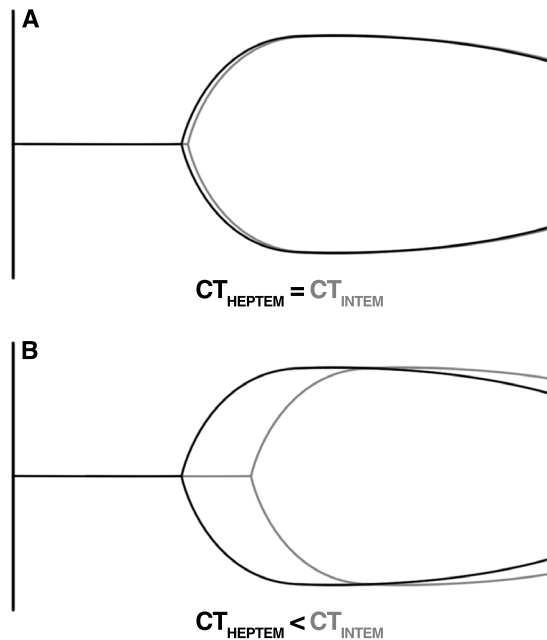
Figure 4. The HEPTEM and INTEM assay before (panel A) and after (panel B) the administration of heparin. (Copyright M.I. Meesters 2017)

Later, the first evaluation of the clinical validity of the rotational thromboelastometry heparinase assay was performed. [99] The in vitro results showed a good neutralizing effect of heparinase on the heparin influence. Interestingly, it was found that protamine overdosing could be detected by the HEPTEM test as clotting times prolonged without affecting the $CT_{INTEM} : CT_{HEPTEM}$ ratio. These results were reproduced in a clinical study of 22 patients undergoing cardiac surgery.

[100] It was suggested that the heparinase assay can be helpful to distinguish between a heparin or coagulopathic cause of a prolonged activated clotting time (ACT) after heparin reversal.

More recently several studies found abnormal results in the HEPTEM assay as fibrinolysis was inappropriately shown. [101,102] Furthermore, it was found that heparin concentrations greater than 0.5 IU ml^{-1} (as during CPB) resulted in a dose depend increase in HEPTEM clotting time, indicating incomplete heparin neutralization by heparinase. [103] However, this was later refuted by others. [104] In clinical practice, we frequently observed unusual thromboelastometric results for the CT_{INTEM} : CT_{HEPTEM} ratio. Therefore, we conducted an analysis of all thromboelastometric test results in cardiac surgery in two centers, shown in **chapter 7**. The CT_{INTEM} : CT_{HEPTEM} ratio frequently showed irrational results. Prior to surgery the heparinase assay was frequently shorter than the INTEM assay while these patients did not receive heparinoids on the day of the procedure. Moreover, before and after surgery the CT_{HEPTEM} was often longer than the CT_{INTEM}. We hypothesized that this might be explained by high protamine dosing, having more effect on HEPTEM than INTEM test results. However, this theory is not supported by previous reports. [99,100] Also, the preoperative difference cannot be explained by this hypothesis. Still, several studies showed an $CT_{INTEM} < CT_{HEPTEM}$. [105-107] Unfortunately, so far no rationale for this finding could been found.

Limitations & Future directions
In line with a previous report we found a dose dependent HEPTEM clotting time increase with protamine dosage. [104] A major limitation of the in vitro part of our study is the lack of INTEM testing to prove the hypothesis that protamine affects HEPTEM without changing INTEM results. The previous study did not find a discrepant increase in HEPTEM compared to INTEM. [104]

Several other studies showed an CT_{INTEM} < CT_{HEPTEM}. [105-107] However, the scarce studies specifically investigating the INTEM : HEPTEM relationship did not find illogical differences between INTEM and HEPTEM. [100,108] Therefore it is difficult to draw conclusions about the validity or need for caution when interpreting the HEPTEM test results. Future studies should investigate if our results can be reproduced. Furthermore, the adequacy of the HEPTEM test should be further investigated to explore on our unusual test results. [101,102]

III. Point-of-care Prothrombin Time
Point-of-care prothrombin time testing (POC-PT) has been developed for rapid outpatient management of patients on vitamin-K-antagonists. [109,110] However,

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these tests might also be useful for the prompt evaluation of patients' coagulation status after cardiopulmonary bypass. The great advantage of the POC-PT test is the short turnaround time of less than 5 minutes whereas sending and testing a blood sample in the central laboratory takes up to 45 minutes. Another advantage of the POC-PT is the testing off whole blood compared to plasma in the laboratory test. This eliminates several preanalytical variables affecting the test results including; insufficient tube filling, delayed transport, centrifugation errors, storage time and temperature. [111]

Still, there are some pre-analytic variables influencing the POC-PT. Anemia (hematocrit < 30%) has been found disturb the test results. [112] Also, severe coagulopathy (INR > 4.0) causes a significant discrepancy between laboratory and point-of-care testing. [113,114] However, the difference at higher INRs does not affect the clinical decision in cardiac surgery as an INR > 1.5 is an indication for coagulation factor replenishment irrespective of the exact INR. Also, a low fibrinogen level disturbs the POC-PT test results. [115] Furthermore, direct oral anticoagulants (DOACs) disturb POC-PT results without influencing the laboratory PT. [116,117] Besides, it is suggested that liver enzyme abnormalities might change the test results. [118] Finally, there is a small, clinically irrelevant difference between capillary and venous blood sample for POC-PT testing. [119]

The applicability of the POC-PT in cardiac surgery has only scarcely been investigated. [120,121] Therefore we investigated the accuracy of the POC prothrombin time measurement after cardiopulmonary bypass. In contrast to previous reports, we found a major discrepancy between POC-PT and laboratory results 3 minutes after protamine administration, as shown in **chapter 8**. Previous studies found a good agreement between the POC-PT and laboratory test at least 10 minutes after protamine administration which might have caused the discrepancy from our results. [120,121] In order to investigate if the difference between the POC and laboratory test persisted over time we conducted another investigation. We evaluated the agreement between the laboratory and point-of-care PT 3, 6 and 10 minutes after protamine reversal in **chapter 9**. In contrast to previous reports the difference between laboratory and POC-PT remained clinically and statistically significant 6 and 10 minutes after protamine administration. We conclude that the POC-PT cannot be used after cardiac surgery with cardiopulmonary bypass. Figure 5 summarizes all (pre)analytic influences on the POC-PT.
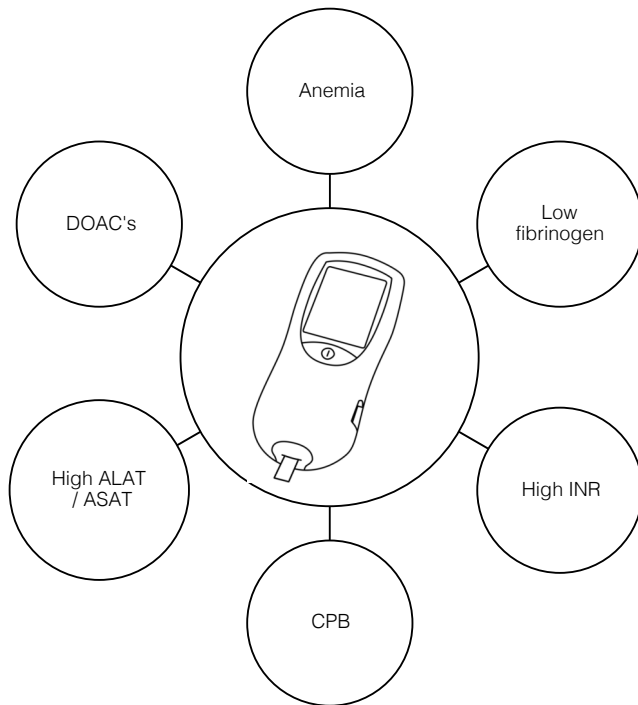
Figure 5. (Pre)analytic influence on the point-of-care prothrombin time device (Coaguchek XS Pro, Roche, Basel, Switzerland). (Copyright M.I. Meesters 2017)

Limitations & Future directions

Most studies inestigating the accuracy of a novel diagnostic device perform Pearson correlation and/or Bland Altman analysis. [122] However, Error Grid analysis as performed in our POC-PT studies is superior as it investigates the diagnostic accuracy of a novel test in the clinical context. [123] As mentioned before, an INR > 4 that highly differs between point-of-care and laboratory testing has a major influence on the Bland Altman and (Pearson) correlation analysis. However, this difference might be clinically irrelevant as an INR >1.5 is an indication for coagulation factor replenishment in the bleeding patient, irrespective of the exact INR. This limitation is overcome by Error Grid analysis which incorporates the clinical consequences in the correlation between the tests.

Besides the discrepancy between the laboratory and point-of-care PT test results, an abnormal PT does not (necessary) mean insufficient thrombin generation and the subsequent need for hemostatic intervention. [43] Besides, the prothrombin time after cardiac surgery does not predict bleeding. [39-41] For this reason

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it is frequently suggested that PT cannot guide hemostatic treatment and thromboelastometry should be used, however neither thromboelastometry predicts bleeding, as shown in chapter 5. Still, thromboelastometry guided coagulation treatment has proven to reduce transfusion, unlike classical coagulation tests. [50] A limitation of the use of POC-PT testing is the lack of information about the other hemostatic components which might cause bleeding, mentioned in the introduction and shown in figure 6. Therefore, an adequate POC prothrombin time test should be combined with a point-of-care aPTT (to detect residual heparin), - fibrinogen and - thrombocyte count to identify the (coagulopathic) source of bleeding.
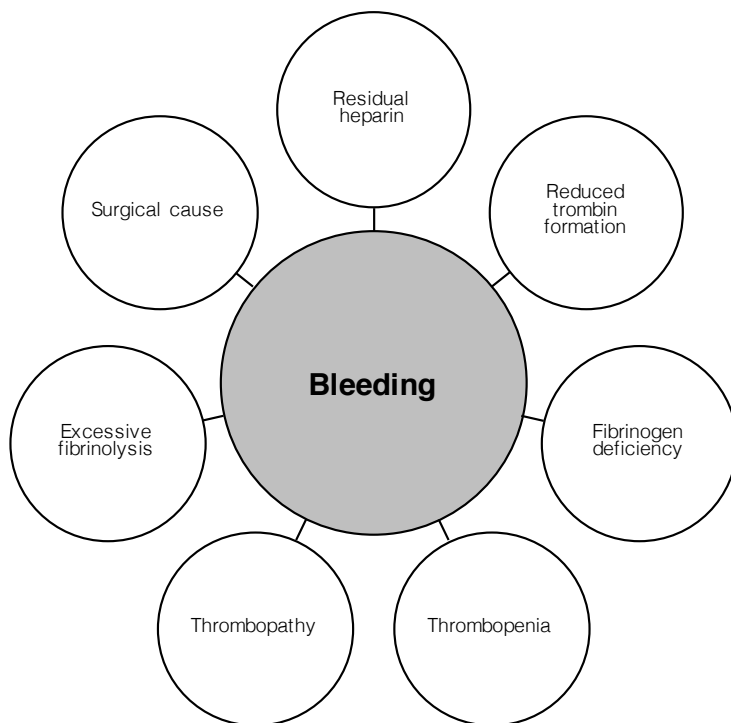
Figure 6. Postoperative causes of bleeding (Based on: Ranucci M. Hemostatic and thrombotic issues in cardiac surgery. Semin Thromb Hemost; 2015 Feb;41(1):84–90.)

PART IV. TREATMENT OF COAGULOPATHY

Does the focus on patient blood management risk the patient for thrombosis?
Patient blood management focusses on the reduction of bleeding and blood transfusion in order to improve outcome. However, as hemostasis is a balance between pro- and anti-coagulatory factors (figure 7) it is of major importance to evaluate the individual patient blood management components for the risk of pathologic thrombogenicity, e.g. the risk for thrombosis and infarction.

A suitable example is the highly procoagulant drug factor VIIa (fVIIa). Recombinant factor VIIa was designed for the treatment of patients with hemophilia who became resistant to coagulation factor replacement due to antibody formation. [124] As the drug has powerful capacity to stop bleeding its use spread to high risk surgery such as cardiac procedures. Activated factor VII showed a reduction of bleeding, rethoracotomies and transfusion in cardiac surgery suggesting a positive effect on outcome. [125] However, a Cochrane review showed that a critical percentage of the patients developed serious adverse thromboembolic events. [126] This highlights the importance of a thorough evaluation of the possible negative effects of an intervention to detect serious adverse events, preferably in several large multicenter randomized controlled trials with sufficient power. Only hereafter the risk benefit balance can be made for each treatment modality. In our interventional studies we did not find an increased incidence of adverse events, other than an effect on bleeding and transfusion. Still, the studies were not powered for this purpose.

| Procoagulant | Anticoagulant |
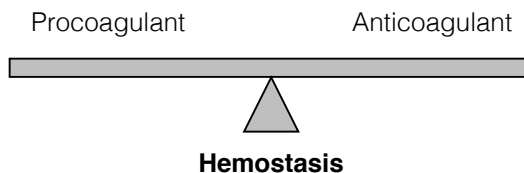
Hemostasis

Figure 7. Physiological hemostasis is the balance between pro- and anticoagulants.

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The problem with many new diagnostic and treatment modalities is that they spread from other fields and are used off-label. This also goes for the use of prothrombin complex concentrate (PCC) which was developed for the acute reversal of vitamin K antagonist therapy. Only two RCTs on the use of PCC for coagulation factor replenishment were performed in the cardiosurgical setting. [127,128] Only one of these was designed for the treatment of postoperative coagulopathy in the bleeding patient. Nevertheless, as mentioned before with

the introduction of thromboelastometry many studies started using PCC for the treatment of coagulation deficiency without available safety data on the factor concentrates in cardiac surgery. [129,130]

It can be argued that evidence regarding the applicability and safety data of a drug can be extrapolated from other fields. [131] However, a recent report showed an increase risk on thromboembolic events with the use of factor concentrates including PCC. [132] More recently, even an absolute increase in adverse events has been observed with PCC use specifically in cardiac surgery. [133] Yet, this might be explained by the increase in PCC use without a relative increase in complications caused by the drug.

The risks of new drugs use should be weight against the risk-benefit ratio of the alternative. In the case of PCC, the alternative is fresh frozen plasma for which neither sufficient evidence is available. Yet, adverse events are well described for FFP, including an increased thrombotic risk, which might justify the use of PCC. [134 -136]

Besides the possible thrombogenic effects of a patient blood management intervention, all possible adverse events should be evaluated. The American Heart Association recently proposed to standardize the outcome of clinical trials in cardiac surgery. [137] They found that there is a high heterogeneity in the reporting of research findings. This hinders the possibility to compare outcome and limits the results of meta-analysis. In 2009 the Society of Thoracic Surgeons (STS) formulated relevant outcome parameters for studies in cardiac surgery. [138] These parameters include mortality, renal failure, stroke, rethoracotomy, prolonged mechanical ventilation, sternal wound infection and mortality. However, despite the formulation of these outcome parameters the adaptation in research is poor. [137] Still, it is important to keep general outcome (morbidity and mortality) in sight when investigating patient blood management strategies. Blood management should not be the sole purpose while overall better outcome for the patient should be.

Is patient blood management being implemented in clinical practice?

In the last two decades, many patient blood management interventions have been developed in cardiac surgery. [139] Especially the combination of several (small) interventions lead to a major reduction in blood loss and blood transfusion. [1,2] Unfortunately, despite the publication of an extensive patient blood management guideline in 2007 (updated in 2011), there is still an increase in blood product utilization in many cardiac surgery centers. [58,140,141] This can partly be explained by the increase in complexity of surgery and change of the surgical

population. However, although increasing blood conservation evidence emerges, general implementation seems to fail. This may be due to the fact that many hospitals do not follow patient blood management guidelines, [142,143] resulting in a large heterogeneity in blood loss between centers. [144-147] Transfusion rates even vary from 0 to 50% for platelet concentrate and 0 to 100% for red blood cell concentrates and fresh frozen plasma between institutions, suggesting room for improvement in many centers. [148]

In 2005 almost 700.000 cardiac surgery procedures were performed in the United States alone. [149] As blood loss and transfusion lead to adverse outcome many blood transfusions, morbidity and mortality can be prevented when the adherence to patient blood management guidelines would increase. This is not the only benefit of patient blood management, as many studies have shown cost effectiveness of implementation of blood conservation methods, including thromboelastometry [150], cell salvage [151], preoperative erythropoietin use [152], tranexamic acid [153], and more.

Currently, a European guideline on patient blood management in cardiac surgery is under development. This guideline is a collaboration between the society of cardioanesthesiology and cardiothoracic surgery, the European Association CardioThoracic Anesthesiologist (EACTA) and European Association CardioThoracic Surgeons (EACTS) respectively. This collaboration hopefully creates more awareness and concern for patient blood management on both sides of the sterile drape, improving health care in cardiac surgery. The publication of the guideline is planned for the end of 2017.

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CONCLUSION

Patient blood management aims to *prevent*, *predict*, *monitor* and *treat* hemostatic disturbances in order to reduce blood loss. Bleeding leads to anemia, blood transfusion and can result in the need for reoperation, all with an independent negative effect on patient outcome. The current thesis aimed to asses, improve and develop strategies in all phases of patient blood management in cardiac surgery.

First, we focused on the *prevention* of postoperative coagulopathy by the optimization of protamine dosing. We demonstrated that protamine, in the absence of heparin, impairs hemostasis in the clinical setting. This highlights the importance of adequate protamine dosing which, as we showed, can be improved by the implementation of a pharmacokinetic model for protamine dosing. Second, we showed that thromboelastometry cannot be used to *predict* which patients are at risk for major hemorrhage. Third, we critically investigated the applicability different point-of-care hemostasis *monitors*. We showed that the applicability of the thromboelastometry HEPTEM test in clinical practice deviates from the findings of previous research reports. Additionally, we noted that the storage time of the citrated blood sample affects the NATEM test results. Furthermore, we found that the POC-PT assay cannot be used in cardiac surgery as test results are inaccurate after cardiopulmonary bypass. Finally, we highlighted the importance of the clinical judgement of the anesthesiologist in patient blood management and evaluated the *treatment* of coagulopathy by fresh frozen plasma and fibrinogen concentrate. These investigations led to the more knowledge in all fields of patient blood management aiming for improved outcome.

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Appendix

English summary

ENGLISH SUMMARY

Cardiac surgery is performed in patients with severe atherosclerosis of the coronary arteries, cardiac valve disease and/or aortic abnormalities. In order to perform most type of surgery on the heart no blood should be flowing through as this would lead to massive blood loss and operating on a beating heart is difficult. For this reason, the blood flow, oxygenation and ventilation are taken over by a heart lung machine (HLM) also known as cardiopulmonary bypass (CPB), shown in figure 1. In order to bypass the heart, blood is removed prior to the heart and pumped into the aorta, which is the major artery suppling blood to the body.
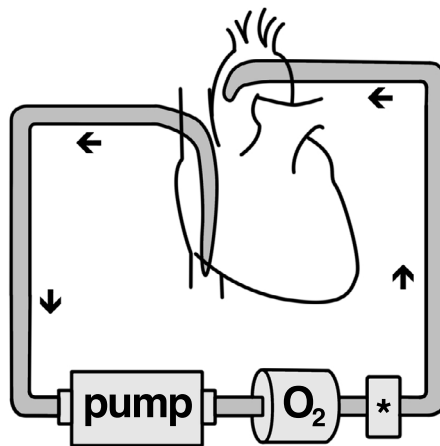
Figure 1. A schematic overview of the heart lung machine. Blood is sucked from the right atrium, oxygenated by an oxygenator and pumped in the aorta, bypassing heart and lungs. (Copyright M.I. Meesters 2017)

When the heart is stopped by the administration of cardioplegia fluid, surgery can safely be performed while oxygen transport is assured by the heart lung machine. In order to prevent clot formation in the heart lung machine, the patients' blood is anticoagulated by administration of heparin. After surgery, the patient is weaned from the heart lung machine, and blood coagulation needs to be restored to prevent blood loss from the surgical wound. This is done by administration of protamine, a drug that binds and inactivates heparin. However, as protamine itself has anticoagulant properties, overdosing of protamine should be avoided. On the other hand, insufficient protamine will lead to residual heparin, which inhibits coagulation. Adequate protamine dosing is required to prevent blood loss due to anticoagulation, as shown in figure 2.

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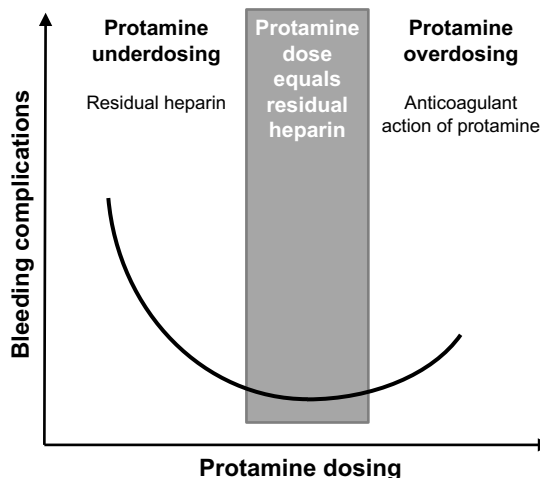
Figure 2. A schematic overview of the importance for adequate protamine dosing.

When the patient is bleeding despite adequate heparine reversal, prompt intervention is necessary as bleeding leads to loss of red blood cells and clotting factors. This might result in blood transfusion and possible reoperation when bleeding does not cease. Bleeding, blood transfusion and reoperation for bleeding are associated with an increased risk of infection and death, highlighting the importance of rapid treatment of coagulation abnormalities.

In case of bleeding due to coagulation abnormalities, adequate supplementations of the deficient coagulation factors is necessary. This can be guided by rapid coagulation tests, such as thromboelastometry or other point of care coagulation assays. Another approach is transfusion based on patient and surgical characteristics guided by the experience of the attending anesthesiologist.

Chapter 1 describes the complexity of all possible modalities to *prevent*, *predict, monitor* and *treat* bleeding in cardiac surgery. These components are usually referred to as 'patient blood management'. There are several ways to prevent bleeding after cardiac surgery including: cessation of drugs that inhibit coagulation before surgery, administration of medication to optimize patients clotting capacity and anemic patients can be treated to improve their red blood cell concentration. The patient at risk for bleeding can be identified by the use of risk scores based on patients and surgical characteristics and advanced clotting tests. In the case of ongoing hemorrhage there are several drugs that can be used to prevent further bleeding, which drug should be used can be guided by the use of rapid clotting monitors.

The thesis starts with methods to *prevent* bleeding, including optimization of protamine dosing. As mentioned before, protamine is used to reverse the anticoagulant properties of heparin after cardiac surgery. However, in laboratory studies it has been found that protamine acts as an anticoagulant in the absence of heparin. **Chapter 2** summarizes what is known about protamine starting from its origin and the discovery of the heparin neutralizing effect. The pharmacological properties and well-known side effects are reviewed, including anaphylaxis and the effect on heart and blood vessels. However, the emphasis is on the negative effect of protamine on coagulation including blood platelets, coagulation factors and clot breakdown. Moreover, the effect of protamine on several coagulation testing methods is discussed. Finally, methods for protamine dosing and alternatives to protamine are reviewed.

In **chapter 3** we investigated if the anticoagulant effect of protamine is clinically significant and influences the amount of blood loss and coagulation status in patients undergoing cardiac surgery. We therefore randomized patients to low or high protamine dosing, all within the spectrum of clinically used dosages. We found that the high dosing group had significantly more blood loss and needed more blood transfusion compared to the low dosing group. This can be explained by the anticoagulant effect of protamine as clotting tests were worse in the high dosing group. This is the first study to prove that too much protamine in patients impairs the coagulation system and has a clinical significant effect on bleeding.

In order to further improve protamine dosing, we investigated a novel dosing regime in **chapter 4**. The method estimates the amount of heparin at the moment that protamine is planned to be administrated. This is realized by a complex pharmacological model that estimates the patient blood volume and takes the administrated dose of heparin and loss of heparin due to its breakdown into account. When the amount of heparin is known, the amount of protamine needed can easily be calculated. We compared the clotting capacity and need for blood transfusion before and after the implementation of the novel dosing strategy. We found that the new protamine dosing method reduced the need for blood transfusion and improved clotting when compared to regular protamine dosing.

Next, the thesis focusses on the *prediction* of bleeding. In **chapter 5** we systematically reviewed the literature on the predictive value of the advanced coagulation monitoring device rotational thromboelastometry (figure 3). This device has been developed for the rapid detection and guidance of coagulation abnormalities at the point of care for the patient in the case of (severe) bleeding. Although rotational thromboelastometry was developed to guide coagulation treatment it remained unclear if abnormal thromboelastometry test results risk

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patients for bleeding. Therefore, we performed an analysis of the predictive value of thromboelastometry in our center and additionally performed a systematic literature search which resulted in 1311 publications of which 10 regarded the predictive value of rotational thromboelastometry in cardiac surgery patients. We combined the results of our study with the results of the 10 publications and concluded that rotational thromboelastometry does not predict which patients are at risk for bleeding.
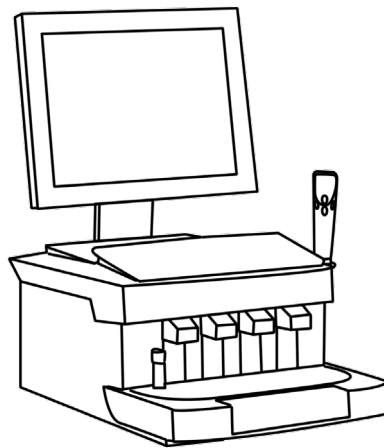

Figure 3.   The rotational thromboelastometry test device. (Copyright M.I. Meesters 2017)

During cardiac surgery patients frequently develop coagulopathy (e.g. coagulation abnormalities) due to blood loss, the surgical wound and the use of the cardiopulmonary bypass system. Several hemostasis monitors have been developed to measure patients' hemostatic capacity and guide hemostatic treatment. We investigated the adequacy of different devices to *monitor* coagulation abnormalities during cardiac surgery.

Thromboelastometry includes several tests to investigate different aspects of the coagulation cascade. These tests measure the coagulation capacity and include assays with (EXTEM and INTEM) and without (NATEM) artificial coagulation activators. Another test focuses on a specific important coagulation factor (fibrinogen), the FIBTEM test. The HEPTEM test is same as the INTEM test with the addition of heparinase. Heparinase is an enzyme that breaks down heparin and thereby investigates the coagulation capacity without the effect of heparin. Heparin prolongs the INTEM test results. The HEPTEM test, without a heparin effect, should only show equal or shorter clotting test results compared

to the INTEM test. However, in clinical practice we found that the HEPTEM test is frequently longer than the INTEM test. Furthermore, we recurrently found a difference in HEPTEM and INTEM test results before the administration of heparin before surgery. In **chapter 6** we investigated the incidence of these abnormal results in cardiac surgery patients of two university hospitals. We found that 15 – 36% of the HEPTEM-INTEM assays in routine cardiac surgery showed abnormal test results. Furthermore, our results suggested these aberrant HEPTEM results might be caused by protamine (over)dosing. However, further studies should elaborate on this hypothesis.

Besides the HEPTEM assay we investigated the accuracy of the thromboelastometric NATEM test. The non-activated rotational thromboelastometric assay (NATEM) does not use any artificial activators and is therefore very sensitive to changes in patients' coagulation status. The test is increasingly used in research due to its high sensitivity. In **chapter 7** we investigated if the storage time of the patients' blood before the start of the test influences NATEM test results. We drew blood in healthy volunteers and intensive care patients and directly performed the NATEM test. We repeated the test after 45 and 90 minutes and evaluated if the test results changed after storage. We found that over storage time the blood clotted quicker as measured by the NATEM test. This suggests that the coagulation cascade gets activated within the blood sample over time. The regular thromboelastometry tests used for clinical practice have previously shown that blood storage does not affect the test results up to 6 hours after blood withdrawal. However, we found that the NATEM test is so sensitive to notice this change. Unfortunately, we could not identify the factor that changed the test results over time. We advise to start the test as soon as possible after blood withdrawal in order to prevent biased test results due to the storage time. Future, research should evaluate the factor(s) responsible for the activation of clotting over time in the stored blood sample.

Besides, thromboelastometry there are other devices which rapidly investigate the coagulation capacity of a patient, an example is the point-of-care (POC) prothrombin time (PT) as shown in figure 4. The PT is a conventional laboratory coagulation assay, however as mentioned before these tests require much time. To overcome this limitation the POC-PT was developed where the test is modified to obtain the test results more rapidly and near the patient, at the 'point-of-care'. As the test was developed for patients on anticoagulation medication the device was not validated for the use in cardiac surgery. Therefore, we investigated the accuracy of the POC-PT in cardiac surgery as described in **chapter 8**. Before and after cardiac surgery we drew a blood sample and investigated if the POC-PT corresponded with the laboratory PT. Before cardiac surgery there was a good

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agreement between the rapid POC-PT and the laboratory, however 3 minutes after protamine administration after cardiac surgery the test results were very different. In order to investigate if after 6 or 10 minutes after protamine administration the test results would agree we performed another investigation, as described in **chapter 9**. However, also 6 and 10 minutes after protamine administration the results remained much shorter in the POC-PT assay. Therefore, we concluded that the POC-PT is not applicable in cardiac surgery.
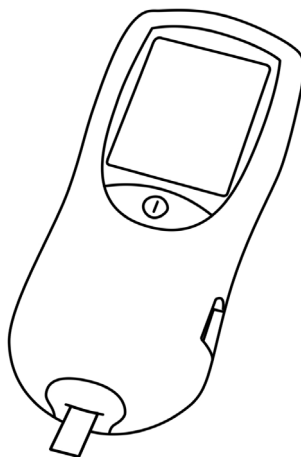
Figure 4. The point-of-care prothrombin time device. (Copyright M.I. Meesters 2017)

Hereafter we focused on *treatment* options for hemostatic abnormalities. In the case of coagulopathy patients might require transfusion of coagulation factors and/or blood platelets. It remains difficult to determine the need for transfusion of coagulation factors, i.e. fresh frozen blood plasma composed from donated blood. Conventional coagulation tests can indicate a deficiency in coagulation factors, however these tests are time consuming and in the bleeding patient the time to wait for the test results is lacking. Therefore, the decision to transfuse coagulation factors is frequently based on clinical signs of bleeding. These signs usually include oozing of blood from the surgical field, long time connection to cardiopulmonary bypass and complex surgery. However, thromboelastometry can help to guide the need for coagulation factor supplementation as test results are rapidly available. In **chapter 10** we investigated if the clinical decision to transfuse blood plasma is confirmed by the findings of thromboelastometry. We found that the clinical decision of the (experienced) cardiac anesthesiologist to transfuse plasma is accurate in 95% of the cases when compared to thromboelastometric

test results.

Another method to treat coagulopathy is the administration of pharmaceutically produced coagulation factor concentrates instead of plasma transfusion. A recent study by Bilecen and colleagues investigated if the administration of fibrinogen concentrate (a specific coagulation factor concentrate) reduced bleeding after cardiac surgery. (*Effect of Fibrinogen Concentrate on Intraoperative Blood Loss Among Patients With Intraoperative Bleeding During High-Risk Cardiac Surgery: A Randomized Clinical Trial. JAMA, 2017 317(7), 738–747*) They concluded that fibrinogen concentrate did not reduce bleeding during cardiac surgery. In our letter to the editor (**chapter 11)** we noted that blood loss during surgery is not a relevant endpoint as most bleeding occurs after surgery and their study showed that fibrinogen concentrate did reduce this amount of blood loss. Furthermore, fibrinogen was administrated to patients who were not deficient in fibrinogen. These notes tone down the strong conclusion drawn by the authors.

In **chapter 12** we describe the limitations of the studies of this thesis. Furthermore, the results from the individual chapters are combined and placed in perspective. First, the results of the protamine dosing studies and directions for future studies are given. Hereafter, predictors for bleeding after cardiac surgery are discussed. Then the implementation of thromboelastometry and the current evidence for its use is elaborated. It is highlighted that most studies on thromboelastometry guided coagulation therapy are limited by the implementation of the device *and* the use of coagulation factor concentrate *and* a transfusion algorithm, making it difficult to determine the additive value of each individual component. Also, the evidence and risks of the use of fibrinogen concentrate are discussed. Next, the disturbing factors in thromboelastometry testing are highlighted in addition to the storage time and use of heparinase. The clinical applicability and validation of point of care coagulation testing is shortly reviewed. Finally, the possible 'dark side' of active patient blood management: thrombosis (excessive coagulation) is examined.

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# Nederlandse samenvatting

NEDERLANDSE SAMENVATTING

Openhartoperaties worden verricht bij patiënten met ernstige aderverkalking van de kransslagaders van het hart en/of afwijkingen aan een hartklep en/of de grote lichaamsslagader (de aorta). Om de meeste vormen van hartchirurgie te kunnen uitvoeren is het van belang dat er geen bloed door het hart stroomt en het hart stilligt. Dit is noodzakelijk omdat er anders veel bloedverlies op zou treden, en het kloppen van het hart het opereren bemoeilijkt. Daarom wordt de functie van hart en longen overgenomen door een hart-longmachine, zoals schematisch weergegeven in figuur 1. Voordat het bloed via de rechter boezem het hart instroomt wordt het naar de hartlongmachine geleid. Aldaar wordt het bloed van zuurstof voorzien en van koolzuur ontdaan. Vervolgens wordt dit bloed in de grote lichaamsslagader gepompt, waardoor het lichaam van zuurstofrijk bloed wordt voorzien. Wanneer de hart- en longfunctie door de hartlongmachine is overgenomen kan het hart stil worden gelegd.
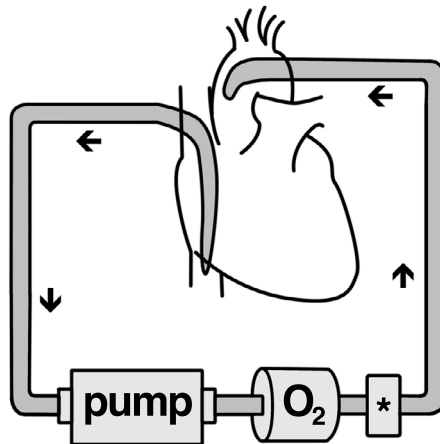
Figuur 1. Een schematische weergave van de hart-longmachine. Bloed wordt voor het hart weggezogen, van zuurstof voorzien en vervolgens het lichaam ingepompt via de grote lichaamsslagader. (Copyright M.I. Meesters 2017)

Als het hart is stilgelegd kan er veilig aan het hart worden geopereerd terwijl het zuurstoftransport wordt gewaarborgd door de hartlongmachine. Om te voorkomen dat er stolsels in de hartlongmachine ontstaan wordt het bloed van de patiënt ontstolt met het medicijn heparine. Als de operatie aan het hart is afgerond en het hart weer zelf het bloed gaat rondpompen moet het bloed weer gaan stollen. Als het bloed ontstolt zou blijven zou er massaal bloedverlies uit de operatiewond optreden.

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De antistolling wordt opgeheven door toediening van protamine, een medicament wat heparine bindt en inactiveert. Omdat protamine zelf ook de stolling kan remmen is het van belang dat protamine juist wordt gedoseerd. Een tekort aan protamine leidt tot resterend heparine in het bloed waardoor de stolling geremd blijft. Een overschot aan protamine belemmert ook de vorming van een stolsel door zijn remmende effecten op de stolling. Adequate protamine dosering is dus van belang voor optimale stolsel vorming, zoals weergegeven in figuur 2.
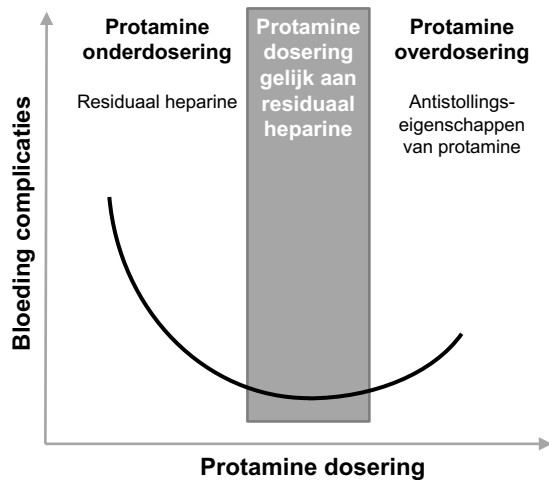
Figuur 2. Een schematische weergave van het belang van juiste protamine dosering.

Als de patiënt bloedt ondanks adequate protamine dosering is snelle interventie nodig aangezien bloeding leidt tot verlies van rode bloedlichaampjes en stollingsfactoren. Door verlies van stollingsfactoren kunnen stollingsstoornissen ontstaan, wat tot een negatieve spiraal van nog meer bloedverlies leidt. Daarnaast kan er zoveel bloedverlies optreden dat bloedarmoede ontstaat en bloedtransfusie of zelfs een heroperatie noodzakelijk is. Bloeding, bloedarmoede, bloedtransfusie en heroperaties zijn allen geassocieerd met een verhoogd risico op infectie en overlijden. Dit benadrukt het belang van tijdige behandeling van stollingsstoornissen om verder bloedverlies te voorkomen.

In het geval van een bloeding ten gevolge van stollingsstoornissen is adequate aanvulling van de tekortschietende stollingsfactoren noodzakelijk. Dit kan worden gedaan op basis van snelle specifieke point-of-care stollingstesten zoals tromboelastometrie. Point-of-care testen zijn snelle mobiele meetmethoden die buiten het laboratorium kunnen plaatsvinden. De noodzaak tot het behandelen van

stollingsstoornissen kan ook worden ingeschat op basis van het medicatiegebruik vóór de operatie, en karakteristieken van de patiënt en operatie gezien door de ervaring van de aanwezige anesthesioloog.

Hoofdstuk 1 beschrijft de complexiteit van alle mogelijkheden om bloeding na openhartoperaties te *voorkomen*, *voorspellen, monitoren* en *behandelen*. Deze vier componenten worden in de literatuur vaak beschreven als patiëntgericht bloed management. Er zijn verschillende methoden om bloedverlies te voorkomen, waaronder het staken van antistollingsmedicatie (bv. sintrom) voorafgaand aan de operatie, toediening van medicatie die de stollingscapaciteit van de patiënt verbeteren en het behandelen van bloedarmoede waardoor meer bloedverlies kan worden verdragen. De patiënten met een hoog risico op een bloeding na de operatie kunnen worden geïdentificeerd met behulp van risicoscores gebaseerd op eigenschappen van de patiënt, het operatietype en specifieke stollingstesten. In het geval van een oncontroleerbare bloeding zijn er verschillende medicijnen die kunnen worden gegeven om bloeding te behandelen. Welk medicijn of bloedproduct moet worden gegeven kan worden gebaseerd op geavanceerde stollingstesten.

Het proefschrift vervolgt met methode om bloeding te voorkomen, waaronder het optimaliseren van protamine dosering. Zoals eerder beschreven wordt protamine toegediend om heparine te binden en daarmee de antistolling op te heffen. Echter, uit laboratoriumonderzoek is gebleken dat protamine in de afwezigheid van heparine ook antistollingseigenschappen heeft. **Hoofdstuk 2** geeft een overzicht over wat er bekend is van protamine, beginnend bij de ontdekking van het medicament en het heparine neutraliserende effect. De eigenschappen en mogelijke bijwerkingen van protamine worden beschreven, waaronder ernstige allergische reactie en het effect op hart en bloedvaten. De nadruk in dit hoofdstuk ligt met name op het negatieve effect op de stolling, waaronder de bloedplaatjes, stollingsfactoren en afbraak van stolselvorming. Ook het effect van protamine op de specifieke stollingstesten wordt beschreven. Tot slot wordt er een overzicht van de mogelijkheden om protamine te doseren en alternatieven voor protamine besproken.

In **hoofdstuk 3** onderzochten wij of het antistollingseffect van protamine relevant is tijdens openhartoperaties en of de protaminedosering invloed heeft op de hoeveelheid bloedverlies en stollingscapaciteit van de patiënt. Dit hebben we onderzocht door patiënten te loten voor een hoge of een lage protamine dosering. Beide doseringen waren binnen het spectrum van klinisch gebruikte doseringen. De groep die een hoge protamine dosering kreeg had meer bloedverlies en bleek vaker bloedtransfusies nodig te hebben in vergelijking met

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de lage doseringsgroep. Dit kon worden verklaard doordat de patiënten in de hoge doseringsgroep meer stollingsafwijkingen vertoonde ten gevolge van een overmaat aan protamine. Dit is de eerste studie die aantoont dat een teveel aan protamine leidt tot stollingsstoornissen met meer bloedverlies tot gevolg.

Om het doseren van protamine verder te verbeteren onderzochten we een nieuwe doseringsstrategie. Deze methode schat de hoeveelheid heparine op het moment dat protamine zal worden gegeven. Deze schatting is gebaseerd op een complex farmacologisch model dat het bloedvolume van de patiënt, de hoeveelheid toegediend heparine en het verlies aan heparine meeneemt in een berekening. Als de geschatte hoeveelheid heparine bekend is, is het eenvoudig de benodigde hoeveelheid protamine te berekenen. In **hoofdstuk 4** vergeleken we de stollingscapaciteit en noodzaak tot bloedtransfusies vóór en na de implementatie van de nieuwe doseringsstrategie. De nieuwe protamine doseringsstrategie bleek de hoeveelheid bloedtransfusies te reduceren en de stollingscapaciteit te verbeteren vergeleken met de reguliere methode van protamine dosering.

Vervolgens richt het proefschrift zich op het voorspellen van bloeding na openhartoperaties. In **hoofdstuk 5** evalueren we systematisch de voorspellende waarde van de moderne snelle stollingstest; tromboelastometrie, weergegeven in figuur 3. Deze test is ontwikkeld voor snelle detectie en ondersteuning van de behandeling van stollingsstoornissen in het geval van hevig bloedverlies. Ondanks dat tromboelastometrie ontwikkeld is om stollingstherapie te begeleiden is het onduidelijk of afwijkende test resultaten een risico vormen voor bloeding. Daarom hebben we in ons ziekenhuis de voorspellende waarde van tromboelastometrie onderzocht. Daarnaast hebben we een systematisch literatuuronderzoek gedaan wat resulteerde in 1311 publicaties waarvan er 10 de voorspellende waarde van tromboelastometrie onderzochten. De resultaten van ons onderzoek hebben we gecombineerd met de resultaten van het literatuuronderzoek en de test bleek niet te voorspellen welke patiënten een verhoogd risico op bloeding hadden.

Tijdens openhartoperaties ontwikkelen patiënten vaak stollingsstoornissen ten gevolge van bloedverlies, de operatiewond en het contact van het bloed van de patiënt met de hartlongmachine. Verschillende stollingstesten zijn ontwikkeld om de stollingsstatus van patiënten te onderzoeken en behandeling daarvan te sturen. We onderzochten de mogelijkheden en betrouwbaarheid van verschillende testen om deze stollingsstoornissen te *monitoren*.

Tromboelastometrie bevat verschillende testen welke ieder een verschillend aspect van de stollingscascade analyseert. Deze testen meten de stollingscapaciteit met (INTEM en EXTEM test) of zonder (NATEM test) de toevoeging van

stollingsactivatoren. Anderen tests richten zich specifiek op de hoeveelheid fibrinogeen (FIBTEM test). De HEPTEM test is een geactiveerde test met de toevoeging van heparinase. Heparinase is een enzym dat zorgt voor de afbraak van het medicijn heparine, gebruikt als antistolling tijdens de openhartoperaties (zie eerste alinea). De HEPTEM test onderzoekt hiermee de stollingscapaciteit zonder het effect van heparine. In **hoofdstuk 6** hebben we onderzoek gedaan naar het voorkomen deze afwijkende HEPTEM-resultaten rondom openhartoperaties in 2 universiteitsziekenhuizen. We vonden dat in 15% tot 36% van de gevallen de HEPTEM test afwijkende resultaten toonde. Mogelijk kunnen deze resultaten worden verklaard door een overmaat aan protamine. Toekomstig onderzoek zal hier verder inzicht moeten geven.
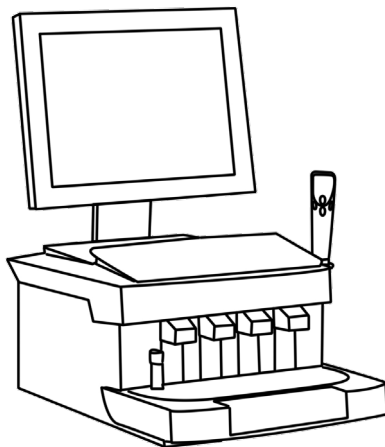
Figuur 3.   De roterende tromboelastometrie machine. (Copyright M.I. Meesters 2017)

Behalve de HEPTEM test hebben we ook de betrouwbaarheid van de NATEM test onderzocht. De NATEM test onderzoekt de stollingscapaciteit zonder toevoeging van een kunstmatige activator aan het bloedmonster. De test wordt in toenemende mate gebruikt in wetenschappelijk onderzoek vanwege de grote gevoeligheid voor veranderingen van de stollingsstatus. Omdat de test zo gevoelig is voor veranderingen onderzochten we in **hoofdstuk 7** of er een effect van de bewaarduur van het bloedmonster is op de NATEM-resultaten. We hebben hiervoor bloed van gezonde vrijwilligers en intensive care patiënten afgenomen en direct na afname de NATEM test hierop verricht. Na 45 en 90 minuten onderzochten wij hetzelfde bloedmonster opnieuw en bekeken of er verandering in de NATEM test resultaten optrad. Een stolsel bleek zich na 45 en 90 minuten sneller te vormen.

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Dit suggereert dat de stolling, gemeten met NATEM, met de tijd geactiveerd raakt. Uit eerder onderzoek bleek dat dit bij de geactiveerde testen (INTEM, EXTEM en FIBTEM) niet het geval was. Helaas konden we de factor verantwoordelijk voor de stollingsactivatie in ons onderzoek niet identificeren. Ons advies is de NATEM test zo snel mogelijk na afname in te zetten om activatie en daarmee verstoring van de testresultaten te voorkomen.

Naast tromboelastometrie zijn er ook andere apparaten welke de stollingsstatus van een patiënt snel kunnen onderzoeken. Een voorbeeld is de point-of-care protrombine tijd (POC-PT), weergeven in figuur 4. De klassieke protrombine tijd (PT) bepaling is een tijdrovende laboratorium stollingstest. De POC-PT is ontwikkeld om sneller resultaten te verkrijgen. De test is oorspronkelijk ontwikkeld om patiënten die antistolling gebruiken zelf de stollingswaarde te laten meten en zo nodig de medicatiedosering aan te passen. Echter, door het kleine formaat kan het apparaat ook op de operatiekamer gebruikt worden.
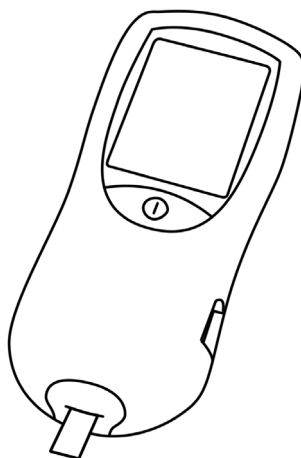
Figuur 4. De point-of-care protrombine tijd meter. (Copyright M.I. Meesters 2017)

Omdat het na openhartoperaties vaak noodzakelijk is snel inzicht in de stollingsstatus te krijgen onderzochten wij in **hoofdstuk 8** of de POC-PT test ook in deze situatie kan worden gebruikt. Voor en na de openhartoperatie namen wij bloed af en vergeleken de resultaten van de POC-PT met de klassieke PT uit het laboratorium. Voorafgaand aan de operatie was er een goede overeenkomst tussen de testen. Echter, 3 minuten na het toedienen van protamine, na afloop van de operatie, bleek er een groot verschil tussen beide testen. Om te onderzoeken of er 6 of 10 minuten na protamine toediening een betere overeenkomst was met

het laboratorium verrichtte wij nog een onderzoek, zoals beschreven in **hoofdstuk 9**. Echter bleek dat er ook 6 en 10 minuten na het opheffen van het heparine effect middels protamine er een groot verschil tussen de resultaten van de POC-PT en het laboratorium bleef bestaan. Derhalve concludeerde wij dat de POC-PT niet geschikt is voor gebruik na openhartoperaties.

Stollingsstoornissen kunnen worden *behandeld* met transfusie van bloedplaatjes en/of stollingsfactoren in de vorm van bloedplasma. Het is lastig in te schatten of er stollingsstoornissen ten grondslag liggen aan een bloeding of dat er een chirurgische voor bloedverlies is. Een chirurgische oorzaak van bloedverlies kan bijvoorbeeld een grote beschadiging van een bloedvat zijn, zonder dat de stolling gestoord is.

Laboratorium testen kunnen uitwijzen of er een tekort aan stollingsfactoren is, echter deze testen nemen veel tijd in beslag en in het geval van een bloeding is er vaak geen tijd om op deze resultaten te wachten. Derhalve berust de beslissing om bloedplasma te geven vaak op klinische aanwijzingen voor een gestoorde stolling. Deze aanwijzingen zijn onder andere het sijpelen van bloed op verschillende plekken van het operatiegebied, een lange duur aangesloten zijn aan de hartlongmachine en complexe operaties. In **hoofdstuk 10** onderzochten we of de beslissing om bloedplasma toe te dienen op klinische aanwijzingen overeenkomt met de bevindingen van tromboelastometrie. We vonden dat de beslissing van de (ervaren) cardioanesthesioloog om bloedplasma toe te dienen op basis van klinische aanwijzingen van stollingsstoornissen in 95% van de gevallen overeenkwam met de tromboelastometrie test resultaten.

Naast bloedplasma zijn er ook andere methoden om stollingsstoornissen te behandelen, waaronder farmaceutisch geïsoleerde stollingsfactoren zoals fibrinogeen concentraat. Een recent onderzoek door dr. Bilecen en collega's onderzocht het effect van fibrinogeen concentraat op bloedingen na openhartoperaties. (Artikel: *Effect of Fibrinogen Concentrate on Intraoperative Blood Loss Among Patients With Intraoperative Bleeding During High-Risk Cardiac Surgery: A Randomized Clinical Trial. JAMA, 2017 317(7), 738–747*) Zij concludeerden dat fibrinogeen concentraat de hoeveelheid bloedverlies tijdens openhartoperaties niet verminderde. In onze ingezonden brief aan de auteurs, in **hoofdstuk 11**, plaatste wij de kanttekening dat bloedverlies tijdens openhartoperaties geen relevante maat is aangezien het meeste bloedverlies na afloop van de operatie optreedt en het toedienen van fibrinogeen dat bloedverlies wel verminderde. Verder hadden de patiënten uit het onderzoek geen tekort aan fibrinogeen in het bloed. Met deze opmerkingen hebben wij gepoogd de sterke conclusies van het artikel wat af te zwakken.

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In **hoofdstuk 12** beschrijven we de beperkingen van de studies uit dit proefschrift. Verder plaatsten we de individuele hoofdstukken in perspectief van de huidige literatuur. Eerst geven we een overzicht van de resultaten van andere protamine doseringsstudies waarna we richting geven aan toekomstig onderzoek naar protamine. Hierna bespreken we voorspellers van bloeding na openhartoperaties. Vervolgens benadrukken we dat in de studies die onderzochten of het gebruik van tromboelastometrie leidt tot betere behandeling gelijktijdig een stroomschema voor stollingsbehandeling én stollingsconcentraten werden geïntroduceerd. Hierdoor is niet te bepalen welk van de drie interventies (tromboelastometrie, het stroomschema of de stollingsconcentraten) de oorzaak van betere resultaten was. Ook wordt het bewijs voor het gebruik van fibrinogeen concentraat uiteengezet. Vervolgens gaan we in op andere verstorende factoren op tromboelastometrie, naast het effect van de bewaartijd van een bloedmonster en het gebruik van heparinase. De klinische toepasbaarheid van point-of-care stollingstesten wordt besproken. Tot slot beschrijven we de mogelijke nadelige kant van het behandelen van stollingsstoornissen: trombose (overmatige stolselvorming) met mogelijk nadelige consequenties.

Acknowledgements

ACKNOWLEDGEMENTS

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Beste Jan de Jong, jouw ideeën over het personaliseren van protaminedosering hebben geleid tot een prachtige publicatie. Ik had graag nog meer met je samengewerkt en geleerd van je enorme anesthesiologische kennis.

Beste Fellery de Lange en Jaap de Vries, zonder jullie was de Ratio-PRO studie niet zo snel tot zo een prachtig resultaat gekomen, waarvoor veel dank!

Beste Evert Jansen, ook jij hebt aan het begin gestaan van mijn wetenschappelijke carrière, ik heb dankbaar gebruik gemaakt van jouw prachtige database en klinische blik.

Marcus Lance, ik heb veel geleerd van jouw talloze presentaties en van het stollingsseminar in Vaals. Onze gezamenlijke studie heeft tot opmerkelijke resultaten geleid.

Alle onderzoeks-studenten: Anne, Anna, David, Emma, Iris, Gerard, Maaike, Pepijn en Robin, ik hoop dat ik jullie enthousiast heb kunnen maken voor wetenschappelijk onderzoek en voor de anesthesiologie. Ik wil jullie enorm danken voor jullie hulp!

Beste cardioanesthesiologen, beste Hans, Suzanne, Peter, Herman, Jenny, Xander, Patrick, Deirdre en Sjoerd, dank voor jullie ruimte en ondersteuning bij het onderzoek tijdens de complexiteit van de cardioanesthesie. Mede door jullie ben ik nog enthousiaster geworden voor de het vak.

Geachte cardiochirurgen en perfusionisten, in het bijzonder Lerau van Barneveld en Reinard Haumann, hartelijk dank voor het mogelijk maken van de studies rondom openhartoperaties. Uw ondersteuning was zeer waardevol.

Beste Hilde en Dana, dankzij jullie hulp heeft de Ratio-PRO studie meer diepgang gekregen. Veel dank voor jullie harde werk.

Peter van de Ven, waar mijn statistische kennis te kort schoot wist jij mij te ondersteunen, veel dank daarvoor.

Beste Jens en Rienk, dankzij jullie steun heb ik in het perifere jaar van mijn opleiding veel onderzoekswerk kunnen verzetten. Alle stafleden uit het Westfriesgasthuis: van jullie heb ik enorm veel geleerd op het klinisch vlak waarvoor veel dank!

Geachte prof. Girbes en dr. Tuinman, door mij ruimte te geven voor mijn wetenschappelijke activiteiten heb ik tijdens mijn IC jaar veel manuscripten kunnen afronden.

Beste Anesthesiologen en collega AIOS, dank voor jullie interesse voor mijn onderzoek en de fijne samenwerking.

Beste Godelieve, Inez, Monique en Noëlle dank voor jullie logistieke steun bij mijn projecten. Wim Janssen, hartelijk dank voor alle financiële afwikkelingen die je in het kader van mijn onderzoek verrichtte.

Beste paranimfen, Nick en Bas, het is erg fijn met jullie de hobbels op de weg van het onderzoek, en combinatie met de opleiding te kunnen bespreken en relativeren. Het is een genoegen te zien dat we altijd voor elkaar klaar staan.

Lieve oma Mariejan, dit is alweer de derde promotie die je van dichtbij meemaakt. Altijd belangstellend naar de voortgang waardeer ik je meeleven zeer. In gedachte zijn Johan, Ella, Jan en Arie er ook bij.

Lieve Maaike en PD, het gevoel dat ik altijd op jullie kan rekenen geeft veel rust. Jullie zijn vol vreugde bij nieuwe publicaties en enorm geïnteresseerd in de voortgang. Het is bijzonder hoe jullie Simon en mij vol liefde de ruimte geven om ons te ontwikkelen en ons daarbij onvoorwaardelijk steunen. Jullie staan altijd voor ons klaar. Jullie zijn geweldig, bedankt voor alles!

Lieve Simon, ondanks dat jouw interesse ver van mijn vak staat en regelmatig vroeg waar ik ook alweer onderzoek naar deed was je altijd enthousiast als er weer een stukje af was. Ik ben enorm trots op je, je maakt prachtig werk en heb je als broer enorm lief.

Allerliefste Katha, er was niemand zo enthousiast als jij wanneer er weer een stuk geaccepteerd was. Jouw steun is geweldig en onbegrensd! Wie had gedacht dat mijn stage in Frankfurt me zoiets prachtigs zou brengen. Je bent alles wat ik mij kan wensen.

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List of Publications

LIST OF PUBLICATIONS

1. Boer C, Meesters MI, Milojevic M, Benedetto U, Bolliger D, von Heymann C, Jeppsson A, Koster A, Osnabrugge RL, Ranucci, M, Ravn HB, Vonk AB, Wahba A, Pagano D. The European Association for Cardio-Thoracic Surgery and the European Association of Cardiothoracic Anaesthesiology Joint Effort Patient Blood Management Guidelines for Adult Cardiac Surgery. J Cardiothorac Vasc Anesth 2017 Accepted for publication.

2. Pagano D, Milojevic M, Meesters MI, Benedetto U, Bolliger D, von Heymann C, Jeppsson A, Koster A, Osnabrugge RL, Ranucci, M, Ravn HB, Vonk AB, Wahba A, Boer C. The European Association for Cardio-Thoracic Surgery and the European Association of Cardiothoracic Anaesthesiology Joint Effort Patient Blood Management Guidelines for Adult Cardiac Surgery. Eur J Cardiothorac Surg, 2017 Accepted for publication.

3. Meesters MI, Burtman D, van de Ven P, Boer C. Prediction of postoperative blood loss by thromboelastometry in adult cardiac surgery: cohort study & systematic review. J Cardiothorac Vasc Anesth (accepted).

4. Meesters MI, von Heymann C, Boer C. Administration of fibrinogen concentrate during cardiac surgery. JAMA. 2017 Jun 27;317(24):2550-2551.

5. Meesters MI, Boer C. A critical note on protamine use in carotid endarterectomy. J Vasc Surg. 2017 Sep;66(3):967-968.

6. Meesters MI, Koning NJ, Romijn JWA, Loer SA, Boer C. Clinical decision versus thromboelastometry based fresh frozen plasma transfusion in cardiac surgery. Br J Anaesth. 2017 Mar 1;118(3):458-459.

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11. <u>Meesters MI</u>, Koch A, Kuiper G, Zacharowski K, Boer C. Instability of the non-activated rotational thromboelastometry assay (NATEM) in citrate stored blood. Thromb Res. 2015 Aug;136(2):481-3.

12. Vonk AB, <u>Meesters MI</u>, van Dijk WB, Eijsman L, Romijn JW, Jansen EK, Loer SA, Boer C. Ten-year patterns in blood product utilization during cardiothoracic surgery with cardiopulmonary bypass in a tertiary hospital. Transfusion. 2014 Oct;54(10 Pt 2):2608-16.

13. <u>Meesters MI</u>, Boer C. Cessation of antithrombotic therapy before surgery: weighing thrombosis and bleeding risks. Neth Heart J. 2014 Sep;22(9):370-1.

14. <u>Meesters MI</u>, Vonk AB, van de Weerdt EK, Kamminga S, Boer C. Level of agreement between laboratory and point-of-care prothrombin time before and after cardiopulmonary bypass in cardiac surgery. Thromb Res. 2014 Jun;133(6):1141-4.

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Biography

BIOGRAPHY

Michael Isaäc Meesters was born on the 16th of August 1988 in Amsterdam, the Netherlands. He attended secondary school at the Fons Vitae Lyceum in Amsterdam. During this time, he developed an interest for both graphical design and medicine. He followed the preparatory course for the academy of arts at the Willem de Kooning academy in Rotterdam. In 2006, he was accepted for the graphical design training. However, his great curiosity in medicine led him to apply for the decentral selection at the VU University in Amsterdam, at which he started his medical training after his high school graduation in 2007.

After his first year of medicine he followed an international tropical medical summer school in Yogyakarta, Indonesia. During the next summer, he worked in a small local hospital near Siem Reap, Cambodia. Throughout his Bachelor, he was active as a student assistant at the physiology department and as a tutor for problem-based learning sessions for (bio)medical students. He actively participated in several committees of the medical curriculum evaluation panel, the Medical Faculty Student Association (MFVU), and the International Federation of Medical Students' Associations (IFMSA).

During his Bachelor, he attended research on hemostasis in cardiac surgery under supervision of prof.dr. Boer at the department of Anesthesiology of the VU University medical center (VUmc). He continued in this line of research during his Master studies, with a 6 months research internship at the department of Anesthesiology at the VUmc in 2010. Hereafter he went for half a year to Frankfurt am Main (Germany) for hemostasis research at the Intensive Care department of the Goethe Clinicum under supervision of dr. Koch and prof.dr. Zacharowski. During this year, he started his PhD training on hemostasis management in cardiac surgery under supervision of prof.dr. Boer, prof.dr. Loer (dept. Anesthesiology, VUmc) and dr. Vonk (dept. Cardiothoracic surgery, VUmc). He performed his PhD training in parallel to his medical training.

After finishing his medicine master in 2014 Michael started his Anesthesiology training at the VUmc, chair prof.dr. Loer. He followed the second year of his training at the Westfriesgasthuis in Hoorn, the Netherlands (drs. Beek and drs. Hering). In 2016, he became an active member of the European Association of CardioThoracic Anesthesiology (EACTA) and worked on the international guideline for the optimization of hemostasis and transfusion in cardiac surgery, a collaboration between the EACTA and European Association of CardioThoracic Surgery (EACTS). The guideline has recently been published.

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