Blood loss in coronary artery bypass surgery: etiology, diagnosis and prevention

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Etiology, diagnosis and prevention

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COLOFON

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‘Control of post-CPB bleeding involved….. packed cells, platelets, plasma, protamine and prayer’

(Hall RI. Can J Anaesth 1998;45:1-5)

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CHAPTER 1

General Introduction

ETIOLOGY OF BLOOD LOSS AFTER CARDIAC SURGERY

Excessive blood loss after cardiac surgery is a relatively common complication, occurring in approximately 20% of the patients (2-4). The normal or generally accepted amount of blood loss after cardiac surgery varies between 300-1500 ml during the first 12 h. In 5% of the operated patients' blood loss requires re-operation (5,6).

The most important predictive indicators for bleeding and consecutive re-operation following cardiac surgery, include: age, obesity, renal insufficiency, heart failure, anti-platelet medication usage, operation- and cardiopulmonary bypass (CPB) time, temperature, number of arterial grafts and intracardiac repair (2,7,8). Postoperative bleeding requiring transfusions and surgical re-exploration is associated with mortality, morbidity (such as increased sternal wound infection, risk of transfusion-related complications) and higher costs (9,10). Consequently, prevention of blood loss and reduction of blood transfusion requirements is a major target in cardiac surgery.

A surgical cause of bleeding (such as due to an unrecognized bleeding vessel, anastomosis or other suture line) is found in approximately half of the patients undergoing re-operation (7). In the remainder of the patients a failure in the pathways involved in hemostasis, due to a pre-existing coagulation factor deficiency or platelet defect, an iatrogenic drug-induced compromised hemostasis or an acquired, operation and CPB related hemostatic defect contributes to bleeding (2,11-13).

PRE-EXISTING COAGULATION FACTOR DEFICIENCIES AND PLATELET DEFECTS

Pre-existing coagulation factor deficiencies and platelet function defects may be either inherited or acquired. There are two distinct groups in genetic coagulation problems leading to blood loss: coagulation factor deficiencies and polymorphic variants in coagulation and fibrinolysis genes. The inherited coagulation factor

deficiencies are relatively rare and severe and, usually, diagnosed before cardiac surgery. Hemophilia's A (factor VIII deficiency) and B (factor IX deficiency) are X-linked recessive diseases. Hemophilia C (factor XI deficiency) is inherited in an autosomal pattern and far less prone to spontaneous bleeding than hemophilia's A and B, however, there is an increased risk for perioperative bleeding (14). Von Willebrand disease is the most common inherited bleeding disorder, occurring in approximately 1% of the population, and is transmitted via autosomal dominant (type 1 and 2) and recessive (type 3) inheritance. There are several other even more rare factor deficiencies, such as factor XIII (fibrin stabilizing factor) and dys- or hypofibrinogenemia's (15). Polymorphic variants cause much less severe changes in coagulation or fibrinolysis, and therefore, often are unrecognized before cardiac surgery (14). Their clinical impact varies and in combination with acquired hemostatic problems they might contribute to excessive blood loss.

Acquired pre-existent coagulation factor deficiencies as result of e.g. cirrhosis, chronic active hepatitis, acute liver failure and biliary tract obstruction should be suspected in patients with unexplained prolongation of routine coagulation tests. Most of these factors can be replaced with factor concentrates to obtain normal preoperative and post-CPB levels in cardiac surgery (16).

Inherited platelet function defects, such as storage pool disease or Glanzmann thrombasthenia, are usually diagnosed prior to cardiac surgery. However, platelet function defects may also be acquired in case of renal or liver failure. Identifying acquired platelet function defects prior to surgery is important to prevent unnecessary bleeding.

DRUG-INDUCED COMPROMISED HEMOSTASIS

There are several groups of medication, frequently used preoperatively, that might contribute to bleeding complications after cardiac surgery.

Antiplatelet medications (such as Aspirin, Carbasalate calcium, Clopidogrel, Prasugrel, Ticagrelor), often prescribed for acute coronary syndromes or for the prevention of stent thrombosis after percutaneous coronary intervention (PCI),

inhibit platelet activation and aggregation. Aspirin (acetylsalicylic acid) and carbasalate calcium (a derivative of acetylsalicylic acid) are salicylate drugs, that have analgesic, antipyretic, anti-inflammatory and anti-platelet effects. The inhibition of platelet aggregation is mediated by inhibition of the production of thromboxane A2 (17,18). Clopidogrel and Prasugrel block the $P2Y_{12}$ subtype of adenosine diphosphate (ADP)-receptor on platelet cell membranes irreversibly and Ticagrelor reversibly (19). After cessation of the irreversible drugs it takes about 7 days to restore normal platelet function, the time needed to produce new platelets. The recovery time of Ticagrelor is shorter. So, when discontinued timely, no extra bleeding risk is expected. *Vitamin K antagonists* (such as Warfarin, Phenprocoumon, Acenocoumarol) reduce blood clotting by inhibiting the recycling of vitamin K epoxide into to the active reduced form of vitamin K (20). When taken timely, vitamin K can effectively reverse the effects of vitamin K antagonists. In acute situations the effect of vitamin K antagonists can be reversed immediately by infusion of prothrombin complex concentrate or plasma.

Low molecular weight heparin (such as enoxaparin, dalteparin, tinzaparin, nadroparin) is frequently used to stabilize acute coronary syndromes and to prevent deep vein thrombosis and pulmonary emboli during operation and perioperative immobilization, by binding to the enzyme inhibitor antithrombin, thereby, inactivating thrombin and factor Xa (21,22). Unfractionated heparin is also used in high doses during cardiac surgery and will be discussed below.

Finally, the more recently developed Argatroban, Dabigatran and Lepirudin, are *direct thrombin inhibitors*, preventing the conversion of fibrinogen to fibrin. Apixaban and Rivaroxaban are *selective, direct factor Xa inhibitors*, prescribed to prevent thrombosis and embolism (23). These medications do not possess an antagonizing agent, which can be given prior to cardiac surgery, potentially, increasing the risk of excessive blood loss when not stopped in time.

OPERATION AND CPB RELATED HEMOSTATIC DEFECTS

Suggested mechanisms for the acquired hemostatic defects in cardiac surgery include the use of high doses of heparin, hemodilution, hypothermia, activation of the coagulation cascade resulting in disseminated intravascular coagulation (DIC), tissue trauma, platelet dysfunction, coagulation factor loss and dysfunction and excessive fibrinolysis (2,3,11,12). The extent of the contribution of each of those factors remains unresolved.

Heparin

During CPB heparin is required to prevent blood clotting within the circuit. Plasma proteins, including fibrinogen and albumin, are absorbed by the extracorporeal surfaces, creating a pro-thrombotic environment (13). Heparin effectively inhibits systemic thrombin but is not able to impede surface-bound thrombin adhering to both fibrinogen and fibrin deposited onto the CPB circuitry. Thrombin activates platelets, which also adhere to specific binding sites on the fibrin- and fibrinogen-coated surfaces, and allows more prothrombin to thrombin activation via prothrombinase complexes on the phospholipid membrane surface of activated platelets (2,24). As a result high-dose heparin is required to prevent the formation of a fibrin-rich thrombus during CPB. Protamine sulfate is the most common agent used to reverse heparin-induced anticoagulation at the end of CPB. However, protamine has a shorter effective half-life than heparin, and after appropriate neutralization of heparin, rebound heparin activity occurs and has shown to be a cause of postoperative bleeding (7). Moreover, heparin inhibits platelet aggregation, an effect not reversed by protamine sulfate, contributing to platelet dysfunction (2).

Hemodilution

Hemodilution during cardiac surgery results from the colloids and crystalloids used for CPB priming and cardioplegia, volume supplementation during cardiac surgery and extensive use of cell saver devices returning red blood cells without platelets and coagulation factors. Hemodilution due to the use of CPB and volume supplementation decreases red blood cell, platelet and coagulation factor con-

centrations, predisposing patients to bleeding. Coagulation factor concentrations are reduced, parallel with hematocrit concentrations, to approximately 25-50% of baseline (25-27). The minimum levels of coagulation factors adequate to support hemostasis often are not surpassed and the reductions in coagulation factor levels that develop during CPB, therefore, generally do not result in excessive bleeding (28-30). However, in combination with a compromised hemostasis hemodilution should not be neglected.

Hypothermia

Hypothermia (defined as a systemic temperature below 35 °C) can cause reduced coagulation factor activity (significant below 33 °C) (31), platelet dysfunction and induction of fibrinolysis (32) and is associated with an increased risk of uncontrolled bleeding. Both coagulation factor activity and platelet function can be restored by normalization of body temperature (33). While hypothermia is sometime unavoidable to protect the body or heart from ischemia in special circumstances, in most cases it should be avoided and treated aggressively at the end of the operation.

Activation of the coagulation cascade

Blood contact with the extensive non-endothelial surface of the CPB circuit and direct blood-air contact lead to the activation of the contact or intrinsic coagulation pathway, via kallikrein activation of factor XII when it binds to the negatively charged surface (13). Factor XII activates factor XI of the intrinsic pathway that finally leads to thrombin formation (2).

The tissue factor (TF) or extrinsic pathway is triggered by exposure to high shear stress and oscillating shear on circulating blood cells in the CPB circuit and by surgical trauma due to e.g. cannulation, coronary arteriotomy, aortic cross clamping and incision of blood vessels (13). Factor VIIa complexes with TF expressed on monocytes, macrophages, fibroblasts and platelets exposed on atherosclerotic plaques or subendothelial constituents within the vessel wall (13). Surgical trauma is considered a greater stimulus for extrinsic pathway activation than the CPB circuit (34). Since TF and factor VII are rapidly generated during operation and bypass, they reach high concentrations, especially in the pericardial sac (due to aortic cross

clamping). TF levels in the pericardial blood can increase to fivefold above systemic levels (35). Recirculation of suctioned blood from the surgical field, therefore, can aggravate the activation coagulation (and fibrinolytic) systems.

The contact activation and tissue factor coagulation pathways converge resulting in activation of factor X which, in combination with factor Va (forming the prothrombinase complex), and calcium, leads to the generation of thrombin (12,13). Since this thrombin is not generated as a physiological reaction to establish clotting, it is called a 'non-hemostatic thrombin generation' and represents dysregulation of the normal hemostatic process. There are two major bursts of 'non-hemostatic thrombin generation'. The first, immediately after going onto CPB when blood first contacts the oxygenator pump circuit, and the second, immediately after reperfusion of the ischemic heart at the end of CPB. Aortic cross clamping, to prevent reflux blood stream to the heart during CPB, further aggravates thrombin generation by mechanical tissue injury (13). The activated thrombin mediates the

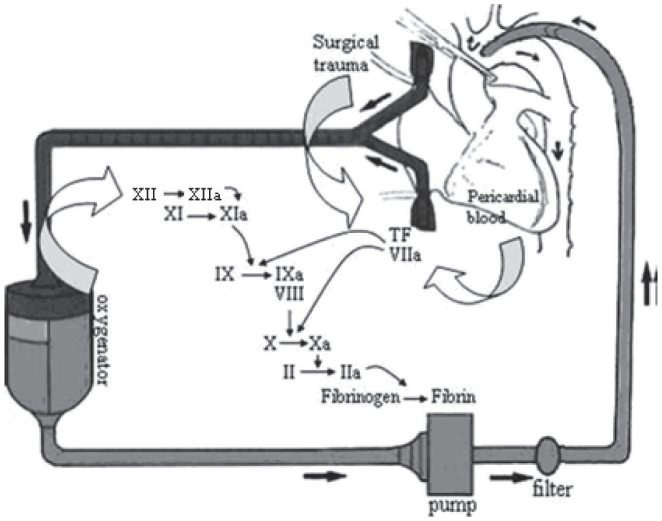

Figure 1. Coagulation and CPB.
The interface of blood with non-endothelial surfaces of the CPB, direct blood-air interface, high shear stresses, cannulation and incision of blood vessels and re-transfusion of blood procured from the surgical field are all triggers for activation of both contact and TF-initiated pathways of coagulation (3).

conversion of fibrinogen to fibrin monomers, activates factors V, VIII, XIII, platelets and the inflammatory cascade, specifically complement.

Activation of the coagulation cascade can ultimately lead to consumption of coagulation factors and platelets (i.e. a disseminated intravascular coagulation (DIC) state), mediated by thrombin and plasmin, and might result in increased blood loss (12). This consumptive process is further enhanced via leukocyte elastase production (36-38). CD11b-expressing monocytes on the surface of oxygenator fibers in the CPB directly activate factor X (39) and complement (40,41). The activated thrombin and generated inflammation factors act synergistic maintaining a vicious circle stimulating each other.

Platelet activation and dysfunction

Platelet counts immediately decrease by approximately 25-60% during CPB (42-45). This decline is larger than expected based on hemodilution alone (46). Furthermore, the absence of a correlation between platelet number and the aggregatory response suggests that factors other than dilution might contribute to thrombocytopenia and platelet dysfunction. During CPB platelets are subjected to two insults. The first is caused by blood contact with surface-absorbed fibrinogen and thrombin on the CPB circuit, activating platelets (11), and the second, is evoked by the oxidizing stress brought about by blood oxygenation and pump-induced hemolysis, causing platelet activation and injury. Increased levels of P-selectin in plasma indicate that the impaired platelet aggregation, as confirmed by Cheung (47) after ECMO (comparable to CPB), is the result of platelet activation, causing a platelet agonist refractory state (7). Platelet activation by e.g. the CPB circuit, surgical trauma and inflammation, can ultimately lead to DIC and platelet exhaustion (47), creating decreased platelet numbers and function. Platelet dysfunction can be further aggravated by hypothermia when temperature is not adequately controlled.

Coagulation factors

Adequate concentrations of coagulation factors are essential to ensure normal coagulation. During surgery (functional) coagulation factor levels decrease due

to blood loss, consumption, acidosis, hypothermia, hypocalcemia and hemodilution (Table 1). Blood loss, consumption and fibrinolysis lead to an actual loss of coagulation factors, whereas acidosis, hypothermia (31) and hypocalcemia cause dysfunction. Hemodilution results in a relative decrease.

Table 1. Effects of intra-operative conditions on coagulation factors.

	Loss of coagulation factors	Dysfunction of coagulation factors	Decrease of coagulation factors
Blood loss	X		
Consumptive coagulopathy	X		
Fibrinolysis	X		
Acidosis		X	
Hypothermia		X	
Hypocalcemia		X	
Hemodilution			X

Fibrinolysis

Activation of fibrinolysis occurs simultaneously with coagulation (48). Thrombin down-regulates hemostasis by stimulating release of tissue factor pathway inhibitor, which inhibits the tissue factor pathway, and by stimulating the release of tissue plasminogen activator (t-PA). t-PA is rapidly released by endothelial cells in response to venous occlusion, exercise, endotoxin and arterial ischemia (49). The distribution of t-PA is further promoted by hypothermia, traumatized endothelial cells and return of suctioned pericardial blood (12). t-PA, like urokinase plasminogen activator (u-PA), activates fibrinolysis by cleaving plasminogen to plasmin. Due to the low levels of u-PA compared with t-PA in blood during CPB, most plasminogen activation during CPB occurs on the fibrin surface by t-PA (50). Fibrinolysis via accelerated plasminogen activation is also stimulated by the fibrin absorbed to the large surface of the pump oxygenator, which is not blocked by heparinisation (50). Activation of fibrinolysis at the onset of CPB is mainly due to this blood contact with the foreign CPB surface and then further becomes extrinsically activated by the release of t-PA from the vascular walls throughout CPB (2).

Plasmin cleaves both fibrinogen and fibrin into fragments, called fibrinogen- and fibrin degradation products, without clotting ability. One of these fragments, D-dimer, is used as a specific marker of fibrin degradation. However, the measured D-dimer levels during and after operation are difficult to interpret, because an increase does not distinguish between amplification of coagulation or primary fibrinolysis. The differentiation can only be made by additional coagulation tests like, prothrombin fragment 1 + 2, plasmin generation and thrombin generation. During early surgery (including sternotomy) prior to the start of CPB, plasmin and D-dimer generation levels do not change from baseline levels (50). This would imply that even relatively large surgical wounds, including sternotomy, by itself do not result in fibrinolysis. However, immediately after starting CPB the t-PA, D-dimer, t-PA-inhibitor complex and plasmin-antiplasmin complex concentrations increase (50,51), suggesting an important role for CPB in inducing fibrinolysis. Valve surgery and longer aortic cross-clamp times are associated with enhanced activation of t-PA and consequently fibrinolysis during CPB (2). Elevated active t-PA during and immediately after CPB is associated with excessive bleeding (49,51).

Plasminogen activator inhibitor (PAI-I) is released by vascular endothelial cells, smooth muscle cells, platelets and hepatocytes after t-PA and u-PA release as a natural endogenous inhibitor of fibrinolysis (2;49). PAI-I is produced by these cells in response to sepsis, exposure to various cytokines, operation and trauma. Normally, PAI-I increases in parallel with t-PA. In cardiac surgery the increase in PAI-I is usually delayed relative to that of t-PA. PAI-I shows a classic acute-phase like response with a slow rise in secretion starting several hours after the beginning of surgery and peaking in the postoperative period (49,51).

Alternative pathways of fibrinolysis may also contribute to hyperfibrinolysis during CPB. These include fibrinolysis secondary to DIC, neutrophil elastase-mediated fibrin digestion and platelet or their excreted microparticles mediated plasmin generation or propagation (11,13).

The relative contributions of heparinisation (and reversal with protamine), hemodilution, hypothermia, surgical trauma, CPB, coagulation factor consumption, decrease in platelet number and function, and fibrinolysis to the development of (excessive) blood loss are yet to be fully established.

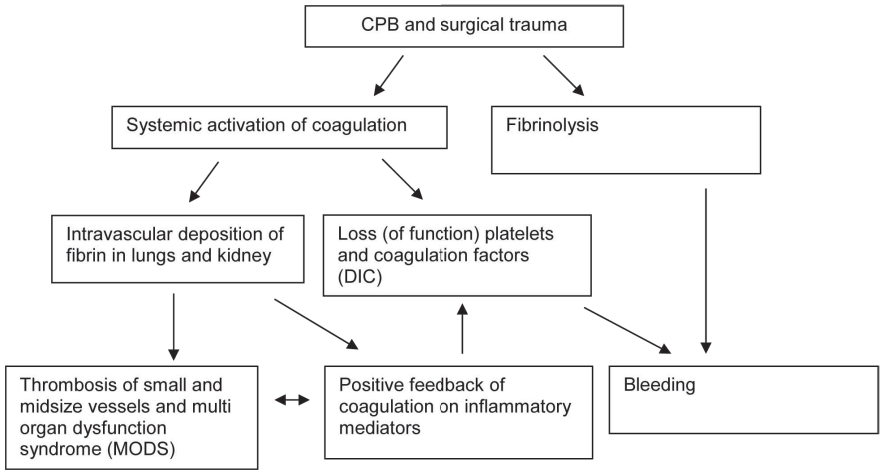

Figure 2. Effects of CPB and surgical trauma on hemostasis.
Overview of the effects of systemic activation of coagulation and fibrinolysis by CPB and surgical trauma.

OUTLINE OF THIS THESIS

The aim of this thesis is to improve our understanding of the development of (excessive) blood loss due to ineffective hemostasis after coronary artery bypass graft (CABG) surgery, in order to support the decision making process concerning medication and blood product substitution and to explore several therapeutic options to reduce the risk of bleeding.

In **Part I** (chapters 2-4) the etiological mechanisms for blood loss after CABG procedures are investigated. Management options to prevent and treat bleeding patients are described in **Part II** (chapters 5-7).

PART I ETIOLOGY AND DIAGNOSIS OF BLOOD LOSS AFTER CABG SURGERY

Fibrinogen plays an essential dual role in coagulation, at one hand enhancing platelet aggregation via binding to the GPIIb/IIIa receptors on platelets and on the other hand by its conversion to fibrin to form an insoluble clot. It is suggested that pre- and postoperative fibrinogen levels are associated with (excessive) blood loss (52-54). To evaluate the role of decreased fibrinogen levels in the development of (excessive) blood loss after cardiac surgery we have performed a systematic review and meta-analysis, as described in **Chapter 2**.

Cardiac surgery can result in a significant reduction of (functional) fibrinogen due to operation trauma and CPB usage. In **Chapter 3** the relative contributions of hemodilution, consumption and degradation are described. Fibrin and fibrinogen concentrations, combined with their degradation products were evaluated in 10 patients before and after isolated CABG.

The laboratory values seen after cardiac surgery resemble both a post-fibrinolytic and a pro-thrombotic state (34,37,51). In **Chapter 4** we evaluate coagulation and fibrinolytic markers at various time intervals during and up to 5 days after CABG procedures, with or without CPB usage, and combined with aortic valve replacement (AVR), to gain more insight in the hemostatic disorders that develop during cardiac surgery.

PART II MANAGEMENT AND PREVENTION OF BLOOD LOSS AFTER CABG SURGERY

Beside etiological consideration of blood loss after CABG surgery, management and prevention are studied in the following chapters.

Antiplatelet medications, such as acetylsalicylic acid and clopidogrel, routinely prescribed to reduce early stent failure after percutaneous transluminal coronary angioplasty (PTCA), improve outcomes after acute coronary syndromes, and decrease cardiovascular morbidity and mortality (55-58). Consequently, these

medications are used regularly by patients prior to CABG surgery (59,60). However, several studies state that administration of clopidogrel prior to cardiac surgery, especially in combination with acetylsalicylic acid, increases the risk of postoperative bleeding (61,62). In **Chapter 5** the optimal stop day, defined as the last day before surgery on which antiplatelet medication was used, for acetylsalicylic acid only or acetylsalicylic acid combined with clopidogrel in relation to the lowest amount of blood loss in the first 48 h after CABG surgery is evaluated.

Locally applied fibrin sealants as an adjunct to hemostasis, wound healing and tissue adhesion have proved to be efficient in reducing blood loss and the need for blood transfusions in many surgical fields, including cardiac surgery (63,64). Nearly all of the commercially available fibrin sealants use either bovine thrombin or human thrombin derived from pooled plasma, raising concerns regarding the antigenicity and antibody formation (65). Recently, CryoSeal was developed, a fibrin sealant produced from allogeneic single donor plasma, without the addition of fibrinolysis inhibitors or bovine proteins. **Chapter 6** describes a randomized, multicentre study in which the safety and cost-effectiveness of CryoSeal in patients undergoing elective isolated CABG surgery was evaluated.

Accumulated blood in the pericardium contains elevated levels of pro- and anticoagulant proteins due to surgical trauma and CPB contact. Blood contact between pericardial and systemic blood represents a significant trigger for activation of the coagulation system, that might result in a DIC state and activation of fibrinolysis, both contributing to excessive blood loss (12,34,49). During re-exploration for postoperative bleeding, removal of accumulated blood and clots by solely irrigating the pericardial space with a warm saline solution often is enough to stop the bleeding instantly in a significant number of cases. In **Chapter 7** a pilot study is described which evaluates the safety, feasibility and effect on blood loss of continuous postoperative pericardial flushing (CPPF) after cardiac.

In **Chapter 8** , the consequences of patient management of the studies described in this thesis will be discussed, as well as future recommendations.

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Part I

Etiology and Diagnosis

CHAPTER 2

The effects of pre- and postoperative fibrinogen levels on blood loss after cardiac surgery: A systematic review and meta-analysis

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ABSTRACT

Objectives

Fibrinogen concentrate is increasingly used in cardiac surgery when bleeding is anticipated or ongoing. As randomized clinical studies to support this are lacking, it is relevant to know whether lower fibrinogen levels are associated with excessive bleeding. We performed a systematic review and meta-analysis to define the association between fibrinogen levels and blood loss after cardiac surgery.

Methods

A database search (January 2013) was performed on publications assessing the association between pre- and postoperative fibrinogen levels and postoperative blood loss in adult patients undergoing cardiac surgery. Cohort studies and case-control studies were eligible for inclusion. The main outcome was the pooled correlation coefficient, calculated via the Fisher's Z transformation scale, in a random effects meta-analysis model stratified for time point at which fibrinogen was measured.

Results

A total of 20 studies were included. The pooled correlation coefficient of studies (n=9) concerning preoperative fibrinogen levels and postoperative blood loss was -0.40 (95% confidence interval: -0.58, -0.18), pointing towards more blood loss in patients with lower preoperative fibrinogen levels. Among articles (n=16) reporting on postoperative fibrinogen levels and postoperative blood loss, the pooled correlation coefficient was -0.23 (95% confidence interval: -0.29, -0.16).

Conclusions

Our meta-analysis indicated a significant but weak to moderate correlation between pre- and postoperative fibrinogen levels and postoperative blood loss in cardiac surgery. This moderate association calls for appropriate clinical studies, whether fibrinogen supplementation will decrease postoperative blood loss.

INTRODUCTION

Excessive blood loss is a frequent complication after cardiac surgery (1,2) requiring reoperation in about 5-10% of patients. Postoperative bleeding obligating transfusions and surgical re-exploration is associated with mortality, morbidity (such as increased sternal wound infection, risk of transfusion-related complications) and higher costs (3-5).

Multiple factors are associated with excessive bleeding after cardiac surgery, but causal pathways have not been elucidated in full detail (5). A surgical cause is only found in approximately half of the patients undergoing reoperation for bleeding. In the remainder of patients an acquired coagulopathy contributes to bleeding. The latter can either be caused by a pre-existing coagulation factor deficiency, a drug-induced platelet inhibition or by an acquired, operation related hemostatic defect (3,6). Suggested mechanisms for the acquired defects include the use of high doses insufficiently neutralized heparin, hemodilution, activation of the hemostatic system resulting in disseminated intravascular coagulation, tissue trauma, platelet dysfunction and excessive fibrinolysis (7,8). Identification of patients at risk for excessive blood loss would offer possibilities to initiate preventive measures. However, diagnostic efficiency of the hemostasis screening tests to identify such indicators is often low.

A suggested predictor of excessive blood loss is fibrinogen, a protein which plays an essential role in coagulation. It has a dual role as it enhances platelet aggregation via binding to the GPIIb/IIIa receptors on platelets and it is converted into fibrin to form an insoluble clot. Cardiac surgery, however, can result in a significant reduction of fibrinogen concentration and function, due to blood loss, hemodilution, platelet activation by cardiopulmonary bypass (CPB), a large wound area for clot formation, hypothermia and acidosis (9,10). The threshold of fibrinogen at which bleeding complications are provoked is difficult to define, as it depends on the status of hematocrit, thrombin formation, platelet number and function, clotting enzyme activities (9), underlying clinical conditions (e.g. hematological malignancy and liver insufficiency (10)) and age. Several reports suggest that a fibrinogen level

of >2 g/l would be sufficient to ensure adequate coagulation even in the presence of moderate thrombocytopenia (10-12).

An inverse association between pre- and postoperative fibrinogen levels, and bleeding risk, even for levels within the normal reference range (1.5-4.0 g/l) was often reported (11). It is suggested that fibrinogen supplementation might decrease blood loss after cardiac surgery (10). Although clinical trials are lacking (1,13), fibrinogen supplementation is increasingly used, also in cardiac surgery. To support such policy a role of pre- and postoperative fibrinogen levels in the development of (excessive) blood loss after cardiac surgery should be unequivocal. We performed a systematic review and meta-analysis to define the association between fibrinogen levels and blood loss after cardiac surgery.

METHODS

Information sources and search

We searched PubMed, Embase, Web of Science, COCHRANE, CINAHL, Academic Search Premier and ScienceDirect using predefined search terms (Supplemental data, Appendix A). The search was performed in January 2013. We used both MeSH terms and free text words. Language restrictions for Dutch, English, French and German articles were set in advance. Full-text articles and meeting abstracts published since 1957 (the year in which the Clauss method to measure fibrinogen was first described) were eligible for evaluation (14).

Study selection and endpoint definitions

To be included in the analysis, studies had to assess the association between pre- and postoperative fibrinogen levels and postoperative blood loss or transfusions in adult patients undergoing cardiac surgery. The study should report either the amount of blood loss or transfusions stratified by levels of fibrinogen or the risk for blood loss (risk ratio or odds ratio (OR)) when comparing fibrinogen levels. Because most articles presented information about the association between fibrinogen level and blood loss we focused on the correlation with bleeding. Only two studies

included in the meta-analysis presented a correlation with blood transfusions. Cohort and case-control studies were considered. Intervention studies with fibrinogen supplementation were excluded.

The definition of (excessive) postoperative blood loss varied between the included articles. Excessive bleeding was defined by the authors of the included articles as total postoperative drain volume of >400 ml in 1 hour, >200 ml for 2 consecutive hours, or >100 ml for 4 consecutive hours, as >200 ml per hour, >150 ml for 2 consecutive hours, or >100 ml for 3 consecutive hours postoperative or as >1500 ml within the first 24 h after surgery. Patients with excessive blood loss (PEBL) were characterised as patients with postoperative blood loss of >1000 ml in the first 24 h after operation, mean blood loss of 1650 ml ± 280 ml, median blood loss of 949 ml, blood loss >1000 ml in the first 16 h after operation, bleeding >600 ml in the first 8 h postoperative, postoperative blood loss >200 ml per hour within the first 4 h after operation with median of 787 ml, requiring re-exploration for bleeding ≥200 ml per hour for over 4 h or experiencing a sudden increase in bleeding after 2 h postoperative, postoperative blood loss >500 ml in the first 24 h after operation or patients in the higher tertile of blood loss with median 1050 ml (700-2550 ml). All definitions for blood loss were accepted and PEBL stated in the article were handled as PEBL for analysis (Table 1).

Table 1. Details of included studies.

Investigators (year of publication)	Design	n	Time point of fibrinogen determination (method)	Clinical characteristics	Outcome measurements
Blome *et al* (2005)	Case-control	98	Preoperative Postoperative (Clauss)	Divided in 3 groups of BL; lower, mid and high tertile Low (I): median 400ml (150- 475) High (III): median 1050ml (700-2550)	BL <12 and 24h fgn median (10^{th} and 90^{th} percentiles) EE: *P*-value
Bolliger *et al* (2008)	Case-control	197	Preoperative Postoperative (not reported)	CABG surgery. PEBL: >1000ml/24h Non-PEBL: <1000ml/24h	BL<12 and 24h fgn range EE: correlation
Davidson *et al* (2008)	Case-control	58	Postoperative (Clauss like)	Primary CABG surgery. PEBL >200ml/h <4h (n=8): median BL 787ml vs. Non-PEBL (n=50): median BL 150ml	BL<4h fgn median (range) EE: *P*-value
Essell *et al* (1993)	Case-control	36	Postoperative (not reported)	All cardiac surgical patients.	BL and BT<24h EE: *P*-value
Faraday *et al* (2002)	Cohort	57	Postoperative (Clauss like)	Elective CABG, (multiple) valve replacement, ascending aortic aneurysm repair and combined valve-CABG procedures*	BL<24 and BT<24h fgn OR EE: correlation
Fassin *et al* (1991)	Case-control	107	Postoperative (not reported)	CABG surgery. Group I (n=70): <1000ml/24h vs Group II (n=33): >1000ml/24h	BL<24h and BT fgn mean (SD) EE: mean differences
Gravlee *et al* (1994)	Case-control	897	Postoperative (Clauss like)	Elective and emergent cardiac surgery. PEBL: BL >1000ml/16h vs. Non-PEBL: BL <1000ml/16h	BL<16h and BT fgn mean (SD) EE: correlation

Table 1. Details of included studies. (continued)

Investigators (year of publication)	Design	n	Time point of fibrinogen determination (method)	Clinical characteristics	Outcome measurements
Hall *et al* (2002)	Case-control	82	Postoperative (Clauss like)	Elective and emergent cardiac surgery. The cases with BL requiring re-exploration (n=82, = 200ml/h 4h or sudden rise BL <2h) and a controls selected random (n=478)	BL <18-24h fgn EE: *P*-value
Josefy *et al* (2011)	Cohort	35	Preoperative (Clauss like)	Patients undergoing CABG surgery not taking clopidogrel*	BT; RBCs, platelets, FFPs and cryoprecipitated AHF fgn EE: correlation
Karkouti *et al* (2010)	Cohort	101	Postoperative (not reported)	Complex cardiac surgery (other than isolated CABG, single valve surgery, or repair of atrial septal defect). Median BL 952 ml/24h	BL<24h fgn mean (SD) EE: correlation
Karlsson *et al* (2008)	Cohort	170	Preoperative (Clauss)	CABG surgery. Median BL 360ml/12h	BL<12h and BT during admission fgn mean (SD) EE: correlation
Liu *et al* (2000)	Cohort	46	Preoperative Postoperative (not reported)	Primary CABG surgery. The median BL was 825 ml/24h	BL<24h fgn (correlation) EE: correlation
Marengo-Rowe *et al* (1979)	Case-control	774	Postoperative (not reported)	CABG surgery. PEBL >600ml/8h (n=164) vs. Non-PEBL <600ml/8h (n=610)	BL<8h EE: *P*-value
Nuttall *et al* (1997)	Case- control	82	Postoperative (Clauss)	CABG, valve replacement or repair, or congenital heart surgery. PEBL (n=30): median BL 949ml vs Non-PEBL (n=52): median BL 547ml	BL and BT <24h fgn mean (SD) EE: correlation

Table 1. Details of included studies. (continued)

Investigators (year of publication)	Design	n	Time point of fibrinogen determination (method)	Clinical characteristics	Outcome measurements
Ozolina *et al* (2011)	Case-control	124	Preoperative Postoperative (not reported)	Cardiac surgery* Group I: <500ml/24h Group II: >500ml/24h	BL<24h EE: correlation
Prohaska *et al* (2008)	Cohort	2831	Preoperative (Clauss like)	CABG surgery, aortic valve replacement, combined CABG and aortic valve replacement and other single or combined cardiac procedures*	BT; RBCs, FFPs and PCs perioperative and <2days fgn mean (SD) EE: OR
Ternström *et al* (2010)	Cohort	59	Preoperative Postoperative (Clauss)	Elective CABG. Median postoperative BL 380ml/12h	BL<12h EE: correlation
Ucar *et al* (2007)	Cohort	97	Preoperative (Clauss)	All cardiac surgical patients.	BL<48h fgn mean (SD) EE: correlation
Wahba *et al* (1997)	Case-control	89	Postoperative (Clauss)	Primary CABG surgery. PEBL (n=14):1.650 ±280ml vs Non-PEBL (n=75): 780 ±250ml	BL until removal of drains (usually day 1) FGN mean (SD) EE: correlation
Welsby *et al* (2006)	Cohort	32	Postoperative (Clauss like)	CABG or valve operations.	BL<4h FGN mean (SD) EE: correlation

BL, blood loss; fgn, fibrinogen; EE, effect estimate; PEBL, patients with excessive blood loss; Clauss like, automated, probably Clauss like, coagulation method; BT, blood transfusions; OR, odds ratio

* Cardiopulmonary bypass (CPB) usage was not mandatory for study inclusion

Data extraction and risk of bias assessment

Selection, data extraction and risk of bias assessment was done by 2 reviewers independently (J.E and C.L.I.G) using a predefined extraction sheet. Concerning our research questions, we collected the following variables: study population, time point at which fibrinogen was measured, level of fibrinogen, amount of perioperative blood loss and transfusions. To assess the risk of bias, we evaluated the study design, measurement of exposure, blinding for fibrinogen level, definition and measurement of outcomes, completeness of follow-up and adjustment for confounders. These variables were used to explore sources of heterogeneity. No summary score for the risk of bias assessment was used for analytical purposes.

Summary measures and synthesis of results

For all studies the main effect-measure was extracted (odds ratio, mean-difference, correlation coefficient, *P*-value) with accompanying measure of uncertainty (95% confidence interval, standard error or *P*-value). We stratified studies based on the time point at which fibrinogen was measured (pre- vs postoperatively).

For analytical purposes, we transformed all effect-measures to correlation coefficients (r). *P*-values were transformed with Fisher's Z transformation scale and via the inverse Fisher's Z transformation to correlation coefficients. Odds ratios were transformed to a correlation scale using the tetrachoric correlation. When the association between fibrinogen and blood loss was characterized by a regression coefficient, the correlation was calculated from the regression coefficient and the standard deviation of the independent variable in the regression. These transformed correlation coefficients were pooled in a random effects model as for instance described by Borenstein (15). Finally, results were transformed back to the correlation scale by the inverse Fisher's Z-transformation. All analyses were performed with STATA release 10 (StataCorp, Texas, USA).

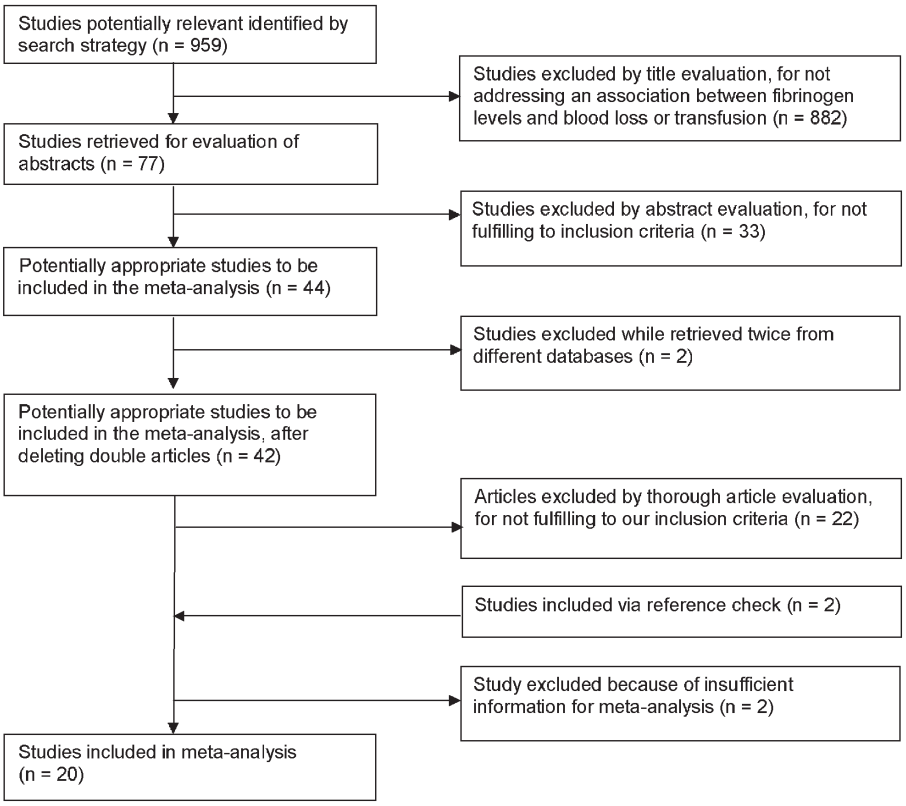

Figure 1. Flow chart of study selection.

RESULTS

Study selection and characteristics

Our search strategy provided 959 articles of which 77 were retrieved for more detailed assessment. The search strategy's flow-chart is shown in Figure 1. A total of 20 articles were included, which incorporated 5972 patients. In Table 1 details of included studies are provided. Nine articles were classified as cohort studies in which fibrinogen was measured before or after operation and the presence of a correlation with blood loss was evaluated. The other 11 studies were nested case-control studies, where cases were defined as PEBL and controls as patients without

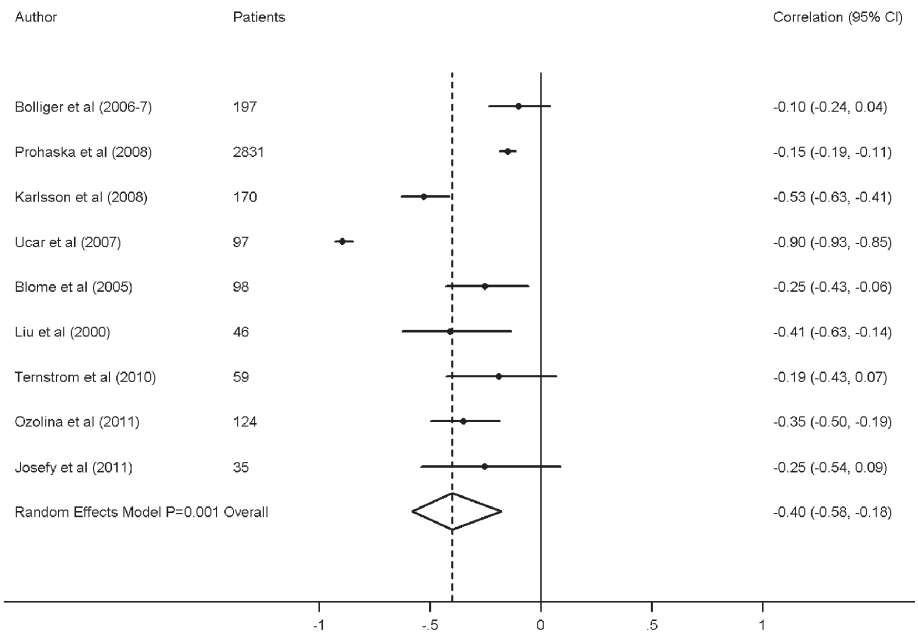

Figure 2. Meta-analysis of the correlation between preoperative fibrinogen and postoperative blood loss.

excessive blood loss (non-PEBL). The definition to classify blood loss (and therefore also case-control status) differed between the articles. Fibrinogen was measured by the Clauss method in 6 papers, 7 papers used an automated, Clauss like, coagulation method and 7 articles did not report the method used. Effect estimates were reported as correlations in 13 studies, as *P*-values in 5 studies, Prohaska reported an odds ratio and the article of Fassin reported mean differences.

Risk of bias

All studies included assessed the association between pre- and postoperative fibrinogen levels and postoperative blood loss or transfusions in adult patients undergoing cardiac surgery. Four articles reported to have performed fibrinogen measurement before patients received any coagulation factors or FFPs. In the majority of articles this form of bias was not reported, 2 stated that they could not exclude it. Furthermore, factors that also might have influenced postoperative

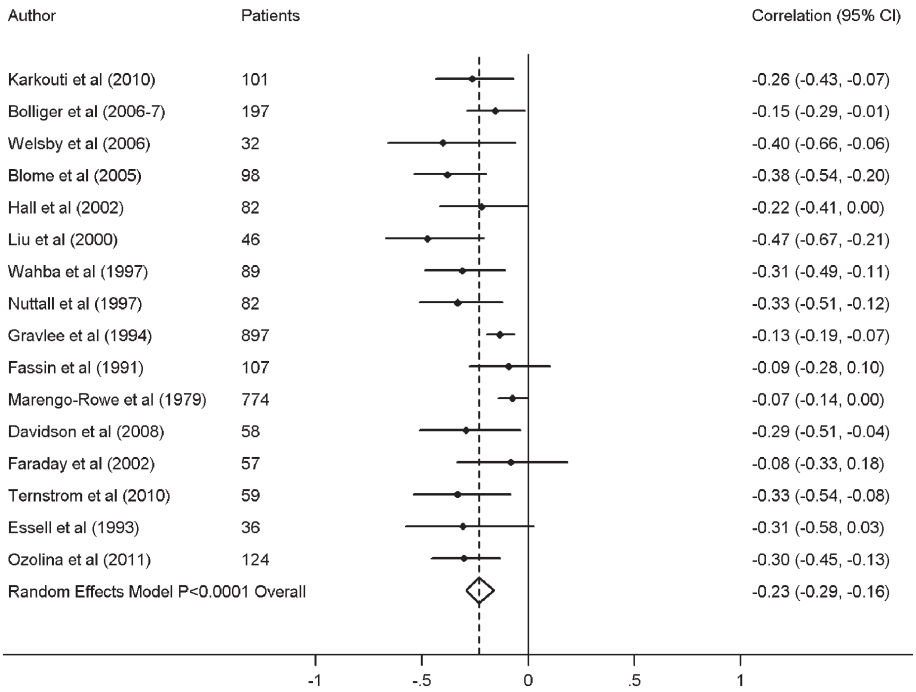

Figure 3. Meta-analysis of the correlation postoperative fibrinogen and postoperative blood loss.

blood loss are drug-induced platelet dysfunction, postoperative administration of low molecular weight heparins, or impairment of von Willebrand factor and factor VIII by hydroxyethyl starch solutions. Most articles do not report how these potential confounding factors were dealt with. None of the articles reported included patients lost in follow up.

Synthesis of results

Nine studies contributed to the meta-analysis on the association between preoperative fibrinogen level and blood loss. Calculated correlations were all below -0.10 (Figure 2). In a random effects model, the pooled correlation for the association between preoperative fibrinogen levels and postoperative blood loss was -0.40 (95% CI, -0.58, -0.18). Clear heterogeneity was shown: I^2 = 95.7%, x^2 = 187.58, *P*<0.0001.

Sixteen studies contributed to the meta-analysis on the association between postoperative fibrinogen level and blood loss. Calculated correlations were all below -0.07 (Figure 3). In a random effects model, the pooled correlation for the association between postoperative fibrinogen levels and blood loss was -0.23 (95% CI, -0.29, -0.16). Tests for heterogeneity showed the following results: I^2 = 54.5%, x^2 = 32.97, *P* = 0.005).

DISCUSSION

The present meta-analysis revealed a significant pooled correlation between pre-operative (r = -0.40, 95% CI, -0.58, -0.18) or postoperative (r = -0.23, 95% CI, -0.29, -0.16) fibrinogen levels on the one hand and blood loss after cardiac surgery on the other hand. This confirms the important role of fibrinogen in the coagulation process. However, as the correlation is not very strong, it is likely that other factors like hematocrit, thrombin formation, platelet number and function and clotting enzyme activities are at least as important in the origination of excessive blood loss.

Despite the presence of statistical heterogeneity among studies, likely reflecting methodological differences between studies, all included studies showed a negative correlation between fibrinogen level and postoperative blood loss. Because of this absence of qualitative heterogeneity, we decided to pool correlation coefficients in a random-effects model, which partly accounts for heterogeneity.

It is important to state that the primary aim of this study was to evaluate the correlation an association between the pre- and postoperative fibrinogen levels and blood loss after cardiac surgery. This may be is most validly represented by the preoperative measurement. Due to operation influences e.g. CPB usage, hemodilution, platelet dysfunction etc. (5,16), the postoperative fibrinogen levels are probably more biased, and therefore, less accurate for this evaluation, even when no FFP or fibrinogen administration is reported in the original article. These surgery related hemostatic defects may have had more impact causing bleeding than those existing prior to the operation (17). Still, the preoperative correlation

found is stronger than the postoperative one, suggesting that surgical procedures also might have a negative, downsizing effect on the correlation. Ternström (18) demonstrated a correlation between pre- and postoperative fibrinogen level (r = 0.80, *P*<0.001), suggesting that the time point at which fibrinogen is measured might be less important.

Hemodilution due to the use of CPB and volume supplementation reduces coagulation factor levels to approximately 25-50% of baseline, parallel with the decrease of the hematocrit level (1,18,19). Each coagulation factor has to be present at a minimum level adequate to support hemostasis, often well below the normal range. Simultaneous reductions in multiple coagulation factor levels that develop during CPB, therefore, generally do not result in bleeding complications (16). However, patients with a lower baseline value are more prone to develop a fibrinogen level insufficient to ensure adequate coagulation. Moreover, if a low fibrinogen level occurs in combination with other factors impairing the strength of clot formation the risk of bleeding may increase. In this context Fenger-Erikson (20) demonstrated that a dilutional coagulopathy due to administration of hydroxyethyl starch (HES) products resulted in less stable clotting assessed by thromboelastometry. Also, patients undergoing urgent surgery while using (dual) antiplatelet therapy may need higher fibrinogen levels to avoid excessive bleeding. Unfortunately, only a few studies in our review included urgent surgery patients and no correlation between fibrinogen levels and bleeding in this population was possible. The lowest average of fibrinogen levels reported in the assessed articles for this meta-analysis is 2.6 ± 0.79 g/l pre- and 1.6 ± 1.18 g/l postoperative, normal reference range is 1.5-4.0 g/l.

Other laboratory parameters, e.g. activated partial thromboplastin time (APTT), prothrombin time (PT), platelet count have also been studied in this context (2,5,7,13), but all these tests, including fibrinogen, have (as single parameter) limited utility for diagnosis of postoperative coagulopathy because their results are only weakly associated with parameters of clinical bleeding or because they are not rapidly available (7,21). Collective assessment of several laboratory values using thromboelastography (TEG) or whole blood hemostatometry may provide

more applicable diagnostic information (5,21), but their association with blood loss remains low.

Although, the correlation between fibrinogen levels and blood loss is not very strong, fibrinogen concentration administration in patients with ongoing bleeding could be considered as a possible treatment option. Indeed, several investigators advocate that fibrinogen can act as first-line therapy to correct postoperative bleeding and reduce the use of allogeneic blood products without evidence of thrombotic complications (10). A recent meta-analysis on the effect of fibrinogen concentrate in patients with various bleeding conditions reported no outcome differences for bleeding, mortality, length of stay at the intensive care unit or side effects. Patients treated with fibrinogen concentrate used significantly less other blood products. The authors concluded that the six included RCTs were of low quality and comprised altogether only 248 patients, not all eligible for evaluation for all outcome effects (22). In this context, it is important to note that fibrinogen is an acute-phase protein which level increases gradually after surgical procedures (1,10,18). Therefore, the use of fibrinogen concentrate, in combination with this physiologic increase postoperative, might enlarge the risk of thrombosis.

Many studies concluding that there is no statistically significant correlation between fibrinogen and postoperative blood loss did not give information about the strength of the correlation or exact *P*-value, and could therefore not be included. This reporting bias could have been further influenced by unpublished studies failing to demonstrate significant correlations. Therefore, the association between fibrinogen and postoperative blood loss that we calculated could be overestimated. Other important limitations of this meta-analysis are the low power and quality of the included studies, the substantial heterogeneity among studies and the variability in the definition of the endpoint blood loss. Systematic reviews on the effect of intervention with fibrinogen concentrate reveal similar lack of quality of the studies (22, 23). However, fibrinogen supplementation is currently solely substantiated on this questionable information. The different assays used among studies, should not be of influence on the analysis of the association with bleeding, while the comparison between PEBL and non-PEBL within the article is performed by the same assay. Also, although the definition of blood loss or a bleeding patient

appeared to be highly divergent among studies, overall, blood loss of >200 ml per hour or > 1 liter in the postoperative period was considered as excessive bleeding. Furthermore, in some of the evaluated studies there is a lack of differentiation between patients who bleed from surgical causes and those that apparently have a severe coagulopathy without a specific surgical source of bleeding (24). Moreover, it is not always clear whether fibrinogen supplementation, transfusions of FFPs or platelets took place before sampling, influencing the results, or after the postoperative fibrinogen measurements. Finally, the usage of other methods to measure fibrinogen, like PT-derived method, or the presence of HES products may lead to an overestimation of the fibrinogen amount (25).

In conclusion, although the results of our meta-analysis support the association between lower fibrinogen levels and a higher bleeding risk after cardiac surgery, the correlation is weak to moderate, the studies evaluated contain substantial heterogeneity, and are of low power and quality. However, this cumbersome available information forms the current base to substantiate fibrinogen supplementation. Fibrinogen supplementation for patients with a lower preoperative fibrinogen level or concomitant factors impairing coagulation such as the use of antiplatelet drugs might have a beneficial effect in reducing the risk of excessive blood loss, but studies of better quality, preferentially randomized, are required before any specific recommendation can be proposed.

CONFLICTS OF INTEREST

None declared.

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APPENDIX A

Predefined search terms for electronic databases

PubMed

((”cardiac surgery” OR ”heart surgery” OR ”cardiac surgical” OR ”heart surgical” OR ”Cardiac Surgical Procedures”[mesh:noexp] OR Cardiomyoplasty OR ”Heart Bypass, Right”[mesh] OR ”heart bypass” OR ”Heart Valve Prosthesis Implantation”[mesh] OR ”Heart Valve Prosthesis Implantation” OR ”Myocardial Revascularization”[mesh:noexp] OR ”Myocardial Revascularization”[tw] OR ”Atherectomy, Coronary”[mesh] OR ”Coronary Atherectomy” OR ”Coronary Artery Bypass”[mesh] OR ”Coronary Artery Bypass” OR cabg OR ”Internal Mammary-Coronary Artery Anastomosis”[mesh] OR ”Internal Mammary-Coronary Artery Anastomosis” OR ”Heart Diseases/surgery”[Mesh] OR ”Cardiovascular Diseases/surgery”[Mesh]) AND (fibrinogen OR fibrinogens OR ”Coagulation Factor I” OR ”Factor I”) AND (”blood loss” OR Hemorrhage OR Haemorrhage OR Hemorrhages OR Haemorrhages OR Hemorrhagic OR Haemorrhagic OR hematoma OR haematoma OR hematomas OR haematomas)) NOT (child NOT adult) AND (English[lang] OR French[lang] OR German[lang] OR Dutch[lang])

Embase

((heart surgery/ or cardiomyoplasty/ or coronary artery surgery/ or heart valve surgery/ or heart ventricle remodeling/ or minimally invasive cardiac surgery/ or mustard operation/ or norwood procedure/ or open heart surgery/ OR (”cardiac surgery” OR ”heart surgery” OR ”cardiac surgical” OR ”heart surgical” OR Cardiomyoplasty OR ”Heart Bypass*” OR ”Heart Valve Prosthe*” OR ”Myocardial Revascularization” OR ”Coronary Atherectom*” OR ”Coronary Artery Bypass*” OR cabg OR ”Internal Mammary-Coronary Artery Anastomosis”).mp OR exp heart disease/su) AND (fibrinogen/ OR fibrinogen*.mp OR ”Coagulation Factor I”.mp OR ”Factor I”.mp) AND (exp bleeding/ OR (”blood loss” OR Hemorrhag* OR Haemorrhag* OR hematoma* OR haematoma*).mp)) AND (adult/ or aged/ or middle aged/ OR adult*.mp) limit 10 to (dutch or english or french or german)

Web of Science

TS=((“cardiac surgery” OR “heart surgery” OR “cardiac surgical” OR “heart surgical” OR Cardiomyoplast* OR “Heart Bypass*” OR “Heart Valve Prosthe*” OR “Myocardial Revascularization” OR “Coronary Atherectom*” OR “Coronary Artery Bypass*” OR cabg OR “Internal Mammary-Coronary Artery Anastomosis”) AND (fibrinogen* OR “Coagulation Factor I” OR “Factor I”) AND (bleeding OR “blood loss” OR Hemorrhag* OR Haemorrhag* OR hematoma* OR haematoma*)) NOT TS=((child* OR infant*) NOT (adult* OR aged*))
Refined by: Languages=(ENGLISH OR GERMAN OR FRENCH OR DUTCH)

Cochrane

ID	Search
#1	MeSH descriptor Cardiac Surgical Procedures, this term only
#2	MeSH descriptor Cardiomyoplasty explode all trees
#3	MeSH descriptor Heart Bypass, Right explode all trees
#4	MeSH descriptor Heart Valve Prosthesis Implantation explode all trees
#5	MeSH descriptor Myocardial Revascularization, this term only
#6	(“cardiac surgery” OR “heart surgery” OR “cardiac surgical” OR “heart surgical” OR Cardiomyoplasty OR “heart bypass” OR “Heart Valve Prosthesis Implantation” OR “Myocardial Revascularization” OR “Coronary Atherectomy” OR “Coronary Artery Bypass” OR cabg OR “Internal Mammary-Coronary Artery Anastomosis”)
#7	(#1 OR #2 OR #3 OR #4 OR #5 OR #6)
#8	MeSH descriptor Fibrinogen explode all trees
#9	(fibrinogen OR fibrinogens OR “Coagulation Factor I” OR “Factor I”)
#10	(#8 OR #9)
#11	MeSH descriptor Hemorrhage explode all trees
#12	(“blood loss” OR Hemorrhage OR Haemorrhage OR Hemorrhages OR Haemorrhages OR Hemorrhagic OR Haemorrhagic OR hematoma OR haematoma OR hematomas OR haematomas)
#13	(#11 OR #12)
#14	(#7 AND #10 AND #13)

#15 ((child* OR infant*) NOT (adult* OR aged*))
#16 #14 AND #15
#17 (#14 AND #15)
#18 (#14 AND NOT #15)

CINAHL

((“cardiac surgery” OR “heart surgery” OR “cardiac surgical” OR “heart surgical” OR Cardiomyoplast* OR “Heart Bypass*” OR “Heart Valve Prosthe*” OR “Myocardial Revascularization” OR “Coronary Atherectom*” OR “Coronary Artery Bypass*” OR cabg OR “Internal Mammary-Coronary Artery Anastomosis”) AND (fibrinogen* OR “Coagulation Factor I” OR “Factor I”) AND (bleeding OR “blood loss” OR Hemorrhag* OR Haemorrhag* OR hematoma* OR haematoma*)) NOT ((child* OR infant*) NOT (adult* OR aged*)))

Academic Search Premier

((“cardiac surgery” OR “heart surgery” OR “cardiac surgical” OR “heart surgical” OR Cardiomyoplast* OR “Heart Bypass*” OR “Heart Valve Prosthe*” OR “Myocardial Revascularization” OR “Coronary Atherectom*” OR “Coronary Artery Bypass*” OR cabg OR “Internal Mammary-Coronary Artery Anastomosis”) AND (fibrinogen* OR “Coagulation Factor I” OR “Factor I”) AND (bleeding OR “blood loss” OR Hemorrhag* OR Haemorrhag* OR hematoma* OR haematoma*)) NOT ((child* OR infant*) NOT (adult* OR aged*)))

ScienceDirect

((“cardiac surgery” OR “heart surgery” OR “cardiac surgical” OR “heart surgical” OR Cardiomyoplast* OR “Heart Bypass*” OR “Heart Valve Prosthe*” OR “Myocardial Revascularization” OR “Coronary Atherectom*” OR “Coronary Artery Bypass*” OR cabg OR “Internal Mammary-Coronary Artery Anastomosis”) AND (fibrinogen* OR “Coagulation Factor I” OR “Factor I”) AND (bleeding OR “blood loss” OR Hemorrhag* OR Haemorrhag* OR hematoma* OR haematoma*)) AND ((child* OR infant*) NOT (adult* OR aged*)

CHAPTER 3

Fibrinogen reduction and coagulation in cardiac surgery: An investigational study

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ABSTRACT

Background

Fibrinogen as precursor of fibrin, plays an essential role in clot formation. There are three main mechanisms associated with a reduction in fibrinogen concentration during cardiac surgery; hemodilution, consumption, and degradation. Moreover, early fibrinogen degradation products (FgDPs) can interfere with normal fibrin formation of intact fibrinogen.

Objectives

The aim of this study was to determine the relative contributions of hemodilution, consumption and degradation to fibrinogen loss in cardiac surgery and to evaluate the effects fibrinogen degradation products on blood clot formation *in vitro.*

Methods

First, fibrin and fibrinogen concentrations, their degradation products, hematocrit, and albumin concentrations were compared in 10 patients before and after isolated coronary artery bypass graft (CABG) surgery with cardiopulmonary bypass. Second, *ex vivo* fibrinogen supplementation experiments were performed. Finally, the effects of purified FgDPs on clotting time and clot firmness were established *in vitro* in whole blood by ROTEM^{TM}.

Results

Fibrinogen plasma concentration decreased 30% during surgery. This drop appears to be mainly caused by hemodilution, since both hematocrit and albumin levels decreased and no relevant increase in D-dimer levels and FgDPs was observed. Furthermore, the coagulation profile normalized after addition of purified fibrinogen. Early FgDPs demonstrated a significant impact on *in vitro* whole blood clotting.

Conclusions

Although early FgDPs have a pronounced effect on blood clot formation *in vitro* and therefore may induce or enhance *in vivo* coagulopathy, the drop of fibrinogen

concentration seen after CABG surgery (using tranexamic acid) is primarily caused by hemodilution.

INTRODUCTION

Fibrinogen, as precursor of fibrin, plays an essential role in clot formation. In contrast with most other coagulation factors that have enzymatic activity and can activate multiple downstream target molecules, fibrinogen is a structural protein that at the final stage of the coagulation cascade has a dual role as it enhances platelet aggregation and is converted into an insoluble fibrin clot. Modest changes in plasma concentration were not considered to be important for the hemostatic capacity of blood. However, recent reports have demonstrated that a decrease in fibrinogen plasma concentration of 25-50%, which resembles a decrease of 1-2 g/l, affects blood clot formation (1,2).

There are several mechanisms via which fibrinogen concentrations or functionality in blood can be decreased during cardiac surgery. Cardiopulmonary bypass (CPB) priming, volume replacement with crystalloids and colloids, and blood transfusions containing low levels of fibrinogen and other coagulation factors (3), can result in hemodilution, and consequently, a significant reduction of fibrinogen (4), hematocrit (Ht), and albumin (Alb) concentrations. Second, excessive activation of the hemostatic system, owing to, for example, blood contact with the surface of the CPB circuit and operation trauma, occasionally instigate a disseminated intravascular coagulation process with consumption of platelets, fibrinogen, and other coagulation factors. This process can be demonstrated by evaluation of the fibrin degradation product D-dimer (FbDP), which also reflects fibrinogen consumption. A third mechanism leading to fibrinogen loss during surgery is fibrinogenolysis or fibrinogen degradation, caused by e.g. plasmin mediated proteolysis after activation of plasminogen through tissue plasminogen activator (t-PA) (5). This mechanism warrants special attention because it can not only reduce the concentrations of normal functional fibrinogen but it can also result in formation of various stages of fibrinogen degradation products (FgDPs) that can interfere with normal fibrin formation of intact fibrinogen. It is unknown to which extent these different mechanisms contribute to the decrease in fibrinogen concentration during cardiac surgery.

Degradation of fibrinogen and FgDPs has direct effects on thrombus formation (6,7). Degradation of fibrinogen starts at the carboxyl terminus of the Aα-chain, a domain that is very sensitive to enzymatic proteolysis (8). Further degradation of fibrinogen takes place at specific sites on the Bβ- and γ-chains and results in the early FgDP designated fragments X and Y. Late stage degradation products are fragment E and fragment D1. FgDPs are considered an important risk factor for bleeding after thrombolysis therapy (9) and have been detected in high levels after coronary artery bypass graft (CABG) surgery performed in the absence of aprotinin (10). However, it is not known what effects various fibrinogen degradation fragments have on rotation thromboelastometry (ROTEM^{TM}) analysis of coagulation.

The present study evaluates the contribution of hemodilution, fibrin(ogen) consumption, and degradation to fibrinogen concentration reduction after isolated CABG surgery with the use of tranexamic acid (i.e. standard procedure in most hospitals in the Netherlands). Furthermore, *ex vivo* fibrinogen supplementation is performed to confirm our findings and finally, the impact of FgDPs on *in vitro* whole blood clotting is studied.

METHODS

Patients

Ten adult patients undergoing elective, isolated CABG with usage of CPB and a preoperative fibrinogen concentration of less than 3.8 g/l (11) were included in this prospective, observational study. Patients that needed emergency surgery, with heart failure (defined as an echocardiographically estimated ejection fraction bi-plane below 35%), a history of bleeding diathesis, or coagulopathy were excluded. Patient characteristics are shown in Table 1. The study protocol was approved by the Medical Ethical commission (MEC number P10.154) of the Leiden University Medical Centre (LUMC, Leiden, The Netherlands) and written informed consent was obtained from each patient.

Table 1. Baseline and clinical characteristics.

Age (years)	68.5 (54-80)
Female	1 (10%)
Logistic EuroSCORE	2.37 (0.88-8.15)
Carbasalate calcium continued before operation	9 (90%)
Clopidogrel continued before operation	3 (30%)
Operation time (min)	297 (215-385)
CPB* time (min)	127 (102-194)
Aorta cross clamp time (min)	102 (79-140)
Minimal pH during surgery	7.35 (7.28-7.44)
Minimal rectal temperature (°C)	34.5 (32.6-35.5)
Tranexamic acid used (mg)	3923 (2000-4927)
Postoperative blood loss witdin 24 h (ml)	780 (200-1240)
Preoperative laboratory values (T0)	
Hemoglobin (mmol/l)	8.7 (7.3-9.0)
Hematocrit (l/l)	0.41 (0.36-0.46)
Fibrinogen Clauss (g/l)	3.2 (2.4-3.7)
FIBTEM MCF† (mm)	14 (10-18)
aPTT (s)	29.7 (25.9-32.4)
PT (s)	15.3 (13.7-16.3)
Platelet count (x109/l)	159 (116-276)

* Cardiopulmonary bypass; † ROTEM™ analysis of the maximum clot firmness (MCF) with FIBTEM for the assessment of fibrinogen under platelet inhibition by cytochalasine D. Data are presented as number (percentage) or median (range).

Clinical management

CABG surgery was performed according to local standardized protocol. All surgical procedures were executed via a midline sternotomy under normothermic CPB machine (Jostra Maquet, Marquet, Hirrlingen, Germany) with intermittent antegrade warm-blood cardioplegia and prevention of acidosis. Heparin was injected as single bolus (300 U/kg in both groups) and the degree of anticoagulation was monitored using the activated clotting time (ACT, Hemochron Signature Elite; ITC; Pleasanton, CA, USA). Additional heparin (5000 U) was given if the ACT fell below 400 s. After surgery heparin was neutralized with protamine sulphate (1000 IE heparin with 10 mg protamine sulphate). Patients were followed until discharge from the hospital. None of the patients received fresh frozen plasma or fibrinogen concentrate trans-

fusion prior to blood sampling. Three patients received cell saver with a median of 474 ml (150-750). Tranexamic acid was used in all patients with a median of 3923 mg (2000-4927).

Coagulation measurements

Of each patient citrated whole blood aliquots with 0.105 mol/l buffered trisodium citrate solution (BD Vacutainer, Plymouth, UK) were sampled preoperative (T0) and directly after sternum closure (T1). All samples were drawn from the central venous line (v. jugularis). Standard blood tests were performed at the hospital laboratory. Within 10 min after obtaining the sample ROTEM™ (Pentapharm, Munich, Germany), analysis was performed and the remaining aliquots were centrifuged at 2700 g for 10 min at 18 ºC to obtain platelet-poor plasma. Plasma was aliquotted and stored at -80 ºC until batch analysis. Albumin concentrations were measured using the ALB plus assay (Cobas, Roche Diagnostics, Mannheim, Germany), to differentiate between a general dilution of the blood or a specific fibrinogen loss (by other cause).

Functional fibrinogen concentrations in plasma were determined according to the method by Clauss (12), using STA fibrinogen reagent (Roche Diagnostics, Almere, The Netherlands). ROTEM™ analysis was performed in two channels, with EXTEM as extrinsic test and FIBTEM for fibrinogen assessment under platelet inhibition by cytochalasine D. For both EXTEM and FIBTEM clotting time (CT), clot formation time (CFT), and maximum clot firmness (MCF) were determined. Total fibrinogen antigen, reflecting all fibrinogen species in plasma, was measured using the Polyclonal Rabbit Anti-Human Fibrinogen ELISA (Dako, Glostrup, Denmark), reacting with all fibrinogen species in plasma. The level of intact fibrinogen, representing only fibrinogen species that contain intact Aα chain, was measured using an ELISA based on two monoclonal antibodies (Mab) that react with the carboxyl terminus (G-8) and amino terminus (Y-18) of the fibrinogen Aa chain (13). Fibrin degradation products, that is, D-dimers (FbDPs), which reflect fibrinogen consumption, were determined using STA Liatest D-dimer (Roche Diagnostics, Almere, The Netherlands). Furthermore, a quantitative evaluation of plasmin-induced fibrinogen degradation was performed by determination of FgDPs using an ELISA based

on 2 Mabs; the Y-18 and the FDP-14. The latter Mab is specifically designated for plasmin degradation products. Qualitative assessment of fibrinogen degradation was performed using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Invitrogen, Carlsbad, CA, USA). To exclude degradation of fibrinogen by proteolytic enzymes other than plasmin, qualitative assessment of fibrinogen degradation using Western blot analysis was performed with Mab Y-18. In this analysis, the gradation profile of the carboxyl terminal region of the fibrinogen Aa chain was used as an indicator for fibrinogen degradation. Fibrinolysis activation was analyzed via t-PA measurement using TECHNOZYM® t-PA Antigen ELISA (Technoclone GmbH, Vienna, Austria). All samples were analysed without any knowledge of any clinical data or patient outcomes.

Fibrinogen supplementation

To determine if indeed the reduction in fibrinogen concentration is causally related to the observed prolonged CT and reduced MCF, and not the dilution of other coagulation factors, we performed an *ex vivo* supplementation study were purified fibrinogen (Haemocomplettan, CSL) was added to blood of the CABG patients sampled after sternum closure (*n*=10).

The purified plasma fibrinogen was dissolved in phosphate-buffered saline to a concentration of 10mg/ml (A280,1% = 15.0). Than 0.18ml of fibrinogen solution or PBS was added to 1.2ml of whole blood. ROTEM™ analysis in the EXTEM mode was performed as described by the manufacturer and the effect of adding purified fibrinogen on CT and MCF was recorded.

Effects of fibrinogen degradation products (FgDPs) on coagulation

Early and late-stage FgDPs were prepared and purified as described by Nieuwenhuizen (6) and kindly provided by M. Voskuilen. To determine the purity and estimate

their molecular weights, the FgDPs were analysed using non-reducing SDS-PAGE (Life Technologies, The Netherlands). Citrated whole blood was diluted 1:1 using ringer lactate that contained various concentrations of the different FgDPs.
The influence of early (fragment X and Y) and late-stage (fragment D1) FgDPs on CT, CFT, and clot firmness (A 25) of the diluted whole blood of healthy volunteers was determined using ROTEM^{TM} analysis in the EXTEM mode.

Statistical analysis

Data is mainly reported as median (range). Differences between pre and postoperative fibrinogen and fibrin concentrations and their degradation products, hemoglobin, hematocrit, albumin concentrations, and platelet count were calculated using Wilcoxon signed rank test. Statistical significance was defined as a $P<0.05$.

RESULTS

Coagulation measurements

Fibrinogen concentrations

The functional Clauss assay revealed a median plasma fibrinogen concentration of 3.2 g/l (2.4-3.7) preoperative and 2.0 g/l (1.5-2.7) after sternum closure, indicating a 34% decrease during cardiac surgery (P=0.005, Supplementary Table 1). MCF decreased significantly in both in EXTEM (10%, P=0.007) and FIBTEM (43%, P=0.005) analysis (Table 2).

Table 2. Pre- and postoperative laboratory values.

	Preoperative (T0)	After sternum closure (T1)	Median Δ (%)	p
Total Fibrinogen (mg/ml)	2.7 (2.0-3.7)	1.9 (1.5-2.7)	-28	0.005
Intact Fibrinogen (mg/ml)	2.7 (2.0-3.5)	1.9 (1.7-2.3)	-29	0.005
Fibrinogen Clauss (g/l)	3.2 (2.4-3.7)	2.0 (1.6-2.7)	-34	0.005
EXTEM				
CT (s)	52 (44-88)	65 (35-94)	23	0.201
CFT (s)	102 (69-130)	152 (126-219)	52	0.005
MCF (mm)	63 (54-67)	55 (50-60)	-10	0.007
ML (%)	5 (1-13)	0 (0-0)	-100	0.027
FIBTEM				
CT (s)	57 (41-101)	64 (46-84)	9	0.283
CFT (s)	NA	NA	NA	NA
MCF (mm)	14 (10-18)	7 (6-14)	-43	0.005
FbDPs (ng FEU/ml)	338 (209-1209)	838 (209-1532)	114	0.059
FgDPs (ng FEU/ml)	157 (70-260)	170 (105-272	14	0.241
t-PA (ng/ml)	0.9 (0.2-1.8)	2.5 (0.5-4.3)	157	0.013
Hemoglobin (g/l)	8.7 (7.3-9)	6.4 (4.7-7.7)	-25	0.005
Hematocrit (%PCV)	0.41 (0.36-0.46)	0.31 (0.23-0.38)	-25	0.005
Albumin (g/l)	36.6 (29.9-38.5)	23.8 (20.6-27.0)	-30	0.005
Platelet count (×109/l)	159 (116-276)	120 (70-194)	-29	0.005

Fibrinogen levels (total and intact ELISA, Clauss); thromboelastometry (EXTEM) assessment of clotting time (CT); clot formation time (CFT); and maximum clot firmness (MCF); not available (NA); maximum lysis (ML); fibrin (FbDP) and fibrinogen (FgDP); degradation products, tissue plasminogen activator (t-PA); hemoglobin, hematocrit, and platelet count immediately before surgery (T0); and directly after sternum closure (T1). Data are median (range).

Total fibrinogen antigen concentrations demonstrated a median preoperative level of 2.7 g/l (2.0-3.7) as compared to 1.9 g/l (1.5-2.7) after sternum closure, indicating a reduction in total fibrinogen antigen of 28% (*P*=0.005).

Intact fibrinogen antigen concentrations demonstrated similar results with a median level of 2.7 g/l (2.0-3.5) preoperative and 1.9 g/l (1.7-2.3) after sternum closure, representing a 29% reduction in intact fibrinogen antigen levels (*P*=0.005).

Hemodilution

The hematocrit level demonstrated a significant reduction of 25% (*P*=0.005) and was decreased to a median of 0.31 (0.23-0.38) after sternum closure compared to 0.41 (0.36-0.46) preoperative (Table 2, Figure 1). Hemoglobin and albumin concentrations in plasma confirmed this finding with a corresponding 25% (*P*=0.005) and 30% (*P*=0.005) decline, respectively. During reperfusion, after releasing the aortic clamp, patients presented the lowest hematocrit levels with a median of 0.29 (0.23-0.32).

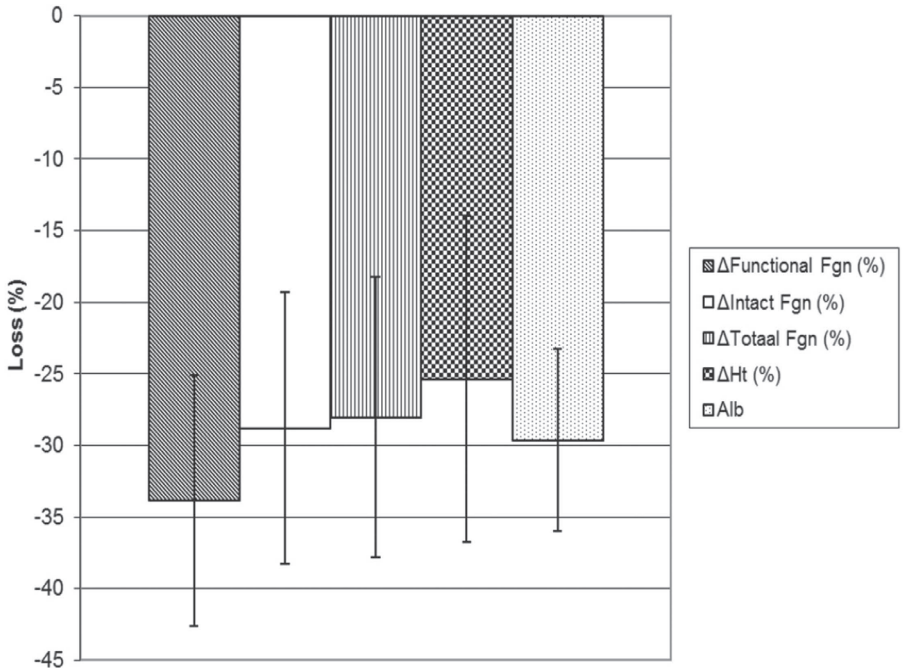


Figure 1. Median postoperative percentage of functional, intact and total fibrinogen (Fgn) loss, as compared to the hematocrit (Ht) and albumin (Alb) levels. Error bar (SD).

Consumption

D-dimer (FbDP) concentrations demonstrated a median rise of 338 ng Fibrinogen Equivalent Units (FEU)/ml (209-1209) in preoperative samples to 838 ng FEU/ml (209-1532) after sternum closure, which was not significant (*P*=0.059, Supple-

mentary Table 2). Five patients revealed an increase ranging from 173 to 343% (*P*=0.043), while other patients demonstrated lower increases or even decreasing FbDPs.

Degradation

Small differences, both increases and decreases, in fibrinogen degradation products (FgDP) concentrations were observed. The median FgDP concentration in preoperative samples was 157 ng FEU/ml (70-260) which remained quite stable after sternum closure (Supplementary Table 2).

Western blot analysis, as presented in Figure 2, did not reveal any differences in the Aa chain profile between preoperative samples and samples after sternum closure, indicating that no detectable proteolytic degradation of fibrinogen has taken place during the surgery. As the detection antibody used , Y-18, specifically detects fibrinogen, and not fibrin, the second band observed in most of the samples suggests the presence of very early degradation products of the Aα-chain of fibrinogen.

t-PA concentrations increased approximately 3 times after sternum closure (*P*=0.013), but there was no clear correlation between t-PA concentrations and FgDP or FbDP products. Two patients revealed almost a tenfold increase in t-PA levels after sternum closure, without (equally) elevated FgDPs and FbDPs relative to the other patients.

Fibrinogen supplementation study

The average CT after sternum closure increased to 65 s (35-94), whereas preoperative samples showed an average CT of 52 s (44-88). The average MCF after sternum closure was decreased to 55 mm (50-60), whereas the preoperative measurement showed an average MCF of 63 mm (54-67). Addition of purified fibrinogen to the samples taken after sternum closure resulted in a shortening of the CT and an increase of the clot firmness (A25, Figure 3). Taking into account the effect of the approximately 10% dilution introduced by adding fibrinogen or PBS solution to whole blood samples, both the CT and clot firmness returned to levels similar to that measured in undiluted preoperative whole blood samples.

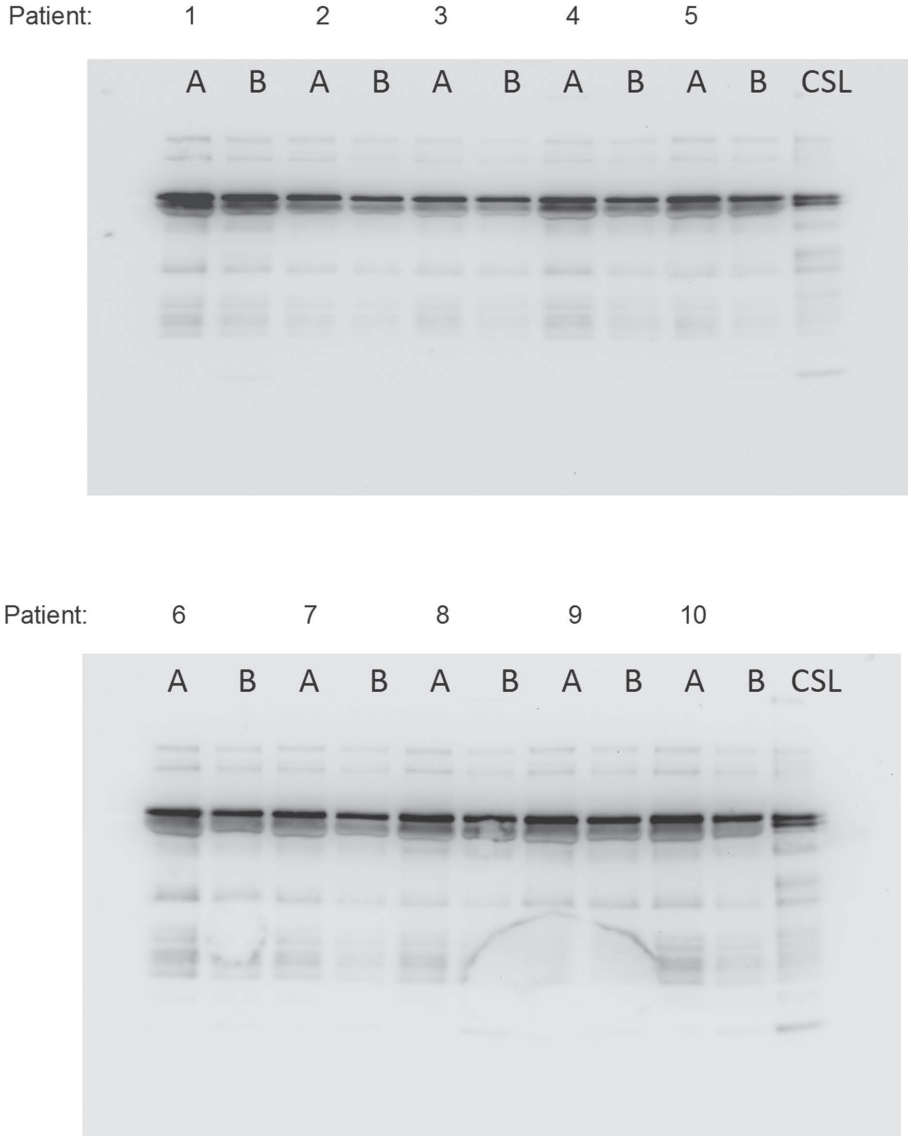


Figure 2. Reduced western blot analysis.
Patient 1 till 10, preoperative (A) and after sternum closure (B), with a positive control (CSL).

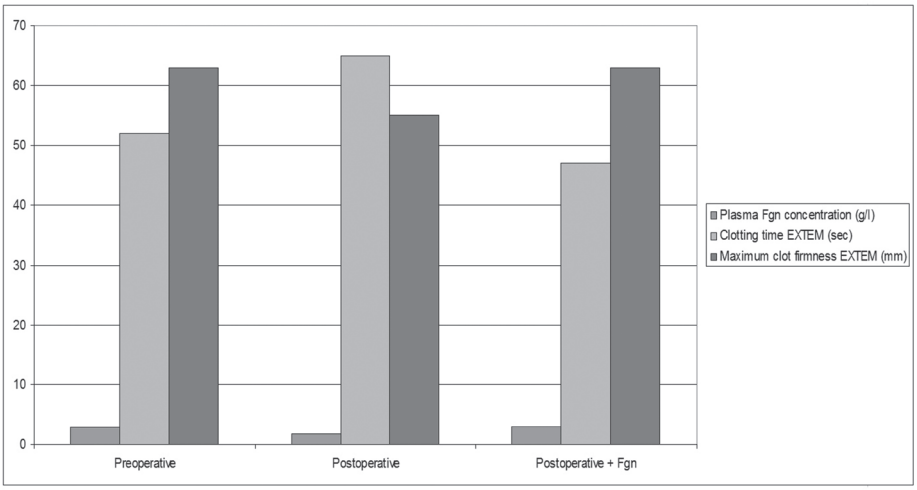


Figure 3. Fibrinogen (Fgn) supplementation study.

Effects of fibrinogen degradation products on coagulation

The purified FgDPs (Figure 4) showed the progressive degradation of fibrinogen with a MW of approximately 340 kDa into fragment X (MW approximately 260 kDa), fragment Y (MW approximately 160 kDa), and fragment D1 (Mw approximately 90 kDa). Estimated purity of the fragments used was >95%.

Figure 5A showed that the early FgDPs fragments X and Y prolonged the CT at concentrations of 0.2-4 µmol/l, reflecting fibrinogen to FgDP ratios in the test samples ranging from approximately 1:0.06 to approximately 1:1. Fragment D1 did not show a significant influence on the CT up to a concentration of 3.6 µmol/l reflecting a fibrinogen to fragment D1 ratio of approximately 1:1. Similar results were obtained when recording the CFT in the same samples. Figure 5B demonstrates the effect of the different FgDPs on the clot firmness, which is represented by the amplitude recorded at 25 min (A25).

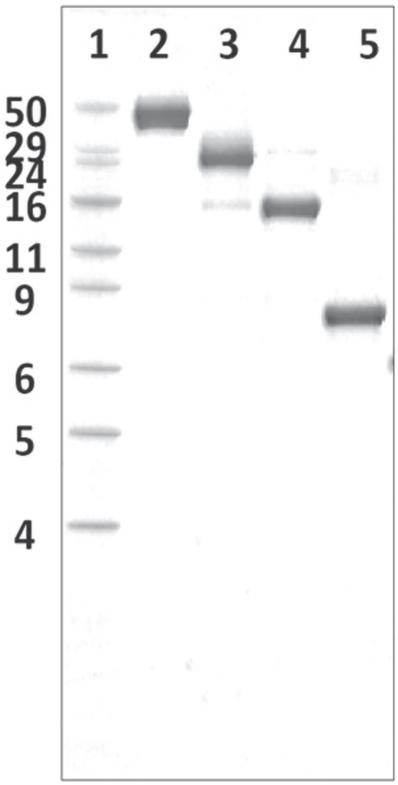
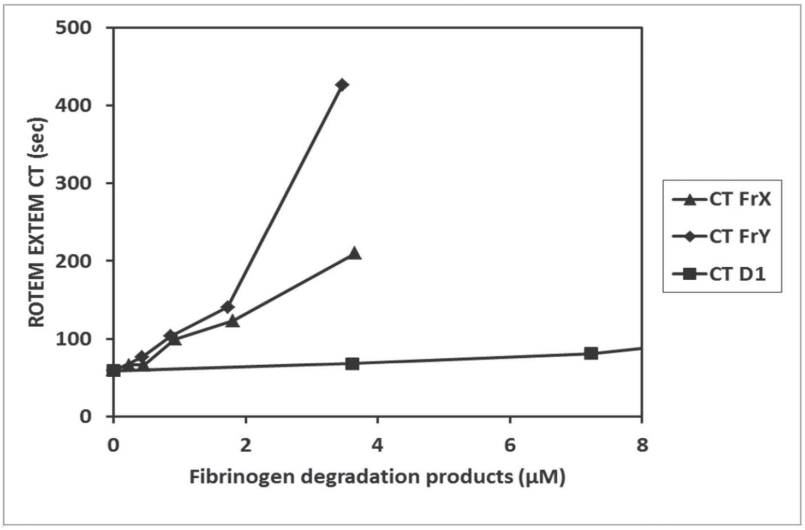


Figure 4. Non reduced SDS-PAGE analysis of fibrinogen degradation products (FgDPs).
Lane 1 HiMark (Life Technologies), Lane 2 HMW Fibrinogen (ERL FIB3) ~ 340 kD, Lane 3 Fibrinogen degradation fragment X ~ 260 kD, Lane 4 Fibrinogen degradation fragment Y ~ 160 kD, Lane 5 Fibrinogen degradation fragment D_1 ~ 90 kD.

A.



B.

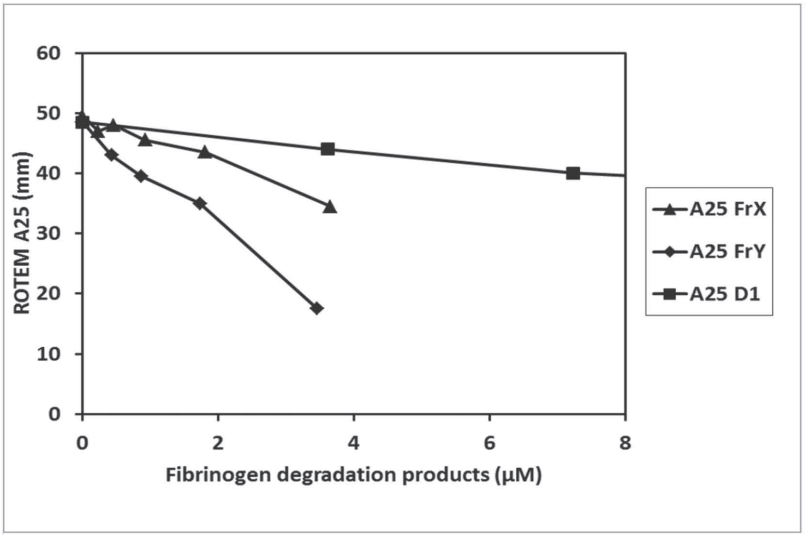


Figure 5. Effects of fibrinogen degradation products (FgDPs) in ROTEM™ EXTEM analysis.
A on clotting time (CT)
B on clot firmness (A25)

DISCUSSION

The 30% decrease in plasma fibrinogen concentration seen during CABG surgery appears to be mainly caused by hemodilution. No relevant consumption or degradation was observed under the condition that tranexamic acid was present. Furthermore, normalization of the clot firmness during ROTEM^{TM} analysis after *ex vivo* addition of purified fibrinogen confirmed that the reduction in fibrinogen concentration is causally related to the observed prolonged CT and reduced MCF, and not the dilution of other coagulation factors. Finally, the early FgDPs had a pronounced effect on blood clot formation *in vitro*.

There seems to be little evidence that fibrinogen consumption plays an important role in the fibrinogen decrease during cardiac surgery, as D-dimer (FbDP) concentrations did not increase. The rise of more than 100% in FbDP concentrations seen in 5 individuals after sternum closure is comparable to an increase of 1 µg/ml in plasma concentration, and therefore, could only account for a decrease of less than 0.1% in fibrinogen concentrations.

FgDPs did not reveal a significant increase after sternum closure, indicating that the contribution of fibrinogen degradation to the decline in fibrinogen concentrations after operation also is limited. Fibrinogen degradation might have occurred by proteases other than plasmin, since the FgDP ELISA uses a monoclonal antibody that is specific for plasmin FgDPs. However, the absence of any alteration in the degradation profile of the Aa chain in Western blot analysis excludes this option. Another explanation may be the administration of tranexamic acid during surgery in order to prevent fibrinolysis via plasminogen activation. Takada (14) demonstrated that tranexamic acid inhibited fibrinolysis as measured by the generation of FbDPs, but that it inhibited fibrinogenolysis only partly. The concentrations of tranexamic acid reported to prevent FgDP generation *in vitro* (15) are 10-fold higher than those applied in clinical settings (5.5 mg/ml relative to 0.2-0.5 mg/ml). Nevertheless, no FgDPs were observed in the plasma of patients, indicating that *in vivo* the relatively low tranexamic acid levels were sufficient to prevent fibrinogenolysis. The rise in t-PA concentration seen after sternum closure possibly resulted as a reaction to surgical manipulation, damaging the endothelial cells and inducing ischemia by

arterial or venous occlusion (16), hypothermia, and blood return from pericardial suction (17), without clinical evidence of fibrinolysis.

As we observed a median drop of 25% in hematocrit level and a corresponding decrease of 30% in albumin concentration between pre- and postoperative measurements, we believe that hemodilution, causing dilutional coagulopathy, is the most important mechanism reducing fibrinogen concentration during CABG procedures with the usage of CPB and tranexamic acid. The parallel decrease in hematocrit and fibrinogen concentration during cardiac surgery with usage of CPB is confirmed by several studies (18,19).

To determine if indeed the reduction in fibrinogen concentration is causally related to the observed coagulopathy as measured by ROTEM (with prolonged CT and reduced MCF), and not the dilution of other coagulation factors, we performed an *ex vivo* supplementation study were purified fibrinogen (Haemocomplettan, CSL) was added to the postoperative blood of the CABG patients. This study showed that both the CT and MCF values measured with ROTEM™ normalized to preoperative values after addition of only purified plasma fibrinogen. This demonstrates that dilution of other blood components like platelets and other coagulation factors have little effect on whole blood clotting, and therefore, that fibrinogen concentration is the rate limiting factor.

FgDPs have been reported to interfere with fibrin formation in plasma through inhibition of fibrin polymer formation. This is first described by Nieuwenhuizen in 1982 (6) who found that fragments Y exhibit twice as much anticlotting activity as fragments X. Using the very same fragments we demonstrated that *in vitro* early FgDP fragment Y and X not only have a significant impact on the CT of whole blood of healthy volunteers, but also on the clot firmness (MA), both measured here for the first time using ROTEM™ analysis and as such created significant coagulopathy in whole blood. Late FgDP fragments D1 showed very little influence on these parameters, suggesting that the effect of FgDPs could be transient and that it can vary with the stage of degradation. These findings suggest that *in vivo* only limited degradation of fibrinogen, resulting in the presence of fragments Y and X, may have a strong effect on coagulation. First by reduction of the fibrinogen concentrations

directly, and secondly, and more importantly, via the generation of the inhibitory fragments Y and X.

There are several limitations to this study. First, it is important to note that all patients in this study received tranexamic acid (according to standard protocol in most hospitals in the Netherlands), a lysine analogue that reduces plasminogen activation by preventing the binding of plasminogen to fibrin and thereby reducing plasmin-mediated degradation of fibrin. Without the use of tranexamic acid, probably, the role of degradation in fibrinogen concentration reduction is more pronounced and the level of FgDPs increased. Second, almost all patients used antiplatelet medication before CABG surgery, possibly influencing ROTEM^{TM} results for functional fibrinogen and fibrinogen supplementation testing. However, Lang (20) demonstrated that acetylsalicylic acid and clopidogrel did not have any influence on ROTEM measurements. While healthy volunteers were used to determine the effects of FgDPs on clot firmness in ROTEM^{TM} analysis, these results were certainly not affected by antiplatelet usage. Furthermore, fibrinogen is an acute phase protein, which is very fast regenerated. This mirrors the chance that the balance of fibrinogen metabolism between consumption and synthesis is towards synthesis. Finally, the study consists of only a few patients, but contains a large amount of measurements.

In conclusion, hemodilution appears to be the primary cause of the 30% plasma fibrinogen concentration drop seen in this investigational study in patients undergoing CABG surgery using tranexamic acid. Normalization of the coagulation profile after *ex vivo* addition of purified fibrinogen confirmed that fibrinogen is the first clotting factor to drop below a critical level and a major contributor to the onset of dilutional coagulopathy. Supplementation of fibrinogen after cardiac surgery could therefore contribute to the treatment of dilutional coagulopathy as suggested by Rahe-Meyer (21,22) and Levy (2). Furthermore, early fibrinogen degradation products (FgDPs) have a pronounced effect on blood clot formation *in vitro* and therefore may induce or enhance *in vivo* coagulopathy, especially without the use of tranexamic acid.

CONFLICTS OF INTERESTS

There are no conflicts of interest.

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Supplementary Table 1. Fibrinogen concentrations.

	Fibrinogeen totaal FE g/l			Fibrinogeen intact FE g/l			Fibrinogeen Clauss g/l		
Patientnr	T0	T1	ΔTotaal Fgn* (%)	T0	T1	ΔIntact Fgn† (%)	T0	T1	ΔFunctional Fgn‡ (%)
1	3.7	2.4	-34	3.5	2.3	-35	3.7	2.4	-30
2	2.2	1.9	-14	2.0	1.7	-14	3.2	1.9	-34
3	2.0	1.8	-10	2.1	1.8	-12	2.9	1.8	-41
4	2.6	1.7	-34	3.1	1.8	-44	3.1	1.7	-42
5	2.4	2.0	-14	2.7	1.9	-28	2.9	2.0	-24
6	3.1	2.0	-36	2.8	1.9	-33	3.2	2.0	-47
7	2.1	1.5	-26	2.4	1.7	-27	2.4	1.5	-33
8	2.7	1.9	-30	2.7	2.0	-29	3.3	1.9	-33
9	3.6	2.7	-25	3.0	2.2	-26	3.7	2.7	-27
10	3.1	2.0	-35	2.7	1.8	-32	3.7	2.0	-51
Median	2.7	1.9	-28	2.7	1.9	-29	3.2	1.9	-34
Min	2.0	1.5	-36	2.0	1.7	-44	2.4	1.5	-51
Max	3.7	2.7	-10	3.5	2.3	-12	3.7	2.7	-24

Fibrinogen levels measured preoperative (T0) and directly after sternum closure (T1) by the Clauss method and ELISAs in grams per liter.
* Difference between T0 and T1 total fibrinogen levels as percentage;
† Difference between T0 and T1 intact fibrinogen levels as percentage;
‡ Difference between T0 and T1 functional fibrinogen levels as percentage.

Supplementary Table 2. Fibrinogen and fibrin degradation products and t-PA.

	FbDP* ng FEU/ml			FgDP† ng FEU/ml			t-PA‡ ng/ml		
Patientnr	**T0**	**T1**	**ΔFbDP (%)**	**T0**	**T1**	**ΔFgDP (%)**	**T0**	**T1**	**Δt-PA (%)**
1	581	209	-64	254	231	-9	0.92	4.27	364
2	338	438	30	70	105	50	1.35	0.95	-30
3	209	312	49	96	113	18	0.28	0.52	86
4	510	1410	176	186	272	46	1.39	1.66	19
5	287	784	173	147	148	1	0.23	2.19	852
6	261	994	281	140	169	21	0.71	1.68	137
7	235	363	54	123	108	-12	0.38	3.7	874
8	338	1498	343	167	265	59	1.21	3.36	178
9	1209	891	-26	260	171	-34	0.86	4.3	400
10	510	1532	200	192	212	10	1.81	2.89	60
Median	338	838	114	157	170	14	0.9	2.5	157
Min	209	209	-64	70	105	-34	0.2	0.5	-30
Max	1209	1532	343	260	272	59	1.8	4.3	874

*Fibrin and †fibrinogen degradation products and ‡tissue plasminogen activator (t-PA) measured preoperative (T0) and directly after sternum closure (T1) and the difference between T0 and T1 as percentage.

CHAPTER 4

Hemostatic alterations during coronary artery bypass grafting

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ABSTRACT

Introduction

The origination of blood loss after cardiac surgery is not fully explained, but is related to operation trauma and use of cardiopulmonary bypass (CPB). However, the extent of their contribution is incompletely known and might differ between distinct operation procedures.

Materials and Methods

Three groups of CABG procedures were studied: 1) off-pump coronary artery bypass surgery (OPCAB, n=11) without CPB, 2) CABG with use of CPB (CABG, n=11) and 3) CABG with use of CPB combined with aortic valve replacement (AVR, n=11). Activation of coagulation and fibrinolysis was measured at various time points by flow cytometry, platelet aggregometry, thrombelastography, the Nijmegen Hemostasis Assay, prothrombin fragment 1+2 and tissue plasminogen activator.

Results and Conclusions

The use of CPB during cardiac surgery decreased platelet counts, clot strength, fibrinogen, hematocrit and albumin concentrations during the procedure. No perioperative platelet activation was observed and functional (collagen induced) platelet aggregation was transiently impaired, but recovered after surgery in all groups. Patients operated with use of CPB showed increased tissue plasminogen activator concentrations after reperfusion followed by minor and transient fibrinolysis. After all types of surgery coagulation parameters and platelet aggregation showed a rebound above preoperative levels. To conclude, no evident platelet activation, dysfunction or consumption was demonstrated. In patients using tranexamic acid the most prominent factor impairing hemostasis after CABG surgery was hemodilution associated with CPB.

INTRODUCTION

Bleeding necessitating blood transfusions or requiring exploration by reoperation is a common complication after cardiac surgery (1,2). A bleeding of surgical origin is found in half of the patients undergoing reoperation (3). In the remainder of patients there is diffuse bleeding. An unambiguous explanation for the origination of bleeding tendency has not yet been established. Ineffective hemostasis can be due to pre-existing coagulation factor deficiencies, drug-induced inhibition of hemostasis or surgery related acquired hemostatic defects (2,4). Suggested causes of acquired hemostatic defects in cardiac surgery include usage of heparin, hemodilution due to priming fluids of the cardiopulmonary bypass (CPB) circuit (5), activation of clotting by the non-endothelial CPB surface, hypothermia, acidosis, tissue trauma, platelet dysfunction and excessive fibrinolysis (4,5-8).

The extent of the contribution of each of those factors remains unresolved.

To gain more insight in the hemostatic disorders that develop during cardiac surgery, while using tranexamic acid, we have measured activation of coagulation and fibrinolysis by the release of prothrombin fragment 1+2 and tissue plasminogen activator at several time points during and up to 5 days after surgery in patients undergoing elective coronary artery bypass grafting (CABG). Furthermore, flow cytometry, platelet aggregometry, thrombelastography (TEG) (5,6) and the Nijmegen Hemostasis Assay (NHA), for simultaneous measurement of coagulation, fibrinolysis and the interplay of both (7), were performed.

METHODS

Patient population

This observational study included a total of 33 adult patients undergoing elective CABG surgery, equally divided into three groups; 1) off-pump coronary artery bypass surgery (OPCAB) without CPB, 2) CABG with use of CPB (CABG) and 3) CABG with use of CPB combined with aortic valve replacement (AVR). These three groups were chosen to compare the hemostatic effects of the surgery and tissue damage

itself (OPCAB group), with the effects of the use of CPB (CABG group) and the additional contribution of longer CPB times (AVR group). Patients needing emergency surgery, with heart failure (left ventricular ejection fraction <35%) or a history of bleeding diathesis or coagulopathy were excluded.

The study was performed in accordance with the Declaration of Helsinki and relevant Dutch laws, and was approved by the hospitals' ethics committee. All patients provided written informed consent.

Clinical and surgical management

All CPB procedures were executed normothermic (CPB machine (Jostra Maquet, Marquet, Hirrlingen, Germany), with intermittent antegrade warm-blood cardioplegia and prevention of acidosis. Heparin was injected as single bolus (300 U/kg in both groups) and monitored using the activated clotting time (ACT, Hemochron Signature Elite; ITC; Pleasanton, CA, USA). Additional heparin (5000 U) was given if ACT fell below 400 s. Tranexamic acid was used during surgery in 32 patients, using a bolus of 15 mg/kg prior to surgery and a continuous infusion of 5 mg/kg during operation. Pump flow rates were settled between 2-2.5 l index with a systemic mean arterial pressure target of 50-70 mmHg and systemic vasodilators or vasoconstrictors were used to maintain pressure between the ranges. After surgery heparin was neutralized with protamine sulphate (1000 IE heparin:10 mg protamine sulphate). All blood from the operative field was filtered, stored in a separate cell saver system (Electa; Sorin Group; Mirandola, Italy) and retransfused according to local standards at the end of the procedure. Blood transfusion practice was based on the transfusion guidelines of the American Society of Anesthesiologists and the Dutch Institute for Healthcare Improvement (CBO). It recommends RBC transfusion when the hemoglobin (Hb) level is <7 g/dl and advices against the use when the Hb is >9 g/dl. In case of the Hb is >7 g/dl and <9 g/dl, the transfusion trigger is determined by blood loss, cardiopulmonary reserve, age, comorbidity, and at the discretion of the anesthesiologist or intensivist. Patients were extubated when hemodynamically stable, rewarmed, awake, without surgical bleeding and with optimal blood gases. Antiplatelet medication and low-molecular-weight heparin were

both (re)started on the first postoperative day. If indicated, vitamin K antagonists were started five days after operation.

Blood loss was determined by drainage from the pleural and mediastinal tubes immediately after surgery until 48 h postoperative. Chest tubes were removed when drainage was less than 20 ml per hour or 200 ml per 24 h.

Blood sample collection

Blood was sampled in citrate (0.105 mol/l buffered trisodium citrate solution, BD Vacutainer, Plymouth, UK), EDTA (BD Vacutainer) and CTAD (containing theophylline, adenosine and dipyridamol, BD Vacutainer) tubes at various time points: preoperative (T0), before CPB or 30 min after start of operation (T1), 30 min into CPB or 30 min after heparinisation administration (T2), 5 min after reperfusion (removal of aortic cross-clamp) or 5 min after completion of the last anastomosis (T3), 1 h after protamine administration (T4), postoperative days 1 (T5) and 5 (T6). Baseline (T0) and T6 samples were obtained via venous puncture. At all other time points blood was drawn from the central venous line.

Standard blood tests hematocrit (Ht), complete blood count, albumin, fibrinogen (Clauss, Roche Diagnostics, Almere, The Netherlands), D-dimer (Roche Diagnostics), activated partial thromboplastin time (aPTT) and prothrombin time (PT) were performed at the hospital laboratory. Citrated whole blood was centrifuged at 2700 g for 10 min at 18 ºC and the CTAD tubes were centrifuged at 4200 g for 15 min at 4 ºC , aliquotted and stored at -80 ºC until batch analysis. All samples were analyzed blinded for clinical data or patient outcomes.

Hemostasis parameters

Platelet tests Immediately after collection, P-selectin expression, spontaneous and induced by adenosine diphosphate (ADP, 10-4 mol/l) and Collagen Related Peptide (10 µg/ml) ,were determined by whole blood flow cytometry (6) (Beckman Coulter FC500 MPL, Beckman Coulter, USA), at all time points.

Light transmission aggregometry (Chrono-Log 490 aggregometer, Chrono-Log Corporation Havertown, PA) with ADP (4 µmol/l), collagen (2.0 µg/ml) and arachi-

donic acid (1.6 mmol/l) as activators was performed at T0, 2, 5 and 6 in platelet rich plasma.

Thrombelastography Within 10 min after sampling whole blood thrombelastography (TEG^{R} 5000 Thrombelastograph® Hemostasis Analyzer System, Hemonetics Corporation, USA) analysis was performed at all time points using kaolin as activator. TEG parameters included the r value (intrinsic coagulation cascade activity), the α angle (speed of solid clot formation) and the maximal amplitude (clot strength, MA). The MA measurement was also separately performed for functional fibrinogen (FF MA), blocking GPIIb/IIIa receptors on platelets.

Coagulation and fibrinolysis prothrombin fragment 1+2 were measured at T0, 3, 5 and 6 by ELISA (Antibodies-Online, Aachen, Germany).

The Nijmegen Hemostasis Assay (NHA) was performed for simultaneous measurement of coagulation and fibrinolysis at T0, 3, 5 and 6 (7). The NHA measures thrombin generation lag time (time between initiation and the start of thrombin generation, TGlt), time to peak thrombin generation (time at which the thrombin generation reached its maximal rate, TTP), thrombin peak height (maximal velocity of thrombin production, TPH) and area under the curve (thrombin generation capacity, AUC) for coagulation and the fibrin lysis time (FLT) and plasmin peak height (height of plasmin generation, PPH) for fibrinolysis.

Tissue plasminogen activator (t-PA) levels in CTAD plasma were determined at T0, 3, 5 and 6, using the Human tPA activity assay (Kordia, The Netherlands) which is not sensitive to tranexamic acid administration.

Statistical analysis

For descriptive purposes we used medians and ranges. Statistical significance was defined as $P<0.05$. For comparison of several outcome variables between the 3 treatment groups at each time point and between time points, we used a linear mixed model with time and group and their interaction as categorical covariates and with compound symmetry as covariance model. All analyses were corrected for the following covariates: blood transfusions (i.e. red blood cells, fresh frozen plasma (FFP), platelet rich plasma and cell saver blood), preoperative acetylsalicylic acid and clopidogrel usage, dilution with colloids and crystalloids, and current value of hematocrit. The statistical analyses were performed using SPSS Statistics 20.0 software (IBM Corporation, Armonk, NY).

RESULTS

Patient characteristics

Patient groups significantly differed on 2 baseline characteristics: logistic EuroSCORE and extracardiac arteriopathy, defined as any one or more of the following: claudication, carotid occlusion or >50% stenosis, previous or planned intervention on the abdominal aorta, limb arteries or carotids (Table 1). All other baseline variables, including laboratory, were comparable among groups.

As expected patients in the AVR group had the longest operation, CPB and aortic clamping time, the lowest minimum temperature and received the highest amount of tranexamic acid and heparin during operation (Table 2). Patients operated on CPB (CABG and AVR) received more colloids during operation, while the amount of crystalloids was similar in the 3 groups.

Table 1. Baseline patient characteristics.

	OPCAB *n=11*	CABG *n=11*	AVR *n=11*	*P*-value
Male	8(73)	8(73)	7(64)	0.866
Age (years)	74(55-88)	66(46-84)	78(53-86)	0.254
Diabetes mellitus	4(36)	4(36)	4(36)	1.000
Systemic hypertension	6(55)	6(55)	7(64)	0.883
Unstable angina	0	2(18)	0	0.207
Previous cardiac surgery	0	2(18)	1(9)	0.333
Previous PTCA	1(9)	4(36)	1(9)	0.160
MI within 3 months before CABG	1(9)	1(9)	0	0.478
Left ventricular ejection fraction				0.543
30-50%	3(27)	1(9)	2(18)	
Extracardiac arteriopathy	4(36)	1(9)	0	0.047
Renal insufficiency	1(9)	0	0	0.357
COPD	1(9)	2(18)	2(18)	0.790
Logistic EuroSCORE	4.02(1.22-11.51)	2.05(0.88-13.07)	4.92(1.51-17.05)	0.031
Acetylsalicylic acid continued	5(45)	6(55)	7(64)	0.537
Clopidogrel continued	4(36)	1(9)	1(9)	0.236
Laboratory values (T0)				
Hemoglobin (mmol/l)	7.8(5.9-9.0)	8.5(6.5-9.1)	7.7(6.7-8.7)	0.155
Hematocrit (l/l)	0.38(0.27-0.45)	0.42(0.34-0.44)	0.37(0.34-0.43)	0.326
Albumin (g/l)	38(33-40)	40(30-48)	39(36-49)	0.301
Fibrinogen Clauss (g/l)	3.7(2.7-4.6)	3.2(2.1-3.7)	3.1(2.4-5.6)	0.087
Functional Fibrinogen MA (mm)	66.5(55.7-73.4)	61.2(51.7-68.2)	65.0(51.3-77.8)	0.057
D-dimer (ng/ml)	668(225-3809)	294(220-1428)	341(223-2237)	0.083
Prothrombin fragments1.2 (pg/ml)	0.21(0.08-0.51)	0.08(0.03-0.37)	0.11(0.04-0.36)	0.051
aPTT (s)	31.2(23.6-39.0)	29.5(24.9-35.3)	30.2(25.1-37.2)	0.559
PT (s)	15.2(13.7-22.7)	15.2(13.3-16.1)	14.8(13.2-22.9)	0.408
Platelet count (x109/l)	202(159-318)	192(138-316)	202(128-371)	0.782
t-PA (UI/ml)	0.01(0-0.05)	0.01(0-0.11)	0.01(0-0.09)	0.998

Median (range) or number (percentage) of patients. Chi-square was used and Kruskal-Wallis testing. OPCAB, CABG surgery without the use of CPB; CABG, CABG surgery with use of CPB; AVR, CABG surgery with use of CPB combined with aortic valve replacement (AVR); PTCA, Percutaneous Transluminal Coronary Angioplasty; MI, myocardial infarction; COPD, chronic obstructive pulmonary disease. TEG functional fibrinogen test, maximal amplitude (MA).

Table 2. Intra- and postoperative variables of study groups.

	OPCAB *n=11*	CABG *n=11*	AVR *n=11*	*P*-value
Intraoperative				
Operation time (min)	177(138-279)	271(195-486)	318(205-467)	0.000*
CPB time (min)	-	105(83-297)	190(104-333)	0.000*
Aortic clamping time (min)	-	78(0-100)	141(87-258)	0.000*
Colloid (ml)	500(0-1500)	1000(0-2150)	1000(500-1500)	0.033*
Crystalloid (ml)	1500(500-1500)	1050(300-2300)	1250(480-1830)	0.636
Red blood cells (ml)	-	0(0-500)	0(0-750)	0.104
Fresh Frozen Plasma (ml)	-	-	0(0-300)	0.127
Platelets (ml)	-	0(0-300)	0(0-300)	0.372
Cell saver (ml)	0(0-1054)	0(0-947)	406(0-1087)	0.108
Patients receiving red blood cells	-	3(27)	4(36)	0.095
Patients receiving fresh frozen plasma	-	-	2(18)	0.119
Patients receiving platelets	-	1(9)	2(18)	0.361
Patients receiving cell saver	5(45)	4(36)	9(81)	0.077
Tranexamic acid (mg)	1560(0-4247)	3873(2340-4433)	4000(2000-5373)	0.000*
Heparin (UI/ml)	18000(10900-25800)	37050(22100-59800)	37400(29200-63000)	0.000*
Minimum pH	7.41(7.29-7.45)	7.36(7.24-7.43)	7.33(7.16-7.46)	0.014*
Maximum ACT (sec)	377(295-423)	473(430-789)	473(435-810)	0.000*
Minimum temperature (°C)	34.0(25.5-35.5)	34.3(29.4-35.5)	32.8(29.5-34.3)	0.045*
Intra-aortic balloon pump use	-	-	-	-
Postoperative				
In hospital mortality	-	-	-	-
Blood loss (ml) 24h	570(170-2828)	680(350-2780)	610(300-950)	0.402
48h	570(170-3325)	680(420-3300)	610(300-1080)	0.250
Colloid (ml)	0(0-1500)	0(0-1250)	250(0-1000)	0.948
Crystalloid (ml)	-	-	-	-
Red blood cells (ml)	0(0-1250)	0(0-1500)	-	0.780
Fresh Frozen Plasma (ml)	0(0-1000)	0(0-1250)	0(0-500)	0.998
Platelets (ml)	0(0-300)	0(0-1300)	0(0-250)	0.324
Cell saver (ml)	0(0-300)	-	-	0.368
Patients receiving red blood cells	1(9)	2(18)	0	0.333
Patients receiving fresh frozen plasma	2(18)	2(18)	2(18)	1.000
Patients receiving platelets	1(9)	3(27)	1(9)	0.390

Table 2. Intra- and postoperative variables of study groups. (continued)

	OPCAB *n=11*	CABG *n=11*	AVR *n=11*	*p value*
Patients receiving cell saver	1(9)	-	-	0.357
Reoperation for bleeding	0(0-1)	-	-	0.368
Myocardial infarction	-	-	-	-
Intensive care unit length of stay (h)	24(20-88)	23(17-306)	23(18-113)	0.689
Total length of stay (days)	4(3-8)	7(3-25)	7(4-10)	0.016*

Data presented as median (range) or number (percentage) of patients. Chi-square (categorical) and Kruskal-Wallis (continuous) test. OPCAB, CABG surgery without the use of CPB; CABG, CABG surgery with use of CPB; AVR, CABG surgery with use of CPB combined with aortic valve replacement (AVR).

The total median amount of blood loss was not different between groups at both 24 and 48 h after surgery. Two patients (1 OPCAB and 1 CABG) endured excessive blood loss of more than 2000 ml in the first 24 h after surgery. One of them (OPCAB) was re-operated for surgical bleeding. Furthermore, the number of patients receiving blood transfusions and the amount of blood products received was equal among groups. Hospital admission was 3 days longer in patients in the CABG and AVR groups. One patient in the CABG group was hospitalized for 25 days due to an inflammatory reaction without a clear focus.

Hemodilution

A pronounced drop in Ht was observed in the CABG and AVR groups during the first 30 min of CPB (T2) and reperfusion (T3) (T0-T3 CABG 28% decrease; AVR 27% decrease, $P<0.001$). The Ht decrease in OPCAB group was minimal (T0-T3 11% decrease, $P<0.001$) and remained above 0.30 l/l (Figure 1). Five days postoperative the Ht level was still below preoperative Ht level in all groups ($P<0.001$).

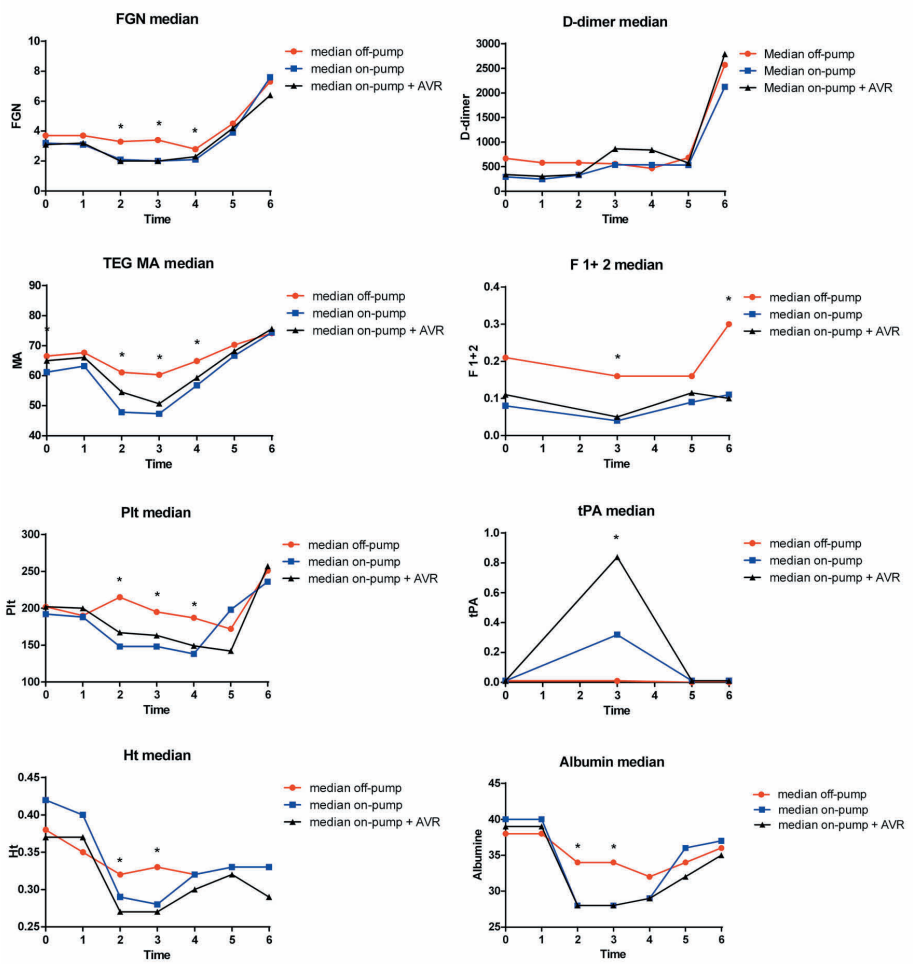

Figure 1. Differences in coagulation and fibrinolysis laboratory values between groups. OPCAB, CABG surgery without the use of CPB; CABG, CABG surgery with use of CPB; AVR, CABG surgery with use of CPB combined with aortic valve replacement; FGN, fibrinogen; TEG MA, TEG functional fibrinogen test; maximal amplitude (MA); F 1+2, prothrombin fragment 1+2; Plt, platelets; tPA, tissue plasminogen activator; Ht, hematocrit. Preoperative (T0), before CPB (T1), 30 min into CPB or 30 min after the start of the first anastomoses in CABG surgery without CPB usage (T2), 5 min after reperfusion or 5 min after the completion of the last anastomosis in CABG surgery without CPB usage (T3), 1 h after reperfusion (T4), 1 day after operation (T5) and 5 days after operation (T6). *P<0.05 between treatment groups.

Albumin concentrations showed the same trend as Ht with a pronounced drop between baseline and reperfusion measurements in patients operated on CPB and a less outspoken in the OPCAB group (Figure 1). At 5 days postoperative albumin was nearly back to preoperative values.

Platelets

Platelet counts dropped during operation until 1 h after reperfusion in patients on CPB, although the decrease was only significant in the AVR group (29% decrease, *P*=0.011; CABG 21% decrease, *P*=0.213). The platelet counts in the OPCAB group remained quite stable during operation (Figure 1). After operation platelets increased above preoperative levels between the first (T5) and fifth (T6) day postoperative *P*<0.001 in all groups.

P-selectin expression and arachidonic acid, ADP and collagen induced aggregation were similar between groups at T0, although aggregation was decreased related to the use of antiplatelet drugs. Collagen induced platelet aggregation was impaired during operation and a rebound of platelet aggregation above preoperative levels was seen postoperative in all patients. Few activated platelets appeared during and 1 h after reperfusion (T3 1.63%; T4 1.29% all groups), while upon agonist P-selectin increased to approximately 70% at both time points.

Coagulation

Plasma prothrombin fragment 1+2 and D-dimer levels remained quite stable throughout operation until the day after surgery, with the OPCAB group demonstrating a higher level of prothrombin fragment 1+2, which was significant at 5 min after the completion of the last anastomosis (T3). Five days after operation fragment 1.2 was further increased in the OPCABG group. D-dimer concentrations increased to approximately four fold at day 5 after operation relative to preoperative concentrations in all groups (T0 vsT6, *P*<0.001).

Fibrinogen concentrations within the OPCAB group remained stable, but the CABG and AVR groups showed a profound decrease during operation (T3: CABG 29% decrease, *P*=0.004; AVR 32% decrease, *P*= 0.003, Figure 1). Postoperatively, fibrinogen doubled compared to preoperative levels in all groups (*P*<0.001).

The TEG and functional fibrinogen MAs demonstrated a similar curve as compared to fibrinogen (and platelets) during CPB and shortly after reperfusion and immediately recovered 1 h after protamine neutralization (T4), whereas fibrinogen and platelet levels remained below baseline until 1 day after operation (T5). The r and α TEG values did not demonstrate significant differences between groups during and after surgery.

None of the changes in the thrombin generation lag time (TGlt),time to thrombin peak (TTP), thrombin generation peak height (TPH) and area under the curve (AUC) in the NHA were above standard error. Patients in the AVR group revealed an overall impaired thrombin generation at 5 days postoperative, whereas the thrombin generation appeared to be increased in the OPCAB and CABG groups, resembling a hypercoagulable state (Figure 2).

APTT levels were increased on CPB relative to off-pump surgery due to higher heparin dosage during CPB (T3, $P<0.001$ (OPCAB vs CABG/AVR). PT was stable at any time point.

Fibrinolysis

t-PA concentrations transiently rose after reperfusion relative to preoperative levels (CABG $P= 0.001$; AVR $P<0.001$) and remained low in the OPCAB group (Figure 1).

The fibrin lysis time (FLT) in the NHA decreased between baseline (T0) and 1 h after reperfusion (T4) in both the CABG and AVR group (only significant for AVR $P=0.016$, Figure 2). Noticeable is the postoperative FLT elevation (impaired fibrinolysis) in all groups.

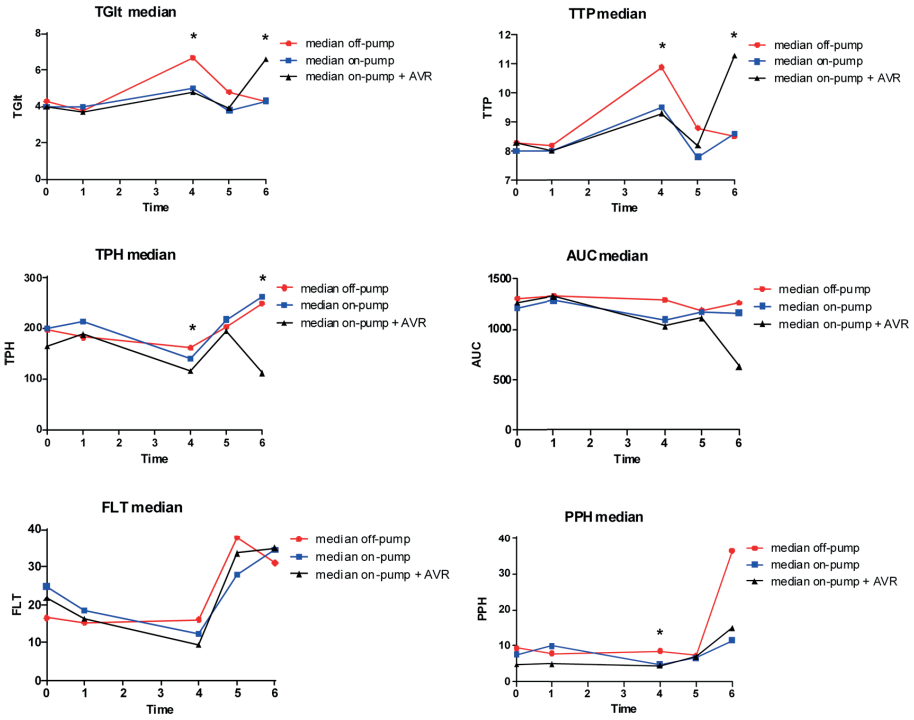

Figure 2. Differences in NHA between groups.
OPCAB, CABG surgery without the use of CPB; CABG, CABG surgery with use of CPB; AVR, CABG surgery with use of CPB combined with aortic valve replacement. Nijmegen Hemostasis Assay (NHA) measures thrombin generation lag time (time between initiation and the start of thrombin generation; TGlt); time to peak thrombin generation (time at which the thrombin generation reached its maximal rate; TTP); thrombin peak height (maximal velocity of thrombin production; TPH) and area under the curve (thrombin generation capacity; AUC) for coagulation and the fibrin lysis time (FLT) and plasmin peak height (height of plasmin generation; PPH) for fibrinolysis. Preoperative (T0); before CPB (T1); 30 min into CPB or 30 min after the start of the first anastomoses in CABG surgery without CPB usage (T2); 5 min after reperfusion or 5 min after the completion of the last anastomosis in CABG surgery without CPB usage (T3); 1 h after reperfusion (T4); 1 day after operation (T5) and 5 days after operation (T6). * P<0.05 between treatment groups.

DISCUSSION

Although previous studies were performed to gain more insight in the development of coagulopathies during cardiac surgery they mainly focused on a restricted number of measurements evaluating coagulation or fibrinolysis in a short period of observation. In our study a quite large number of laboratory values was measured including the novel Nijmegen Hemostasis Assay (NHA) for the evaluation of both coagulation and fibrinolysis simultaneously and the interplay of both. Our study revealed that the most prominent changes after initiation of CPB were: decreased levels of hematocrit, albumin, platelets and fibrinogen, combined with impaired clot strength. No perioperative platelet activation was observed and functional (collagen induced) platelet aggregation was transiently impaired, but recovered after surgery in all groups. Furthermore, plasma prothrombin fragment 1+2 and D-dimer levels remained quite stable throughout operation and the changes demonstrated in thrombin generation were not above standard errors. Finally, an increased t-PA release was demonstrated in patients on CBP.

The absence of perioperative platelet activation combined with the normally expressed P-selectin after activation upon agonists, makes *in vitro* platelet dysfunction by activation in the CPB circuit unlikely. The decreased platelet aggregation observed during operation might be explained by colloid usage and a decreased platelet count, although a decrease of 29% should not affect aggregation testing. The stable prothrombin fragment 1+2 plasma levels and lack of significant differences at any time point in the D-dimer/fibrinogen ratio virtually exclude disseminated intravascular coagulation. Furthermore, stabilization of aPTT after reperfusion indicated that coagulation factor consumption is negligible.

Previous papers have reported a massive thrombin generation during CPB (9-11), however we could not confirm this, despite the use of comparable amounts of heparin.

The t-PA burst after reperfusion seen in patients on CPB probably partly results from surgical tissue damage, as t-PA is rapidly released by endothelial cells in response to venous occlusion, manipulation and arterial ischemia (12). Furthermore, a major t-PA burst during reperfusion is created by accumulation in the heart during

aortic cross clamping (4), return of pericardial blood during cardiotomy suction (13) and the use of cell saver (14), without necessarily resulting in a clinically relevant fibrinolysis. Probably, without the use of tranexamic acid (standard procedure in most hospitals in the Netherlands), the role of fibrinolysis in bleeding might be more pronounced (15).

Coagulation tests, including platelet aggregation, showed a postoperative rebound above baseline levels in all patients. This rebound is probably attributed to an acute phase response to surgical trauma and CPB usage (9,16). The high fibrinogen, D-dimer and prothrombin fragments 1+2 concentrations, combined with an increased thrombin generation, suggest a hypercoagulable state, primarily seen in patients undergoing isolated CABG (OPCAB and CABG). This hypercoagulable state in combination with the postoperative impaired fibrinolysis in all groups, may promote the risk of thromboembolic complications (9,17,18). The NHA measurements of patients in the AVR group are largely influenced by medication usage (vitamin K antagonists), which are prescribed accoring to protocol, causing slower and less thrombin generation.

Preoperative differences in laboratory values (e.g. elevated D-dimer level) may be a consequence of the operation indication for OPCAB surgery, more frequently performed in older or sicker patients with higher inflammatory markers that may be associated with activation of coagulation (19).

Our study was neither intended, nor powered to detect causes of blood loss, but rather to obtain insight in the sequence of events that may lead to the development of coagulopathy and blood loss after cardiac surgery. Although only two patients endured excessive blood loss of more than 2000 ml in the first 24 h after surgery, and so, no conclusive adjudication can be made concerning excessive blood loss, the bleeding tendency in 'normal bleeding patients' appears to be mainly influenced by hemodilution. The parallel decrease in Ht and fibrinogen during cardiac surgery with CPB is congruent with several previous studies (1,4,8). Scrascia , who compared coagulation, fibrinolysis and intraoperative red blood cell transfusions between 36 off-pump CABG patients and 36 CABG patients in whom a closed, phosphorylcoline-coated CPB system with a closed-collapsible venous reservoir (Sorin Group, Mirandola, Italy) was used, concluded that priming volume

reduction is required in order to decrease intraoperative red blood cell transfusion (20). However, as hemodilution is not the only difference in this patient model we cannot exclude interference of other processes in the development of coagulopathy. The differences measured by the laboratory assays are affected by the concentration of the tested analyte (e.g. platelets or fibrinogen), and therefore, do not confirm a qualitative defect. This effect might aggravate the decreases seen in these analytes in patients on CPB relative to those operated without CPB and fortify the outcome towards hemodilution.

The use of tranexamic acid might have affected the results, especially, the minimal differences demonstrated in NHA. However, as all patients used tranexamic acid, the differences between groups are not assumed to be affected by this factor. Another limitation of this study is that due to the use of heparin, which is known to be of great influence on the different laboratory measurements, we were not able to perform all laboratory tests at every time point. Especially T2 and T3 are strongly influenced by heparin. The decreased MA as measured by TEG is probably pronounced by heparin administration. Furthermore, the drop in thrombin generation peak height (TPH) and area under the curve (AUC) seen between preoperative and 1 h after reperfusion (T4) in the AVR group is probably caused by the higher amount of heparin administered in those patients.

Conclusion

No evident platelet activation, dysfunction or consumption was demonstrated. In patients undergoing CABG surgery with use of tranexamic acid, only minor and transient fibrinolysis was observed after reperfusion in patients operated on CBP. The most prominent factor impairing hemostasis was hemodilution associated with CPB. Restricting hemodilution, by reducing the CPB circuit and priming volume, may reduce postoperative blood loss.

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DISCLOSURE OF CONFLICT OF INTERESTS

No conflict of interests to declare.

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Part II

Management and Prevention

CHAPTER 5

Stopping antiplatelet medication before coronary artery bypass graft surgery: Is there an optimal timing to minimize bleeding?

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ABSTRACT

Objectives

As the indication for antiplatelet medication expands, patients may be exposed to an increased risk of excessive blood loss when cardiac surgery is required. The optimal timing to stop acetylsalicylic acid (ASA) or ASA combined with clopidogrel (ASA+Clo) before surgery is subject of controversy.

Methods

A total of 1,065 patients were selected from a prospective randomized study on the effect of a fibrin sealant application in coronary artery bypass graft surgery (Fibrin sealant Induced Blood Exposure Reduction study; Registration number: NTR1386 (http://www.trialregister.nl)), and divided into three groups according to the use of antiplatelet medication within 10 days prior to surgery: (1) ASA only (n=662), (2) ASA+Clo (n=290) or (3) no antiplatelet medication (n=113). To investigate if an optimal stop day could be established we fitted a series of multiple linear regression models, one for each preoperative day (running from day -10 up to -1). The specific day corresponding to the best fitting model (highest adjusted R^2, with blood loss in the first 48 h postoperatively as the dependent variable) was considered as the best estimate for the optimal stop day. Bootstrap analysis (1000 times) was performed to calculate the corresponding confidence interval. Furthermore, major adverse cardiovascular and cerebral events (MACCE) were evaluated.

Results

We could not estimate an optimal stop day for patients using ASA or ASA+Clo prior to their operation. Last use of ASA on day -2 or earlier significantly decreased the percentage of patients receiving platelet transfusions compared with continuation until surgery (7% vs 13 % for day -1, *P*=0. 007). In patients using ASA+Clo, this percentage was reduced from 41% to 10% (*P*<0.001). There was no association between stop day and the occurrence of MACCE.

Conclusions

There is no clinically relevant effect on blood loss indicating an optimal stop day for ASA alone or in combination with clopidogrel. Last use on day -2 resulted in a reduction of percentage of patients receiving platelet transfusions, especially in the ASA+Clo group.

INTRODUCTION

Bleeding necessitating blood transfusions or even requiring re-exploration is a common and serious complication after cardiac surgery (1,2). Especially patients undergoing coronary artery bypass graft (CABG) surgery are at increased risk for postoperative blood loss as these patients often use antiplatelet medication prior to their operation (3). Acetylsalicylic acid (ASA) and clopidogrel (Clo) are platelet aggregation inhibitors prescribed to improve outcomes after acute coronary syndromes and to reduce early stent failure after percutaneous intervention (PCI) (4,5). ASA is an irreversible platelet inhibitor via acetylation of the cyclo-oxygenase enzyme and thereby blocking the synthesis of prostaglandin thromboxane A2 in the platelet. Clo inhibits platelet activation and thereby aggregation via irreversible blockade of the adenosine diphosphate chemoreceptor P2Y on the platelet membrane. As platelets lack the ability for protein synthesis, the only way aggregation can be restored is by *de novo* synthesis of platelets. The platelet lifespan is approximately 8-10 days, thus after 4-5 days half of the platelet pool is replenished, which is considered sufficient to normalize bleeding time (6).

To reduce the amount of blood loss after CABG surgery, it is important to consider if and when the use of preoperative ASA and Clo should be terminated (6-8). However, solid data on the optimal timing is lacking.

The present study investigates the effect of different stop days on blood loss within 48 h after isolated CABG surgery in patients who preoperatively used ASA only or ASA combined with Clo (ASA+Clo). Furthermore, we included patients using no preoperative antiplatelet medication to evaluate differences in 48 h blood loss, platelet transfusions received and incidence of major adverse cardiovascular and cerebral events (MACCE) between groups.

METHODS

Patient population

The 1,065 patients involved in this study were retrieved from the Fibrin sealant Induced Blood Exposure Reduction (FIBER) study, a multicentre randomized controlled study to investigate the cost-effectiveness of the use of a new fibrin sealant in preventing blood loss in patients undergoing isolated CABG. The study involved seven cardiac surgery centres in The Netherlands. Exclusion criteria were: exclusive use of venous grafts, any concomitant procedure (including atrial fibrillation ablation), emergency surgery, participation in any study involving an investigational drug or device, Jehovah's witness and a history of bleeding diathesis or coagulopathy. The trial was performed in accordance with the Declaration of Helsinki and relevant Dutch laws, and was approved by the hospitals' ethics committees. The trial was registered with http://www.trialregister.nl (NTR1386). All patients provided written informed consent before entering in the study. The FIBER study did not define a predetermined mandatory stop day for preoperative antiplatelet medication use.

For the current study, patients were divided into three groups according to the use of antiplatelet medication within 10 days prior to surgery: (1) ASA only (ASA, n=662), (2) ASA+Clo, n=290), or (3) no antiplatelet medication because it was stopped for more than 10 days or was never used (None, n=113). Excluded were patients using preoperative vitamin K antagonists or dipyridamol, and patients for whom the stop date for preoperative antiplatelet medication was not available. Twenty-seven patients who used Clo as monotherapy within 10 days before surgery were excluded from analysis because of consequent small patient numbers per stop day. For analytical purposes, the ASA+Clo group consisted only of patients with the same stop date for ASA+Clo (Figure 1).

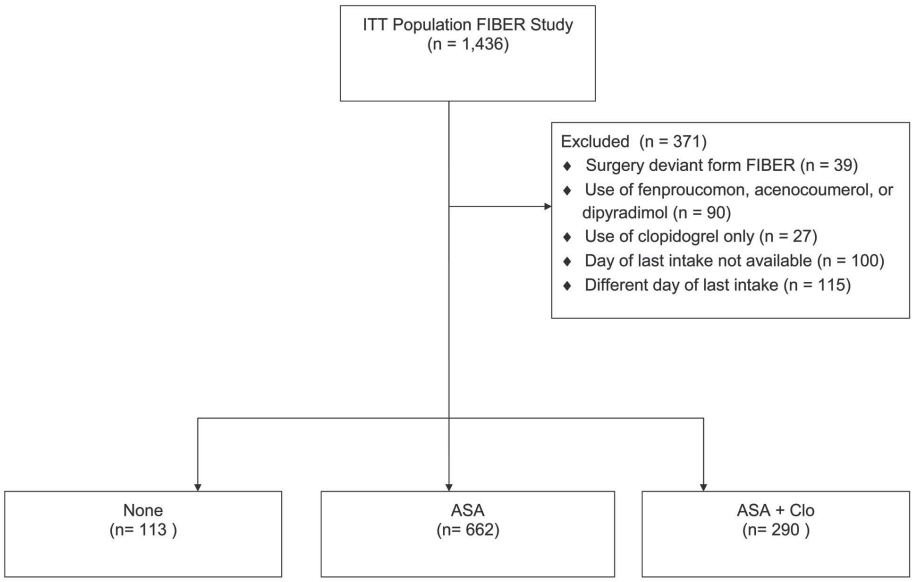

Figure 1. Study flow chart.
FIBER, Fibrin sealant Induced Blood Exposure Reduction; ITT, intention to treat analysis; CABG, coronary artery bypass grafting; None, no antiplatelet medication within 10 days before surgery or never used; ASA, use of acetylsalicylic acid within 10 days prior to surgery; ASA+Clo, use of ASA and Clo within 10 days prior to surgery.

Clinical and surgical management

Surgical techniques were used according to the local standards. Tranexamic acid was administered during surgery in 916 patients, according to local standards. In general, a bolus of approximately 15 mg/kg prior to surgery was followed by continuous infusion of approximately 5 mg/kg/hour during operation with some variation among study sites. After surgery, heparin was neutralized with protamine sulphate (1000 IE heparin:10 mg protamine sulphate). The fibrin sealant CryoSeal, produced from an allogeneic single non-remunerated volunteer donor without the addition of antifibrinolytics, was randomly applied in 50% of the patients during surgery, according the FIBER study protocol, i.e. 5 ml mandatory per internal thoracic artery bed, and on anastomosis, cannulation sites, and other parts in the surgical field at the discretion of the surgeon. Furthermore, blood from the operative field was filtered, stored in a separate cell saver system, and retrans-

fused according to local standards. The number of units transfused red blood cells (RBCs), fresh frozen plasma and platelet or fibrinogen concentrates were the primary endpoints of the FIBER study. Blood transfusion practice was based on the transfusion guidelines of the American Society of Anesthesiologists (9) and the Dutch Institute for Healthcare Improvement (10). It recommends RBC transfusion when the hemoglobin (Hb) level is <7 g/dl and advices against the use when the Hb is >9 g/dl. In case of the Hb is >7 g/dl and <9 g/dl, the transfusion trigger is determined by blood loss, cardiopulmonary reserve, age, comorbidity and at the discretion of the anesthesiologist or intensivist. Chest tubes were removed when drainage was less than 20 ml per hour or 200 ml per 24 h. Antiplatelet medication and low-molecular-weight heparin were both (re)started on the first postoperative day. If indicated, vitamin K antagonists were started five days after operation.

End-points

The primary end-point of this study was the amount of blood loss within 48 h after operation. Blood loss was measured from the chest tube output starting immediately at arrival at the intensive care unit and continued until the chest drains were removed.

The secondary endpoint was MACCE during hospital admission, consisting of the parameters: in-hospital mortality, myocardial infarction (defined as presence of at least two of the following criteria: (1) ischemic chest pain lasting for more than 20 min, (2) changes in serial electrocardiogram (ECG) tracings, and (3) Troponine T >1 µg/l), reintervention for ischemia (defined in case the need for re-intervention was based on changes in serial ECG tracings and Troponine T >1 µg/l), and stroke (defined as a new persistent cerebrovascular event leading to neurological defaults being diagnosed by a neurologist).

Furthermore, the percentage of patients undergoing platelet transfusions given during and directly after surgery was evaluated.

Statistical analysis

Data are expressed as mean (standard deviation) or number (percentages), where appropriate. Because the variable 48 h blood loss failed to satisfy the assumption

of normality, data for this variable are presented as median (interquartile range (IQR)). The logarithm of 48 h blood loss was calculated and used in all analyses. Patient characteristics and study outcomes were compared between the three treatment groups using analysis of variance for continuous variables and Pearson chi-square test or Fisher's exact test, where appropriate, for categorical variables. In case $P<0.05$, post hoc analysis was used for comparisons between two groups.

Stop day was defined as the last day before surgery on which antiplatelet medication was used (operation day is day 0). Last use on day -1 was considered as continuation. We defined the optimal stop day as the day shortest before operation such that stopping on an earlier day did not decrease blood loss any more. To investigate if an optimal stop day could be established from the data, we fitted a series of multiple linear regression models, one for each day 'i' (running from day -10 up to day -1) before the operation. In these models, the logarithm of 48-h blood loss was the dependent variable and the effect of the variable stop day was modelled. For the stop day before day 'i', the level of blood loss was assumed to be constant and for the stop day after day 'i', a linear increase in blood loss was assumed (this model is referred to as a 'broken stick'). All these 10 regression analyses were adjusted for the hereunder mentioned covariates. The day 'i' corresponding to the best fitting model (highest adjusted R^2) was considered as the best estimate for the optimal stop day. Bootstrap analysis (1000 times) was performed to calculate the corresponding confidence interval (CI).

Binary logistic regression analysis using the above covariates was used to calculate adjusted odds ratios (ORs) and 95% CIs for the association of stop day with the occurrence of MACCE. Statistical significance was defined as a $P<0.05$.

RESULTS

Patient baseline and surgical characteristics

The treatment groups significantly differed on two baseline characteristics: myocardial infarction within three months prior to surgery and previous PCI (Table 1). Post hoc analysis revealed that in the ASA+Clo group, the number of patients

Table 1. Baseline and surgical patient characteristics.

	None *(n=113)*	ASA *(n=662)*	ASA+Clo *(n=290)*	*P-value*
Baseline characteristics				
Age, years; mean(SD)	65(10)	66(9)	65(10)	0.43
Male/female, n/n	95/18	531/131	242/48	0.38
Diabetes mellitus; n(%)	38(33.6)	160(24.2)	71(24.5)	0.095
Hypertension; n(%)	62(54.9)	378(57.1)	160(55.2)	0.81
CCS IV; n(%)	10(8.8)	82(12.4)	43(14.8)	0.36
MI <3 months before CABG; n(%)	17(15.0)	84(12.7)	91(31.4)	<0.001
Previous cardiac surgery; n(%)	1(0.9)	13(2.0)	6(2.1)	0.89‡
Previous PCI; n(%)	14(12.4)	109(16.5)	78(26.9)	<0.001
LVEF; n(%)				0.087‡
30-50%	12(10.6)	81(12.2)	51(17.6)	
<30%	5(4.4)	15(2.3)	5(1.7)	
Extracardiac arteriopathy; n(%)	13(11.5)	75(11.3)	31(10.7)	0.95
Renal insufficiency; n(%)	7(6.2)	40(6.0)	11(3.8)	0.35
COPD; n(%)	15(13.3)	63(9.5)	24(8.3)	0.31
Neurological dysfunction disease; n(%)	1(0.9)	21(3.2)	9(3.1)	0.46‡
Logistic Euroscore, %; mean(SD)	3.3(3.3)	3.1(3.0)	3.5(3.6)	0.34
Laboratory values				
Hemoglobin, mmol/l; mean(SD)	8.7(0.9)	8.8(0.9)	8.7(0.9)	0.15
Hematocrit, l/l; mean(SD)	0.42(0.04)	0.42(0.04)	0.41(0.04)	0.073
Erytrocytes, 1000 billion/l; mean(SD)	4.7(0.7)	4.7(0.6)	4.6(0.5)	0.065
Fibrinogen Clauss, g/l; mean(SD)	4.6(1.0)	3.7(0.8)	4.4(1.5)	0.085
aPTT, seconds; mean(SD)	31.16(3.48)	30.86(5.84)	31.88(7.77)	0.10
PT, seconds; mean(SD)	12.91(1.95)	13.00(2.00)	13.74(1.97)	<0.001
Platelet count, x109/l; mean(SD)	249(74)	242(71)	252(78)	0.15
Surgical characteristics				
Beating heart CABG; n(%)	13(11.5)	77(11.6)	41(14.1)	0.54
Number of arterial grafts, n; mean(SD)	1.7(0.9)	1.7(0.9)	1.6(0.7)	0.63
Operation time, min; mean(SD)	223(68)	220(63)	241(71)	<0.001
CPB time, min; mean(SD)	88(47)	84(42)	90(51)	0.14
Aorta cross clamp time, min; mean(SD)	67(33)	64(29)	69(38)	0.061
CS transfusion, ml; mean(SD)	234(320)	259(315)	245(310)	0.66
FL use; n(%)	65(57.5)	329(49.7)	153(52.8)	0.26
Tranexamic acid use, ml/mg; mean(SD)	3089(1693)	2825(1714)	3204(1516)	0.004
IABP; n(%)	1(0.9)	2(0.3)	0	0.31‡

ASA, acetylsalicylic acid; ASA+Clo, acetylsalicylic acid and clopidogrel; SD, standard deviation; CCS, Canadian Cardiovascular Society Angina Pectoris classification; MI, myocardial infarction; CABG, coronary artery bypass grafting; PC, percutaneous coronary intervention; LVEF, left ventricular ejection fraction; COPD, chronic obstructive pulmonary disease; CPB, cardiopulmonary bypass; CS, cell saver; FL, fibrin sealant; IABP, intra-aortic balloon pump.
‡Fisher's exact test.

enduring a myocardial infarction within three months prior to operation was significantly higher compared with the None and ASA groups (P=0.001 and P<0.001, respectively). PCI procedures were significantly more frequently performed in the ASA+Clo group than in the None and ASA groups (P=0.002 and P<0.001, respectively).

Operation time was significantly longer in the ASA+Clo group compared with the None and ASA groups (P=0.015 and P<0.001, respectively), although CPB time and aorta cross clamp time were not significantly different between the groups. Consistent with the longer operation time, the amount of tranexamic acid administered in the ASA+Clo group was significantly higher compared with patients receiving ASA only (P=0.001).

Blood loss and transfusions

In the ASA+Clo group, the median amount of blood loss 48 h after surgery was approximately 30-70 ml higher than in the None and ASA groups (overall test: P=0.037; post hoc: ASA vs None, P=0.073 and ASA+Clo vs None, P=0.012). The ASA+Clo group also received more blood transfusions after operation, especially platelet transfusions (Table 2).

In total, 29 patients (None=3; ASA=13; ASA+Clo=13) underwent reoperation for bleeding with a median blood loss of 1885 ml (IQR: 998-2703 ml). Only in two patients (None=1; ASA=1) a surgical cause for the excessive bleeding during reoperation was found.

Table 2. Patient outcomes.

	None *(n=113)*	ASA *(n=662)*	ASA+Clo *(n=290)*	*P-value*
Blood loss <48 h, ml; median(IQR)	620(416-905)	660(490-950)	695(500-1013)	0.037
Blood transfusions <48 h				
Patients receiving PRBC; n(%)	28(24.8)	144(21.8)	85(29.3)	0.044
Patients receiving FFP; n(%)	12(10.6)	59(8.9)	57(19.7)	<0.001
Patients receiving PT; n(%)	10(8.8)	70(10.6)	88(30.3)	<0.001
Ventilation time >48 h; n(%)	1(0.9)	7(1.1)	1(0.3)	0.59‡
Reoperation for bleeding; n(%)	3(2.7)	13(2.0)	13(4.5)	0.10‡
Length of ICU stay; h; mean(SD)	29(26)	32(54)	33(53)	0.79
Length of hospital stay, days; mean(SD)	9.0(7.8)	8.0(4.4)	9.1(6.1)	0.007
MACCE	5(4.4)	22(3.3)	19(6.6)	0.078
Hospital mortality; n(%)	0	5(0.8)	3(1.0)	0.76‡
MI; n(%)	4(3.5)	14(2.1)	13(4.5)	0.12‡
Reintervention for ischemia; n(%)	0	3(0.5)	1(0.3)	1.00‡
Stroke; n(%)	1(0.9)	1(0.2)	2(0.7)	0.18‡

ASA, acetylsalicylic acid; ASA+Clo, acetylsalicylic acid and clopidogrel; IQR, interquartile range; PRBC, packed red blood cells; FFP, fresh frozen plasma; PT, platelet transfusions; ICU, intensive care unit; MACCE, major adverse cardiovascular and cerebral events; MI, myocardial infarction.
‡Fisher's exact test.

Acetylsalicylic acid

The median amount of blood loss within 48 h in the ASA group was 660 ml (IQR: 490-950 ml; Table 2). The median amount of 48-h blood loss relative to the different stop days is shown in Figure 2-1A. The best fitting broken stick model had an adjusted R^2 of 0.129 (weak correlation) and corresponded to day -10 as the estimated optimal stop day. The model yielded an estimated increase in blood loss of 2% (*P*=0.018) for each day stopped later than day -10. Last use of ASA on day -10 resulted in a blood loss reduction of 25% compared with continuation of ASA until surgery. However, the bootstrap CI of the optimal stop day was very wide, running from day -1 to -10. Last use of ASA on day -2 or earlier (day -2 to -10) resulted in

a significant reduction of median blood loss 48 h after operation (630 ml, IQR: 480-850 ml) compared with continuation of ASA until surgery (day -1, 690 ml, IQR: 496-1004 ml, *P*=0.007; Figure 2-1B).

Platelet transfusions given during and directly after surgery were associated with blood loss 48 h after operation (*P*<0.001). Last use of ASA on day -2 or earlier resulted in an almost 50% reduction of the percentage of patients receiving platelet transfusions (7%) compared with continuation of ASA until surgery (day -1, 13%, *P*=0.007; Figure 2-2B). Earlier stop days than day -2 did not result in an additional decrease in platelet transfusions.

Furthermore, multivariable analysis revealed that a lower preoperative Hb level was associated with more blood loss within 48 h after operation in patients using ASA before surgery (*P*=0.012).

ASA and Clopidogrel

The median amount of blood loss within 48 h was 695 ml (IQR: 500-1013 ml; Table 2). In all fitted (broken stick) regression models, the effect of stop day was not significant. Last use of ASA+Clo on day -2 or earlier resulted in a significant reduction of median blood loss 48 h after operation (623 ml, IQR: 485-913 ml) compared with continuation of ASA+Clo until surgery (day -1, 715 ml, IQR: 513-1078 ml, *P*=0.005; Figure 2-1B).

Also for patients in the ASA+Clo group, platelet transfusions given during and directly after surgery were associated with blood loss 48 h after operation (*P*<0.001). Last use of the combination of ASA+Clo on day -2 or earlier resulted in a 30% reduction of patients receiving platelet transfusions (10%) compared with continuation of both antiplatelet medications until surgery (day -1, 41%, *P*<0.001; Figure 2-2B). Last use on day -3 or earlier even reduced the number of patients undergoing platelet transfusions to 5%.

Other factors associated with more blood loss within 48 h after operation in the ASA+Clo group were a lower preoperative Hb level (*P*=0.024) and a higher amount of intra-operative colloid usage (*P*=0.007).

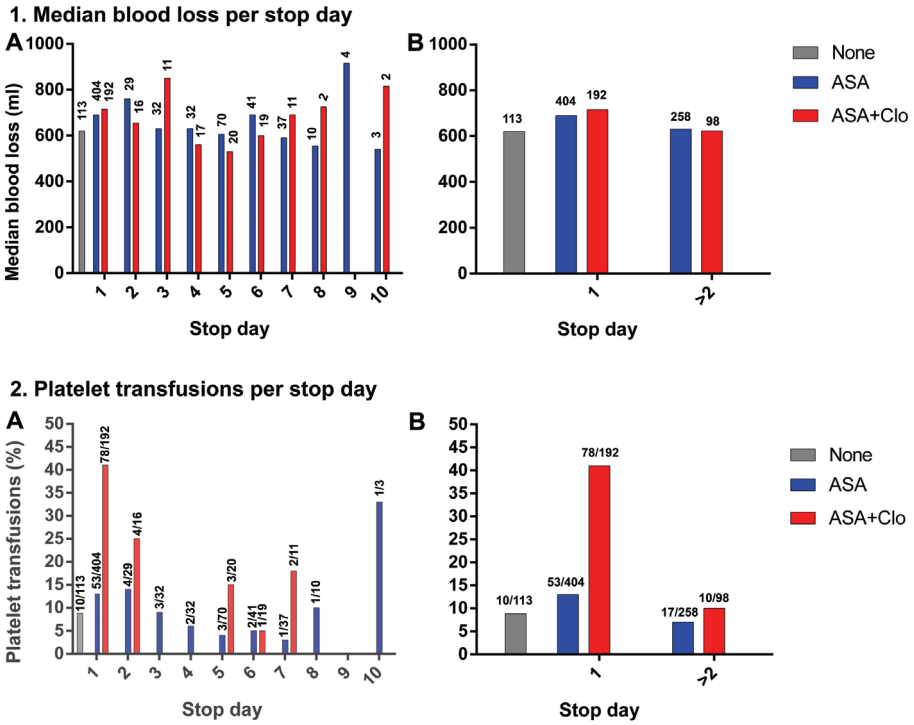

Figure 2. Median blood loss and platelet transfusions within 48 h after surgery.

1. Median blood loss (ml) within 48 h after surgery;
   1A for each stop day until day -10
   1B divided in stop day -1 and days -2 until -10 (>2)

The numbers above the bar charts represent the amount of patients that stopped their antiplatelet medication on that particular stop day.

2. Platelet transfusions (percentage of patients transfused) within 48 h after surgery;
   2A for each stop day until day -10
   2B divided in stop day -1 and days -2 until -10 (>2)

The numbers above the bar charts represent the amount of patients transfused relative to the total amount of patients that stopped their antiplatelet medication on that particular stop day. None, no antiplatelet medication within 10 days before surgery or never used; ASA, use of acetylsalicylic acid within 10 days prior to surgery; ASA+Clo, use of ASA and Clo within 10 days prior to surgery.

Major adverse cardiovascular and cerebral events

There was no significant association between the moment of last use of ASA before surgery and MACCE (OR=0.990, 95%CI=0.817-1.200, *P*=0.92) or the moment of last use of ASA+Clo and MACCE (OR=0.849, 95%CI=0.635-1.135, *P*=0.27).

DISCUSSION

The FIBER study in which no fixed stop day for preoperative antiplatelet medication was demanded provided an outstanding opportunity to study the effects of stop day on bleeding and transfusion requirements in isolated CABG patients. In the present study, no clinically relevant difference in blood loss per stop day was demonstrated for preoperative use of only ASA or dual antiplatelet therapy with ASA+Clo using multiple linear regression models. Last use on day -2, however, resulted in lower amounts of blood loss and percentage of patients receiving platelet transfusions, especially in patients using the combination of ASA+Clo. There was no association between stop day and the occurrence of MACCE.

ASA and Clo are widespread used drugs among patients undergoing CABG surgery. Several large, randomized, double-blind, multicenter studies, such as the CAPRIE (11), CURE (12), and CREDO (13) trials, have stressed the beneficial effects of prophylactic ASA and Clo therapy in patients at risk of ischemic events. The 2011 update of the American College of Cardiology Foundation/American Heart Association (ACCF/AHA) guidelines for CABG surgery states that preoperative ASA use reduces operative morbidity and mortality rates (14). Yet, at the same time, several studies have shown that administration of Clo prior to cardiac surgery, especially in combination with ASA, increases the risk of postoperative bleeding (4,15,16). Optimal timing for discontinuation of antiplatelet medication prior to surgery is thus important.

Both the ACCF/AHA guidelines for CABG surgery and the European Society of Cardiology/European Association for Cardio-Thoracic Surgery (ESC/EACTS) guidelines on myocardial revascularization published in 2014 (17) recommend preoperative ASA to be administered without discontinuation (Class I recommendation, Level of Evidence: B) and preoperative Clo to be discontinued at day -5 (Class I recommendation, Level of Evidence: B) in order to reduce bleeding and the need for transfusion. Furthermore, the ESC/EACTS guidelines append that bedside platelet function testing is to be preferred as it is a more precise option for guiding interruption of treatment, rather than use of an arbitrary, specified stop period.

In this study, regression analysis demonstrated that last use of ASA on day -10 was optimal and resulted in an approximately 25% (corresponding with 250 ml) blood loss reduction within the first 48 h after isolated CABG surgery compared with continuation of ASA until surgery. However, results at this extreme stop day apparently were influenced by small patient numbers. Bootstrap analysis revealed a CI from stop day -1 till -10 and the correlation with blood loss 48 h after operation was weak. Correspondingly, Kwak (18) demonstrated no association between stop day of ASA before off-pump CABG and the amount of postoperative blood loss. Furthermore, Hijazi (5) found that continuation of low-dose (80 or 100mg per day) ASA until CABG surgery did not appear to increase postoperative bleeding or the need for allogeneic blood transfusion. For patients who used dual antiplatelet therapy with ASA+Clo, regression analysis also revealed no optimal stop day. Probably, other factors, like a low preoperative Hb level, as revealed by multivariable analysis, have a greater influence on 48-h blood loss than interruption time of preoperative antiplatelet medication usage.

In patients who last used their antiplatelet medication on day -1, especially in patients who used ASA+Clo, the received platelet transfusions appeared to reduce 48-h blood loss to an amount that it is comparable with the amount of blood loss associated with earlier stop days (-2 till -10). Last use of especially the combination of ASA+Clo on day -2 or earlier was associated with a lower percentage of patients receiving platelet transfusions. This is congruent with the ACCF/AHA guidelines which conclude that the amount of blood loss prevented by discontinuation of Clo on day -5 relative to later stop days (-4 till -1) is not life threatening, but only decreases the amount of blood transfusions required. Several studies reported that treatment with Clo within 3 to 4 days preoperatively increased blood transfusion requirements, but if discontinued 3 days before surgery, patients did not suffer from significantly more blood loss (7,16,19,20).

Because nearly all platelet transfusions were given during and directly after surgery, this variable was included in the list of covariates that was controlled for in the performed regression analysis to establish an optimal stop day for blood loss reduction after surgery. When performing the analysis without correction for platelet transfusions, almost similar results were found for patients using only ASA.

For patients using the combination of ASA+Clo, without his correction stop day -4 appeared most optimal for the reduction of 48-h blood loss. However, since the amount of blood loss in this patient group seemed to rise from stop day -5 till -10, stop day -4 was considered clinically not plausible.

The main, clinically relevant blood loss reduction appeared to occur between stop day -1 and -2 in both treatment groups, but especially in the patients on dual antiplatelet therapy with ASA+Clo, indicating that the combination of ASA+Clo should not be taken on the day before surgery. This is in accordance with an earlier study by Mahla (21) who found that bedside platelet function testing, as suggested by the ESC/EACTS guidelines, resulted in an overall 46% shortening of the recommended preoperative stop period for Clo-treated patients (mean 2.7 days versus 5 days). For this reason, the 2012 update of the Society of Thoracic Surgeons guideline on use of antiplatelet drugs in patients having cardiac or non-cardiac operations (22) recommends for patients with acute coronary syndrome who require urgent surgery an interruption of dual antiplatelet therapy of 2 days in order to decrease bleeding and thrombotic risk (Class IIa recommendation; Level of Evidence: B).

The fact that we found no association between stop day and the occurrence of MACCE might be due to the fact that the decision to preoperatively continue antiplatelet medication was possibly influenced by the perceived thrombotic risk. Recently, large series have suggested that patients are at highest risk of stent thrombosis when both antiplatelet agents are discontinued (23,24). We therefore cannot exclude that last use of a combination of ASA+Clo on day -2 might result in an increased risk of MACCE.

A weakness of this study is that despite standard surgical and anesthetic principles regarding transfusion strategy were used, most surgeons and anesthesiologists knew when a patient last used antiplatelet drugs prior to operation. It is conceivable, therefore, that the threshold for blood transfusion in patients who continued antiplatelet therapy, especially dual antiplatelet therapy with ASA+Clo, was lowered. Furthermore, this study is limited in that we cannot report on the effect of last intake of Clo alone due to low patient numbers on this therapy in the FIBER cohort. Isolated Clo use until CABG surgery has been associated with a

higher incidence of hard-to-manage intra-operative bleeding and blood transfusions (25). Finally, newer and more potent antiplatelet inhibitors (e.g., dipyridamol (adenosine reuptake inhibitor), prasugrel, ticagrelor (both adenosine diphosphate receptor inhibitors), and thromboxane inhibitors) are not analyzed in this study. These medications will probably demonstrate a greater effect of stop day on 48-h blood loss but were rarely prescribed within the FIBER study that was conducted between March 2009 and January 2012.

In conclusion, an optimal stop day for preoperative use of ASA alone or dual antiplatelet therapy with ASA+Clo minimizing 48-h blood loss after isolated CABG surgery could not be established using multiple linear regression models. Yet, last use on day -2 resulted in a reduction of blood loss and percentage of patients receiving platelet transfusions, especially with combined preoperative ASA+Clo use, and should be considered as the best compromise in the vast majority of these patients.

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CONFLICTS OF INTEREST

None declared.

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CHAPTER 6

Multicentre randomized controlled clinical trial to investigate the cost-effectiveness of an allogeneic single-donor fibrin sealant in coronary artery bypass grafting (FIBER) Study

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ABSTRACT

Background

Reduction of blood transfusion in cardiac surgery is an important target. The aim of this study was to investigate the cost-effectiveness of the use of CryoSeal, an allogeneic single-donor fibrin sealant, in patients undergoing coronary artery bypass grafting (CABG).

Methods

This randomized controlled study involved seven cardiac surgery centres in the Netherlands. Patients undergoing elective isolated CABG with the use of at least one internal thoracic artery (ITA) graft were randomly assigned to receive either CryoSeal (5 ml per ITA bed) or no CryoSeal. Primary efficacy endpoints were units transfused red blood cells, fresh frozen plasma and platelet concentrates, and length of Intensive Care Unit stay. Secondary efficacy endpoints were 48-h blood loss, reoperation for bleeding, mediastinitis, 30-day mortality and length of hospital stay.

Results

Between March 2009 and January 2012, 1,445 patients were randomized. The intention-to-treat (ITT) population comprised 1,436 patients; the per-protocol (PP) population 1,292. In both the ITT and the PP analysis, no significant difference between the treatment groups was observed for any of the primary and secondary efficacy endpoints. In addition, no significant difference between the groups was seen in the proportion of transfused patients. Estimated CryoSeal costs were €822 per patient (95%CI €808 to €836), which translated to €72,000 per avoided transfusion (with unbounded 95%CI).

Conclusion

The use of the fibrin sealant CryoSeal did not result in health benefits. Combined with the high cost per avoided transfusion, this study does not support the implementation of routine CryoSeal use in elective isolated CABG. Registration number: NTR1386 (http://ww.trialregister.nl).

INTRODUCTION

Transfusion of blood products is a common intervention in cardiac surgery but is not without risk. It is associated with increased in-hospital morbidity, including increased rates of renal failure, serious infection, prolonged ventilator support, atrial fibrillation and stroke, as well as increased in-hospital mortality (1). In addition, it has been associated with increased long-term mortality (2). The relationship with early risk for adverse outcome appears to be dose-dependent, with transfusion of no more than 1-2 units of red blood cells (RBCs) already increasing risks after isolated coronary artery bypass grafting (CABG). Any reduction in blood transfusion during cardiac surgery is thus an important target to improve outcome and reduce health care costs (3).

During the past two decades, fibrin sealants have gained popularity as an adjunct to achieve hemostasis and reduce RBC transfusion in many surgical fields, including cardiac surgery (4). Fibrin sealants are indicated for control of surgical bleeding when conventional interventions such as compression, ligation, clipping, suturing or electrocoagulation are insufficient. The application of fibrin sealants is generally considered clinically safe (5). However, there are still concerns regarding the antigenicity of bovine thrombin or aprotinin used in the majority of the commercially available fibrin sealants (6). Furthermore, most fibrin sealants are produced of pooled plasma, which is associated with an increased viral or prion risk. The use of an autologous fibrin sealant in cardiac surgery, on the other hand, may be associated with variable quality arising from co-morbidities.

The fibrin sealant CryoSeal (Sanquin Blood Supply Foundation, Amsterdam, The Netherlands) is produced from an allogeneic, single, non-remunerated volunteer donor without the addition of antifibrinolytics, and may therefore have potential advantages (7). CryoSeal fibrin sealant has not been studied in cardiac surgery previously.

In CABG, use of the left internal thoracic artery (ITA) is universally accepted. Evidence from several studies suggests that bilateral ITA grafting prolongs survival (8,9). However, not only has the use of a single ITA graft been associated with increased mediastinal drainage compared to the exclusive use of saphenous vein

grafts (10), but also bilateral ITA grafting has been associated with increased post-operative blood loss compared to single ITA grafting (11). The aim of this study was to investigate the cost-effectiveness of the use of CryoSeal in patients undergoing elective isolated CABG with the use of single or bilateral ITA.

METHODS

The FIBER (Fibrin sealant Induced Blood Exposure Reduction) Study was a multicentre randomized clinical study involving seven cardiac surgery centres in the Netherlands. The trial was performed in accordance with the Declaration of Helsinki, the ethical principles of the International Conference on Harmonization Good Clinical Practice and relevant Dutch laws, and was approved by the ethics committee of the Leiden University Medical Centre, followed by the local ethics committees. All patients provided written informed consent before entering in the study. A steering committee was responsible for organizing the study, executing data analysis and writing the paper.

Inclusion and randomisation

Patients undergoing elective isolated CABG (either on-pump or off-pump) with the use of at least one ITA graft were eligible. Exclusion criteria were: CABG with exclusive use of saphenous vein grafts; any concomitant procedure including ablation for atrial fibrillation; emergency surgery; history of bleeding diathesis or coagulopathy; Jehovah's Witness; participation in any other study involving an investigational drug or device; and inability of the patient to understand the study information. Once a patient had signed a consent form, they were randomized by calling the trail manager. In this telephone call, information about the intended procedure (the use of single or bilateral ITA) was requested in order to receive a sufficient amount of CryoSeal fibrin sealant (5 ml for each ITA bed) if randomized to CryoSeal treatment. Patients were assigned randomly at a ratio of 1:1 to either treatment with CryoSeal fibrin sealant or no CryoSeal treatment (control group), stratified according to the study site.

Investigational product

CryoSeal is an allogeneic single-donor fibrin sealant produced from fresh frozen quarantined plasma from donations of non-remunerated volunteers. It is produced by Sanquin Blood Bank (12) using disposables provided by Recuperate Medical (Recuperate Medical BV, Haren, The Netherlands). CryoSeal consists of two components: cryoprecipitate and thrombin. Cryoprecipitate is the fraction of human plasma that contains concentrated coagulation factors, such as fibrinogen, fibronectin, plasminogen, factor VIII, factor XIII an von Willebrand factor. Owing to the low levels of plasminogen, CryoSeal does not require an artificial fibrinolysis inhibitor (such as aprotinin, sourced from bovine lung tissue, or tranexamic acid).

Safety and toxicology

The single-donor plasma was routinely tested on hepatitis C virus, hepatitis B virus, human immunodeficiency virus 0-2, human T-limphotropic virus, *Treponema pallidum* hemagglutination assay, and parvovirus B19 at donation. It was retested after a quarantine period of at least 6 months before processing to CryoSeal, thereby virtually excluding the plasma as a source of infectious disease transmission to the recipients.

Given the origin of CryoSeal (no pooled plasma and no addition of bovine or chemical fibrinolysis inhibitors), no toxicity was expected. However, CryoSeal is a blood product and adverse transfusion reactions may occur.

Feasibility in CABG

To study the feasibility of CryoSeal use in CABG, a pilot-study was performed in the Department of Cardiothoracic Surgery, Leiden University Medical Centre. The study included 40 consecutive patients who had elective isolated CABG, either on-pump or off-pump. It was concluded that CryoSeal can be best applied to the ITA bed in this population. No side-effects or postoperative complications were reported in the feasibility study.

Surgical procedure and application of cryoSeal

Surgical techniques were used according to the local standards. After sternotomy, harvesting of the ITA, pedicled or skeletonised, was carried out simultaneously with saphenectomy (if applicable). Cardioplegic arrest, when applicable, was obtained either by use of cold crystalloid solution or by warm or cold blood cardioplegia. The surgeon was blinded to treatment group allocation until nearly the end of the operation. For each randomized patient, a cooling box was delivered to the operating room containing either CryoSeal or no CryoSeal. The handling of CryoSeal was taught by video instruction before the study. The cooling box was not opened until 30 min before use of CryoSeal, which had to be applied after the administration of protamine. In this way, the routine of achieving hemostasis during surgery was influenced minimally. CryoSeal had to be thawed at 40 ºC for at least 20 min before use. After the administration of protamine, patients assigned to the study group were treated with a maximum of 15 ml CryoSeal each. For each ITA bed, 5 ml CryoSeal was used. CryoSeal was applied with the use of a spray tip mounted on a 5-ml syringe. Any remaining fibrin sealant could be used on anastomoses, cannulation sites and other part of the surgical field, at the discretion of the surgeon. The chest was then closed routinely. All CryoSeal not used during the operation was returned to the local Hospital Blood Transfusion Services to record the amount of fibrin sealant applied to each patient. Surgeons had the discretion to use additional tranexamic acid, but use of another fibrin sealant was not allowed.

Transfusion policy

Blood transfusion practice was based on the transfusion guidelines of the American Society of Anesthesiologists (13) and the Dutch Institute for Healthcare Improvement (14). The Dutch guidelines support a quite restrictive transfusion policy. It recommend RBC transfusion when the hemoglobin level is less than 7 g/dl, and advices against transfusion when the level is above 9 g/dl. When the hemoglobin is above 7 g/dl and less than 9 g/dl, the transfusion trigger is determined by blood loss, cardiopulmonary reserve, age, and/or comorbidity. Management was at the discretion of the treating physician.

Efficacy end-points

The primary efficacy endpoints were number of transfused blood products (RBCs, fresh frozen plasma(FFP) and platelet concentrates (PCs)) up to 48 h after surgery and duration of stay in the Intensive Care Unit (ICU). Secondary efficacy end-points included the amount of blood loss within 48 h after surgery, reoperation for bleeding, mediastinitis, 30-day mortality and length of hospital stay.

Safety and safety reporting

Prespecified serious adverse events (SAEs) were myocardial infarction, stroke, mediastinitis, re-operation for ischemia and 30-day mortality. Myocardial infarction was defined as presence of at least two of the following: (1) ischemic chest pain lasting for more than 20 min; (2) changes in serial electrocardiogram (ECG) tracings; and (3) troponin T level above 1 µg/l. Stroke was defined as a new persistent cerebrovascular event leading to neurological defaults being diagnosed by a neurologist. Diagnosis of mediastinitis required positive substernal tissue cultures. Reoperation for ischemia was defined in case the need for reoperation was based on changes in serial ECG tracings and troponine T level above 1 µg/L. Each SAE had to be recorded on a SAE Form and sent to the Coordinating Principal Investigator. SAEs were reported to the local Ethics Committee, according to local requirements.

Other collected adverse events (AEs) included low cardiac output syndrome, duration of ventilation of more than 48 h, sepsis, pneumonia, renal insufficiency, atrial fibrillation, ventricular fibrillation, transient ischemic accident, superficial wound infection and reoperation for bleeding.

Data safety monitoring board

An independent Data Safety Monitoring Board (DSMB) was established to protect the safety and welfare of the patients. The DSMB was blinded to group allocation when assessing the interim report data.

Statistical analysis

To demonstrate CryoSeal to be cost-effective, a treatment effect of a 50% reduction in the number of RBC transfusions and a 0.4 day reduction in duration of ICU stay

was needed. The power calculation based on this treatment effect, a power of 90%, and an α level of 0.05 (two-sided test), showed that the minimum size per treatment group should be 500 patients. It was planned to enrol approximately 750 patients per group to allow for violations of the protocol, non-evaluable patients, patient withdrawal from the study, and reliable preplanned subgroup analysis.

A planned interim analysis was conducted by the DSMB after 1,000 patients had been randomized and complete ICU data were achieved in at least 90% of those patients. The interim analysis was intended as both a safety assessment and superiority analysis. For statistical stopping boundaries, the Haybittle-Peto approach was used, which requires $P < 0.001$ as evidence required to consider stopping the trial (15). A futility analysis was not considered in order to provide for an adequate sample size to perform planned subgroup analyses.

Efficacy and safety analyses

Efficacy and safety analyses were conducted in all randomized patients (intention to treat (ITT) population) and for randomized patients who completed the study according to the protocol (per-protocol (PP) population).

Heterogeneity in treatment effect across the seven study sites was examined for all primary efficacy endpoints using analysis of variance or logistic regression analysis, as appropriate. These analyses were conducted by including interaction terms for treatment and study site. Heterogeneity was assumed to be present in case of a statistically significant treatment x study site interaction effect. Once no heterogeneity had been demonstrated, differences in efficacy outcomes between the two treatment groups were tested for continuous variables using the Student's t test for independent samples. Comparison on categorical variables was performed with Pearson chi-square test or the Fisher's Exact test, as appropriate.

The robustness of results for alternative population specifications (subgroup analysis) was tested in a similar way as for study site. There were four predefined subgroup variables: sex, preoperative use of antiplatelet medication within 5 days of surgery (yes or no), use of cardiopulmonary bypass (CPB; yes or no), and use of single versus bilateral ITA. When a significant treatment x subgroup interaction was

detected, a subgroup analysis was conducted for the respective primary efficacy endpoint.

For safety analysis, the treatment effect on safety measures was tested by univariable binary logistic regression analysis. Odds ratios and 95% CI were computed with the no-CryoSeal treatment group as a reference.

All statistical tests were two-sided. $P < 0.05$ was considered statistically significant. The statistical analyses were performed using SPSS software version 20.0 (IBM, Armonk, New York, USA).

Economic evaluation

The economic evaluation consisted of a cost-minimization analysis, from the hospital perspective and with a time frame from the operation day to discharge. No detectable costs differences were expected in non-hospital or other societal costs, or byond the duration of hospitalization. Included cost categories were CryoSeal use, blood products, hospitalization and reoperations.

Costs of CryoSeal use were estimated at €705 and €1,080 for surgery with one and two ITAs respectively (based on the use of 9 and 14 ml of CryoSeal, a market price of €75 per ml, and two additional min of operating time, valued at €15 per min (16)). The product costs were counted regardless of whether the patient received the CryoSeal; additional operating time was included only if the patient actually received the CryoSeal.

Costs of blood products were estimated using unit prices in the Netherlands in 2012: €215 for RBCs, €185 for FFP and €519 for PCs (17-18). To account for compatibility tests and hospital handling costs, the unit price for RBCs was multiplied by a factor four, and the unit prices of FFP and PCs by a factor two (19).

Costs of hospitalization were estimated using standard prices, at €2,288 per ICU day and €490 per non-ICU day (13). Reoperation for ischemia, reoperation for bleeding and treatment of mediastinitis were valued at €4,000, €2,000, and €3,000 respectively.

Average costs were compared according to ITT, using unequal-variance t-test, multiple imputations to account for missing data (rendering slight differences compared to the efficacy analysis), and at 2012 price level. A cost-effectiveness

analysis was performed using cost-effectiveness acceptability curves, comparing the difference in the proportion of transfused patients to the difference in costs (20).

RESULTS

Between March 2009 and January 2012, a total of 1,445 patients were included in the study (Figure 1). Nine patients withdrew consent or had incomplete data. Thus, the ITT population comprised 1,436 patients: 722 patients (50.3%) were randomized to CryoSeal, 714 patients (49.7%) to no CryoSeal. There were 144 protocol violations: 113 in the CryoSeal group and 31 in the no-CryoSeal group; resulting in a PP population of 1,292 patients: 609 and 683 repectively. Protocol violations included treatment with less than the required amount of fibrin sealant (n = 98), treatment with CryoSeal although randomized to no sealant (n = 6), other than isolated CABG surgery (n = 32) and other reasons (n = 8). The two groups were similar with respect to baseline demographic, clinical and surgical characteristics, except for the use of CPB (*P*=0.001 and *P*=0.005 in the ITT and PP population respectively) (Table 1).

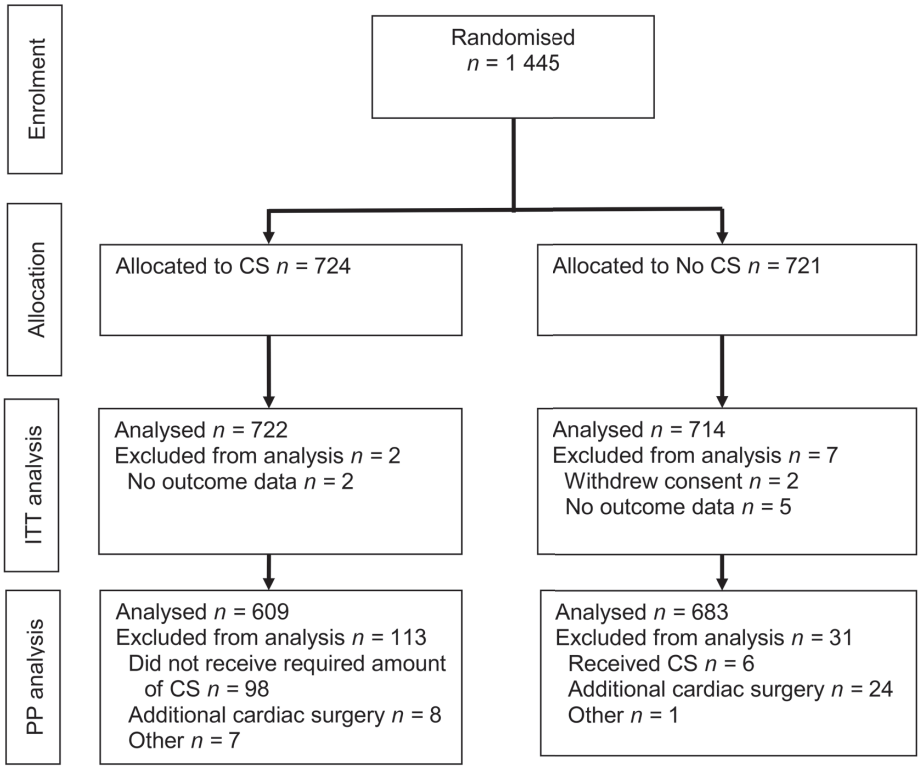

Figure 1. CONSORT diagram for the trial.
ITT, intention-to-treat; PP, per-protocol

Table 1. Baseline demographic, clinical and surgical characteristics.

	Intention to treat		Per protocol		
Variable	**CryoSeal (n = 722)**	**No CryoSeal (n = 714)**	**CryoSeal (n = 609)**	**No CryoSeal (n = 683)**	**P-value[§]**
Demographics					
Age (years) *	65.7 (9.7)	65.1 (10.0)	65.4 (9.8)	65.1 (9.9)	0.554[‡]
Female gender	126 (17.5)	136 (19.0 %)	110 (18.1 %)	128 (18.7 %)	0.754
Comorbidity					
Myocardial infarction	277 (38.4)	272 (38.1)	232 (38.1)	255 (37.3)	0.778
LV function <30%	17 (2.4)	17 (2.4)	16 (2.6)	17 (2.5)	0.875
Previous cardiac surgery	18 (2.5)	13 (1.8)	13 (2.1)	12 (1.8)	0.623
Previous PCI	148 (20.5)	133 (18.6)	127 (20.9)	125 (18.3)	0.248
Hypertension	409 (56.6)	398 (55.7)	344 (56.5)	378 (55.3)	0.680
Diabetes	192 (26.6)	174 (24.4)	160 (26.3)	169 (24.7)	0.529
COPD	65 (9.0)	67 (9.4)	54 (8.9)	66 (9.7)	0.623
Renal insufficiency	45 (6.2)	37 (5.2)	36 (5.9)	37 (5.4)	0.701
Logistic EuroSCORE*	3.4 (3.5)	3.3 (3.3)	3.4 (3.5)	3.3 (3.3)	0.634[‡]
Preoperative medication					
Anticoagulants within 5 days	20 (2.8)	12 (1.7)	14 (2.3)	11 (1.6)	0.370
Antiplatelets within 5 days	463 (64.1)	460 (64.4)	395 (64.9)	439 (64.3)	0.826
Preoperative laboratory data					
Hemoglobin (mmol/l)*	8.7 (0.9)	8.7 (0.9)	8.7 (0.9)	8.7 (0.9)	0.799[‡]
Hematocrit (%)*	41.5 (4.2)	41.4 (4.1)	41.4 (4.1)	41.5 (4.1)	0.767[‡]
Thrombocytes (10^9/l)*	243.5 (73.6)	243.6 (72.5)	245.0 (73.7)	243.5 (72.3)	0.702[‡]
Prothrombin time (sec)*	13.6 (3.2)	13.4 (2.7)	13.6 (2.8)	13.4 (2.6)	0.179[‡]
aPTT (sec)*	31.5 (7.4)	31.0 (5.8)	31.7 (7.4)	31.0 (5.8)	0.112[‡]
Surgery					
Use of 2 ITAs	292 (40.4)	298 (41.7)	242 (39.7)	294 (43.0)	0.228
Use of CPB	615 (85.2)	640 (89.6)	514 (84.4)	612 (89.6)	0.005
CPB time[a] (min)*	97.6 (33.9)	100.2 (36.4)	97.4 (31.6)	99.2 (34.8)	0.380[‡]
Use of tranexemic acid	610 (86.2)	620 (88.7)	517 (86.3)	593 (88.6)	0.210

Values in parentheses are percentages unless indicated otherwise;
* values are means (SD).
[§] Pearson chi-square test except
[‡] Student's t-test.
[a] In case of use of CPB.
aPTT, Activated Partial Thromboplastin Time; COPD, Chronic Obstructive Pulmonary Disease; CPB, Cardiopulmonary Bypass; ITA, Internal Thoracic Artery; LV, Left Ventricular.

Primary and secondary efficacy outcomes

In both the ITT and the PP analysis, no significant difference between the treatment groups on any of the primary and secondary efficacy endpoints was observed. In addition, no significant difference between the two groups was seen in the proportion of patients transfused with blood products (Table 2).

Table 2. Primary and secondary efficacy endpoints.

	Intention to treat			Per protocol		
Variable	**CryoSeal (n = 722)**	**No CryoSeal (n = 714)**	***P*- value**	**CryoSeal (n = 609)**	**No CryoSeal (n = 683)**	***P*-value**
Primary Endpoints						
Blood product usage						
RBC (units)*	0.57 (1.38)	0.59 (1.52)	0.977[a]	0.57 (1.37)	0.54 (1.38)	0.688[a]
RBC (pts transfused)	179 (24.9)	179 (25.1)	0.915	153 (25.2)	166 (24.4)	0.730
FFP (units)*	0.27 (0.82)	0.32 (1.10)	0.947[a]	0.25 (0.77)	0.28 (0.97)	0.869[a]
FFP (pts transfused)	93 (12.9)	92 (12.9)	0.994	75 (12.4)	81 (11.9)	0.800
Platelets (units)*	0.19 (0.50)	0.20 (0.55)	0.966[a]	0.20 (0.50)	0.18 (0.48)	0.507[a]
Platelets (pts transfused)	117 (16.3)	115 (16.2)	0.951	105 (17.3)	107 (15.7)	0.444
Any blood product	249 (34.6)	252 (35.4)	0.763	212 (34.9)	237 (34.8)	0.963
Length of ICU stay (hours)*	31.4 (46.9)	34.5 (58.9)	0.267	31.2 (44.7)	34.5 (60.0)	0.251
Secondary Endpoints						
Blood loss within 48 hours (ml)*	809 (542)	817 (692)	0.796	802 (508)	810 (684)	0.823
Reoperation for bleeding	21 (2.9)	23 (3.2)	0.731	19 (3.1)	19 (2.8)	0.719
Mediastinitis	10 (1.4)	5 (0.7)	0.203	9 (1.5)	5 (0.7)	0.197
30-day Mortality	7 (1.0)	8 (1.1)	0.779	6 (1.0)	8 (1.2)	0.747
Duration of postoperative hospital days*	5.99 (3.95)	6.07 (4.21)	0.710	5.81 (3.44)	6.01 (4.23)	0.362

Values in parentheses are percentages unless indicated otherwise;
* values are means (SD).
[a] Mann-Whitney U test.
FFP, Fresh Frozen Plasma; ICU, Intensive Care Unit; RBC, Red Blood Cells.

Subgroup analysis

Analysis of variance or logistic regression analysis for the primary efficacy outcomes revealed only significant treatment x subgroup interaction effects for the subgroup variable use of CPB. For this subgroup variable, significant interaction

effects were found for the proportion of patients transfused with FFP (*P*= 0.027), units of transfused PCs (*P*= 0.038), the proportion of patients transfused with PCs (*P* = 0.014) and length of ICU stay (*P* = 0.035). In the subgroup of patients operated on off-pump, significant differences between treatment groups were found for the proportion of patients transfused with FFP (*P* = 0.020), units of transfused PCs (*P* = 0.036) and the proportion of patients transfused with PCs (*P* = 0.014) in the ITT analysis. In the PP analysis, however, these significant differences disappeared. No significant differences between the treatment groups were found in the on-pump CABG subgroup.

Safety

Except for the duration of mechanical ventilation exceeding 48 h in the ITT analysis (*P* = 0.032), there were no significant differences between the groups in the occurrence of SAEs and AEs (Table 3).

Economic evaluation

Of 722 patients randomized to CryoSeal, 642 (88%) actually received this intervention. Estimated CryoSeal costs were €822 (95% CI €808 to €836) per patient. Other cost categories showed no statistically significant differences, in line with the lack of impact that CryoSeal use had on clinical outcome (Table 4). The overall cost difference was estimated at €461 (95% CI €-247 to €1170) per patient.

Table 3. Serious Adverse Events and Adverse Events.

Variable	Intention-to-treat analysis				Per-protocol analysis			
	CryoSeal (n = 722)	No CryoSeal (n = 714)	OR (95% CI)	*P*-value	Cryoseal (n = 609)	No CryoSeal (n = 683)	OR (95% CI)	*P*-value
Serious Adverse Events								
Myocardial infarction	18 (2.5)	26 (3.6)	0.68 (0.37-1.24)	0.208	14 (2.3)	24 (3.5)	0.65 (0.33-1.26)	0.199
Stroke	4 (0.6)	3 (0.4)	1.32 (0.29-5.91)	0.718	4 (0.7)	3 (0.4)	1.50 (0.33-6.71)	0.599
Mediastinitis	10 (1.4)	5 (0.7)	1.99 (0.68-5.85)	0.212	9 (1.5)	5 (0.7)	2.03 (0.68-6.09)	0.197
Reoperation for ischemia	2 (0.3)	4 (0.6)	0.49 (0.09-2.70)	0.451	2 (0.3)	4 (0.6)	0.56 (0.10-3.07)	0.503
30-day Mortality	7 (1.0)	8 (1.1)	0.86 (0.31-2.40)	0.779	6 (1.0)	8 (1.2)	0.84 (0.29-2.43)	0.747
Adverse Events								
Low cardiac output syndrome	15 (2.1)	18 (2.5)	0.82 (0.41-1.64)	0.573	13 (2.1)	18 (2.6)	0.81 (0.39-1.66)	0.555
Ventilation time >48 hours	12 (1.7)	3 (0.4)	4.00 (1.12-14.24)	0.032	7 (1.1)	3 (0.4)	2.63 (0.68-10.22)	0.162
Sepsis	2 (0.3)	2 (0.3)	0.99 (0.14-7.03)	0.990	1 (0.2)	2 (0.3)	0.56 (0.05-6.18)	1.000
Pneumonia	16 (2.2)	15 (2.1)	1.06 (0.52-2.15)	0.884	11 (1.8)	13 (1.9)	0.95 (0.42-2.13)	0.894
Renal insufficiency	19 (2.6)	13 (1.8)	1.46 (0.71-2.97)	0.302	16 (2.6)	13 (1.9)	1.39 (0.66-2.91)	0.385
Atrial fibrillation	212 (29.4)	213 (29.9)	0.98 (0.78-1.23)	0.845	167 (27.5)	201 (29.5)	0.91 (0.71-1.16)	0.427
Ventricular fibrillation	11 (1.5)	4 (0.6)	2.75 (0.87-8.67)	0.085	9 (1.5)	4 (0.6)	2.55 (0.78-8.31)	0.121
Transient ischemic accident	0	3 (0.4)	-	0.122[a]	0	3 (0.4)	-	0.252[a]
Wound infection, superficial	5 (0.7)	9 (1.3)	0.55 (0.18-1.64)	0.279	3 (0.5)	9 (1.3)	0.37 (0.10-1.37)	0.137
Reoperation for bleeding	21 (2.9)	23 (3.2)	0.90 (0.49-1.64)	0.731	19 (3.1)	19 (2.8)	1.13 (0.59-2.15)	0.719

Values in parentheses are percentages. [a]Fisher's Exact test. CI, Confidence Interval; OR, Odds ratio

Table 4: Estimated postoperative hospital costs (by randomization and intention-to-treat anlaysis).

	Volumes of care		Costs (€)		
	CryoSeal (n=722)	No CryoSeal (n=714)	CryoSeal (n=722)	No CryoSeal (n=714)	*P*-value
Use of CryoSeal	642 (89)	6 (1)	822	0	<0.001
Blood products					
RBC	181 (25.1)	180 (25.2)	1,200	1,208	0.896
FFP	95 (13.2)	93 (13.0)	443	459	0.342
Platelets	119 (16.5)	116 (16.3)	1,123	1,141	0.380
Proportion of transfused patients	252 (34.9)	253 (35.5)			
Hospitalization (days) *					
ICU care	1.32	1.45	3,011	3,307	0.272
Non-ICU care	7.04	7.09	3,455	3,479	0.818
Interventions during hospitalization					
Reoperation for ischemia	2 (0.3)	4 (0.6)	11	22	0.407
Reoperation for bleeding	21 (2.9)	23 (3.2)	58	64	0.731
Mediastinitis	10 (1.4)	5 (0.7)	42	21	0.201
Total costs (€)			10,163	9,702	0.202

Values in parentheses are percentages unless indicated otherwise; * values are means. RBCs, red blood cells; FFP, fresh frozen plasma; ICU, intensive care unit.

Whether use of CryoSeal in CABG is cost-effective depends on the willingness to pay to avoid blood transfusion. Use of CryoSeal resulted a 0.6% estimated decrease in the proportion of transfused patients (from 35.5% to 34.9%), which translates the estimated cost difference to €72,000 per avoided transfusion (with unbounded 95%CI). Figure 2 shows the probability that use of CryoSeal is cost-effective compared with no CryoSeal, conditional on the willingness-to-pay per avoided transfusion. For willingness-to-pay up to €10,000 per avoided transfusion, CryoSeal is, at most, 21% likely to be cost-effective. Restricting the cost analysis to only the costs of CryoSeal (the only significantly different cost category), CryoSeal use is even less likely to be cost-effective (Figure 2).

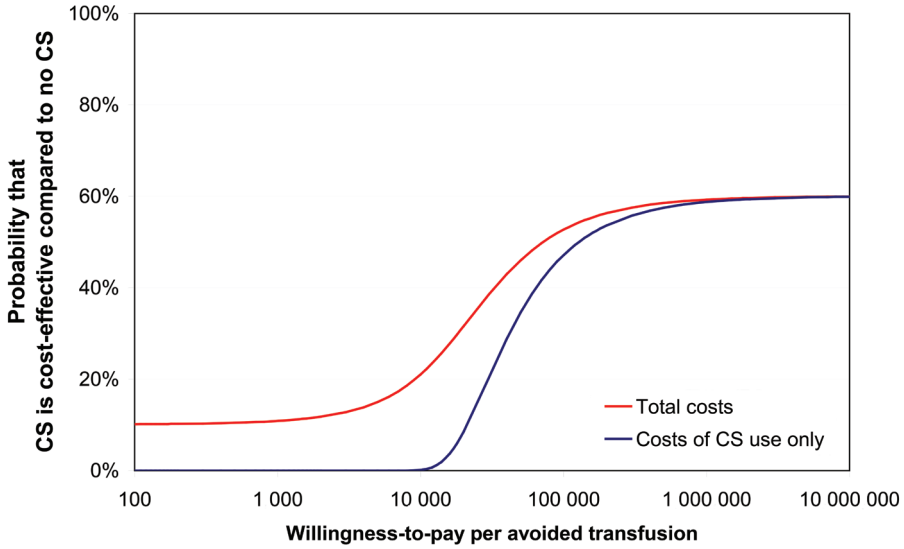

Figure 2. Cost-effectiveness acceptability curves: probability that CryoSeal is cost-effective compared to no CryoSeal, conditional on the willingness-to-pay per avoided transfusion.

DISCUSSION

In this multicentre study performed in the Netherlands, use of the fibrin sealant CryoSeal as an adjunct to achieve hemostasis in patients undergoing elective isolated CABG with ITA grafting was not associated with a reduction in blood transfusion. The numbers of transfused units of RBCs, FFP and PCs were not significantly different between the patients randomized for treatment with CryoSeal and patients randomized to no fibrin sealant. The duration of ICU stay and the secondary efficacy outcomes also showed no significant differences between the two treatment groups. In line with this lack of impact of CryoSeal on outcomes, there was no significant difference between the groups on any of the predefined cost categories (blood product usage, hospitalization, reoperation for ischemia or bleeding, and treatment of mediastinitis) other than the costs of the sealant itself.

The sample size calculation was based on a treatment effect of a 50% reduction in RBC transfusion. The control group, however, showed a mean number of only

0.6 RBC units transfused per patient. The mean numbers of units of transfused FFP and PCs were even lower. Studies on blood transfusion usage in CABG continue to show a considerable variability. An average of approximately 2 units of RBCs transfused per patient has been reported in studies (21-23) from different continents, whereas the mean blood loss in these studies appeared to be similar to that in the present study. This obvious difference in transfusion practice in CABG may reflect an enhanced implementation of blood conservation techniques in the Netherlands. Here, intraoperative blood salvage and blood-sparing interventions as well as restricted blood transfusion algorithms have become common practice recently. This raises the question how the complementary use of a fibrin sealant could be cost-effective when already effective blood conservation strategies are used. It should be noted, though, that the effectiveness of the CryoSeal could also not be demonstrated by comparing postoperative blood loss between the treatment groups in this study.

Risk factors for postoperative blood loss and blood transfusion in CABG include female sex, use of antiplatelet drugs within 5 days before surgery, use of CPB and use of bilateral ITA (24,25). Planned subgroup analyses were undertaken in subgroups based on these four variables. Only in the subgroup of patients who had surgery off-pump did the ITT analysis show significant differences in blood transfusion between the treatment groups, in favour of CryoSeal. This potential effectiveness of the fibrin sealant in off-pump CABG procedures might be explained by the fact that during CPB the coagulation profile is more severely affected. CPB is known to be associated with hemodilution by the pump prime, resulting in anemia, reduced fibrinogen and platelet levels, and platelet dysfunction (26). Furthermore, an increase in thrombin formation has been described after the release of the aortic cross-clamp by liberating blood from the myocardium and pulmonary vascular bed (27). The coagulation profile in on-pump patients might be so disturbed that CryoSeal use cannot compensate. In off-pump patients, on the other hand, the coagulation profile is changed minimally changed (28), and the use of fibrin sealant may have contributed to preventing postoperative hemorrhagic complications. It should be noted, however, that the significant effects in the ITT analysis were not confirmed by the PP analysis.

The use of fibrin sealants in cardiac surgery is in generally considered to be safe, and their use has increased substantially since their commercial introduction 20 years ago. Recently, however, concerns about early graft occlusion after CABG have been reported based on clinical studies. In these studies (29,30), an increased risk of myocardial infarction and even 30-day mortality after the use of Tissucol® fibrin sealant (Baxter, Vienna, Austria) was observed. These deleterious effects were thought to be mainly associated with an inadequate application of Tissucol®, insufficient mixing of its components and spray application directly on bypass graft anastomoses. The retrospective character of these studies, however, precluded a definitive conclusion on the safety of Tissucol®. Although safety was not the main purpose of the current study, no significant differences were found between the treatment groups in the occurrence of SAEs and AEs, except that with CryoSeal the duration of mechanical ventilation more frequently exceeded 48 h in the ITT analysis.

Related specifically to the use in CABG, the application site of fibrin sealant and the amount given are often not clearly documented in the literature. In the present study a standard treatment regimen was followed, requiring 5 ml CryoSeal per ITA bed. The rationale for this was that the ITA graft is a risk factor for hemorrhage after CABG. It has been reported (31) that, in patients having an ITA harvested, the ITA or its bed was the main cause of bleeding in 43%. Not only has single ITA grafting been linked to an increased bleeding risk compared to the use of only saphenous vein grafts (10), but also bilateral ITA grafting increases the risk of bleeding compared to single ITA grafting (11). Furthermore, based on our pilot study, it was established that the application of 5 ml of CryoSeal was the optimal amount to cover the entire ITA bed.

Whether a strategy in patient blood management is cost-effective depends on willingness to pay to avoid transfusion. The present costs analysis was performed from a hospital perspective, including costs of CryoSeal, blood products, hospital stay and complications related to postoperative bleeding. Prices vary by country and by centre; the present study prices for blood products and hospitalization that are specific tot the Dutch context, which may not be representative for elsewhere. Yet, our study showed no differences between the treatment groups on the respec-

tive types of care; therefore, the conclusion of our analysis would not change when other prices were used. For the CryoSeal fibrin sealant, a price was used that may underestimate the true costs: it included a realistic price for the product itself, but only a relatively small surcharge of €30 to account for the approximately 2 min additional surgery time. Implementation and other in-hospital handling costs were neglected, so the economic evaluation is biased in favour of CryoSeal. For reasonable willingness-to-pay per avoided transfusion, the fibrin sealant CryoSeal is unlikely to be cost-effective.

A limitation of this study was the lack of blinding with the possible risk of surgeons' performance bias. This limitation seems unavoidable, as the use of a placebo fibrin sealant is hardly feasible. However, to minimize the influence on routine hemostasis, a cooling box was used in every patient which was instructed to open only until 30 min before the end of surgery. Furthermore, although number of blood transfusions required is an objective efficacy measure, it is influenced by transfusion policy. Using a restrictive transfusion policy, as has become common practice in the Netherlands, effectiveness of CryoSeal treatment may not be detected. However, even if blood loss was used as an endpoint in this study, there would be no evidence for the effectiveness of CryoSeal.

There is limited research on fibrin sealants in cardiac surgery. The majority of controlled studies suggest that fibrin sealants are efficacious in reducing postoperative blood loss and RBC transfusion. However, previous large studies in this area were retrospective, and prospective studies had small numbers (32). Evidence of efficacy is only a first step in evaluating whether fibrin sealants are appropriate for clinical use (33). So far there are no large randomized controlled studies on the cost-effectiveness of a fibrin sealant in CABG.

Conclusion

This multicentre randomized controlled clinical trial demonstrated that the use of the fibrin sealant CryoSeal in elective isolated CABG procedures was not cost-effective. There were no health benefits. In combination with the high costs per avoided transfusion, this study does not support the implementation of routine CryoSeal use in elective isolated CABG.

COLLABORATORS

Local Principal Investigators in the FIBER study were: G. Tavilla (Leiden University Medical Centre, Leiden; 565 randomized patients; Radboud University Medical Centre; 275 patients), R.C.A. Meijer (University Medical Centre Utrecht; 199 patients); M.A. Keyhan-Falsafi (Haga Hospital, The Hague; 179 patients), P.S. Eggens, M. Bentala (Amphia Hospital, Breda; 141 patients), J.G. Maessen (University Hospital Maastricht; 77 patients), and R. Cocchieri (Academic Medical Centre, Amsterdam: 9 patients).

Members of the DSMB were: H.R. Büller (chair); R.J. de Haan, and A.P. Kappetein. For interim-analysis only: R. Brand (statistician).

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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CHAPTER 7

Continuous postoperative pericardial flushing: A pilot study on safety, feasibility and effect on blood loss

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ABSTRACT

Background

Prolonged or excessive blood loss is a common complication after cardiac surgery. Blood remnants and clots, remaining in the pericardial space in spite of chest tube drainage, induce high fibrinolytic activity that may contribute to bleeding complications. Continuous postoperative pericardial flushing (CPPF) with an irrigation solution may reduce blood loss by preventing the accumulation of clots. In this pilot study, the safety and feasibility of CPPF was evaluated and the effect on blood loss and other related complications were investigated.

Methods

Between November 2011 and April 2012 twenty-one adult patients undergoing surgery for congenital heart disease (CHD) received CPPF from sternal closure up to 12 h postoperative. With an inflow Redivac drain that was inserted through one of the chest tube incision holes, an irrigation solution (NaCl 0.9% at 38 ºC) was delivered to the pericardial cavity using a volume controlled flushing system. Safety aspects, feasibility issues and complications were registered. The mean actual blood loss in the CPPF group was compared to the mean of a retrospective group (n = 126).

Results

CPPF was successfully completed in 20 (95.2%) patients, and no method related complications were observed. Feasibility was good in this experimental setting. Patients receiving CPPF showed a 30% (*P*=0.038) decrease in mean actual blood loss 12 h postoperatively.

Conclusions

CPPF after cardiac surgery was found to be safe and feasible in this experimental setting. The clinically relevant effect on blood loss needs to be confirmed in a randomized clinical trial.

INTRODUCTION

Prolonged or excessive bleeding is one of the most common complications after cardiac surgery. Postoperative bleeding requiring transfusions and surgical re-exploration remains an important complication because it is associated with short- and long-term postoperative mortality, morbidity, prolonged hospitalization and higher societal healthcare costs (1).

The mechanisms involved in perioperative bleeding are complex and involve disturbances in various physiologic systems including primary hemostasis, coagulation and fibrinolysis. This may be caused by several surgical factors including cardiopulmonary bypass (CPB) and operative trauma. Together with primary fibrinolysis, platelet dysfunction and hemodilution these mechanisms contribute to dysfunction of the coagulation, fibrinolytic, and inflammatory systems with postoperative coagulopathy and bleeding as a result (2,3). Consequently, the normal or generally accepted amount of blood loss after cardiac surgery is higher than most other surgical specialties and varies between 300-1500 ml during the first 12 h.

The standard operating procedure is to insert chest tubes in order to evacuate this blood from the pericardial cavity postoperatively. However, if blood loss or clot formation is excessive the chest tubes often fail due to partial or complete blockage. The resulting stasis of blood and clots in the pericardial cavity leads to high fibrinolytic activity and consequently, maintenance of blood loss (3-7). This is also supported by the finding that during re-exploration for postoperative bleeding, removal of accumulated blood and clots by solely irrigating the pericardial space with a warm saline solution is enough to stop the bleeding instantly in a significant number of cases[8]. Following on from this, a method of preventing blood and clots from accumulating in the pericardial space could hypothetically stop postoperative bleeding at an earlier stage and reduce bleeding complications. Continuous postoperative pericardial flushing (CPPF) was developed for this purpose.

CPPF works by continuously flushing the pericardial cavity with a warm saline irrigation solution starting towards the end of surgery just before sternal closure. Continuous flushing will result in a lower viscosity mixture that will prevent chest

tube blockages and promote the evacuation of blood and clots from the pericardial cavity. This pilot study evaluates the safety and feasibility of CPPF. In addition, its effects on blood loss and other related complications are investigated and discussed. To our knowledge, the continuous pericardial flushing method post-cardiac surgery has not been used or described before.

LIST OF ABBREVIATIONS

ACT	Activated clotting time
AMC	Academic Medical Centre, Amsterdam, The Netherlands
CHD	Congenital heart disease
CPB	Cardiopulmonary bypass
CPPF	Continuous postoperative pericardial flushing
CRP	C-reactive protein
FFP	Fresh frozen plasma
ICU	Intensive care unit
IU	International unit
MCTD	Mediastinal chest tube drainage
METC	Medical ethics committee
PRBC	Packed red blood cell
TTE	Transthoracic echocardiogram

METHODS

Study design

In this pilot study, a prospective cohort of patients who underwent surgery and CPPF between November 2011 and April 2012 (n = 21, over a 6-month inclusion period) was compared to a retrospective group of patients who underwent surgery and no CPPF (non-CPPF group) between January 2010 and December 2011 (n = 126). The study protocol was approved by the internal review board of the Aca-

demic Medical Centre (AMC) Amsterdam, protocol number METC 2011_270. All adult patients undergoing surgical correction for congenital heart disease (CHD) were eligible to participate in this prospective cohort study. Exclusion criteria were emergency surgery, a history of bleeding diathesis or coagulopathy, participation in any study involving an investigational drug or device and the inability to understand the study information or give informed consent. Informed consent was obtained one day preoperatively from all patients in the CPPF group and data were gathered prospectively. Data from the non-CPPF group were obtained retrospectively by analysing consecutive patient records before the use of CPPF for a period of 24 months. All patients underwent cardiac surgery at the AMC, which is a quaternary care university hospital. A single surgeon performed surgery on all included patients in the CPPF group and almost all of the patients in the non-CPPF.

Continuous Postoperative Pericardial Flushing

The method of inserting chest tubes in the CPPF group was the same as routinely used in the non-CPPF group; one chest tube (Ch30 Redivac drain, Medica Europe, Oss, The Netherlands) in the pericardial space, one chest tube (Ch30 Redivac drain, Medica Europe, Oss, The Netherlands) in the anterior mediastinum and in case of opened pleural spaces they were each drained separately (Ch30 Redivac drain, Medica Europe, Oss, The Netherlands). In the CPPF group an extra infusion tube (Ch10 Redivac drain, Dispo Medical, Hattemerbroek, The Netherlands) was inserted through the incision hole of one of the standard chest tube incisions and positioned in the pericardial space. This extra infusion tube was directly connected to the CPPF system that comprises a bag of irrigation solution (NaCl 0,9%) connected to infusion line that runs through a volumetric pump (Infusomat® Space by B. Braun, Melsungen, Germany) and through a fluid heating device (Enflow® fluid warmer by GE Healthcare, Hoevelaken, The Netherlands). CPPF was started at sternal closure and continued for 12 h postoperatively. The irrigation solution NaCl 0.9%, was delivered to the pericardial cavity at a flow rate of 500 ml/h for the first two postoperative hours. It was then set to volume controlled for the next ten hours so that the volume corresponded with the patients' actual blood loss at a 1:1 ratio, with a minimum flow rate of 100 ml/hour. The irrigation solution was delivered to

the patient at a constant temperature of 38 ºC to avoid changes in patient core temperature. Irrigation solution volume and total mediastinal chest tube drainage (MCTD) volume were monitored every 15 min for the first two postoperative hours and thereafter hourly until chest tube removal. Actual blood loss was calculated at the same time intervals by subtracting irrigation solution volume from the total MCTD volume. In this way, a secondary manually written record was kept to monitor actual blood loss and trace fluid accumulation. If MCTD volume was >200 ml less than the infused irrigation solution volume, the CPPF system was stopped to prevent accumulation of fluid in the pericardial and/or pleural spaces. One and the same research assistant facilitated both preparation of the CPPF system and the safeguarding and control of the system during the CPPF window.

Operative procedures and cardiopulmonary bypass management

Routine anesthetic procedures were employed making circumstances standardized and equal for both groups. All patients underwent full median sternotomy; CPB (Stöckert S5, Sorin Group, Italy) was instituted in all patients using mild hypothermia (30-32 ºC). Before initiation of CPB, all patients received a standard heparin dosage of 150 IU/kg bodyweight with an additional optional bolus of 50 IU/kg before cannulation. Anticoagulation was monitored by serial measurements of the activated clotting time (ACT), which was kept above 450 s at all time during CPB. The heparin was reversed by administration of 1ml protamine for each 1000 IU heparin and ACT return to baseline served as confirmation.

Transfusion policies

A standardized transfusion protocol was followed in all patients. Intraoperative blood management included reinfusion of residual blood from the cardiotomy reservoir and packed red blood cell (PRBC) transfusion during CPB were given at hemoglobin levels <4.0 mmol/l. Postoperative blood management on the ICU included PRBC transfusions for hemoglobin level <5.0 mmol/l; platelet concentrate for platelet count $<50\times10^9$/l or $<100\times10^9$/l if blood loss exceeded 150 ml/h and fresh frozen plasma (FFP) was transfused if the activated partial thromboplastin time and prothrombin time were prolonged >150% during active bleeding.

Primary end-points

The primary end-points of this pilot study were to evaluate the safety and feasibility of CPPF procedure and its effect on actual blood loss. Safety end-points during hospitalization were defined as chest tube competence, pericardial and/or pleural fluid accumulation, infection and adverse events. Adverse events between discharge from hospital until most recent follow-up were also recorded. Feasibility end-points were defined as system functionality and labour-intensiveness. Transthoracic echocardiograms and chest radiographs were the imaging modalities of choice that were used to evaluate pericardial and pleural effusions on arrival in ICU, at 5-7 days postoperatively and on discharge. Pericardial or pleural effusions that were clinically significant and required drainage were registered.

Secondary end-points

Secondary end-points included transfusion requirements, time to chest tube removal, time to extubation and length of ICU and hospital stay. Transfusion requirements were defined as total PRBC, total FFP and total platelet concentrate transfusions as well as the proportion of patients requiring blood product transfusion. The time frames between operation and chest tube removal and between operation and extubation were recorded in postoperative hours.

Statistical analysis

Variables are presented as mean (± standard deviation) or as number (percentage), unless otherwise noted. An independent-samples t-test was conducted to compare mean actual blood loss between the CPPF and non-CPPF group; a $P<0.05$ was considered statistically significant. All data analyses were performed using SPSS 20.0 for Macintosh (IBM® SPSS® Software). Mean actual blood loss curves were plotted using GraphPad Prism® 6.0 software for Macintosh.

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RESULTS

Demographic and preoperative clinical data

Preoperatively we registered there were two significant differences between the CPPF and the non-CPPF group of patients with respect to demographic and clinical characteristics as shown in Table 1.

Table 1. Clinical characteristics of the CPPF and non-CPPF group.

	CPPF	non-CPPF	P-value
	n=21 (%)	n=126 (%)	
Mean age (y ± SD)	43.8 ± 13.6	40.5 ± 15.0	0.353
Male / Female	11 / 10	73 / 53	0.637
Body mass index (mean ± SD, (kg/m^2))	27.5 ± 7.0	24.3 ± 4.6	0.057
Diagnoses:			
Tetralogy of Fallot	6 (28.6)	14 (11.1)	0.109
Transposition of the Great Arteries	1 (4.8)	8 (6.3)	0.781
Univentricular heart	0 (0.0)	3 (2.4)	0.478
Connective tissue disease	0 (0.0)	16 (12.7)	< 0.001 *
Fabry syndrome	1 (4.8)	1 (0.8)	0.420
Factor V Leiden	1 (4.8)	1 (0.8)	0.420
Associated diseases:			
BMI >30 kg/m^2	5 (23.8)	11 (8.7)	0.142
Diabetes	1 (4.8)	4 (3.2)	0.518
Renal insufficiency (at least moderate)	6 (28.6)	18 (14.3)	0.189
Chronic obstructive lung disease	2 (9.5)	7 (5.6)	0.486
Urgent/emergency surgery	0 (0.0)	1 (0.8)	0.685
Left ventricular ejection fraction:			
>50%	14 (66.6)	106 (84.1)	0.127
30-50%	7 (33.3)	18 (14.3)	0.096
<30%	0 (0.0)	2 (1.6)	0.564
Euroscore I (logistic) (mean ± SD)	6.14 ± 7.27	7.51 ± 6.67	0.389
Euroscore II (mean ± SD)	2.90 ± 2.97	2.84 ± 2.63	0.916
Preoperative anticoagulant use: [a]			
Acetylsalicylic acid	6 (28.6)	23 (18.3)	0.274
Vitamin K antagonists	4 (19.0)	10 (7.9)	0.235
Other	0 (0.0)	1 (0.8)	0.685

Table 1. Clinical characteristics of the CPPF and non-CPPF group. (continued)

	CPPF	non-CPPF	P-value
	n=21 (%)	n=126 (%)	
Preoperative laboratory values:			
Hemoglobin (mmol/l)	8.8 ± 0.7	8.9 ± 0.9	0.831
CRP (mg/l)	4.0 ± 4.4	3.6 ± 6.4	0.903
Leukocytes ($\times 10^9$/l)	7.5 ± 2.1	6.4 ± 1.7	0.009 *
Platelet count ($\times 10^9$/l)	221 ± 70	219 ± 55	0.869
INR (median;Q1;Q3;IQR)	1.08 (0.98;1.62;0.64)	0.99 (0.96;1.08;0.12)	0.160

[a] Use of all antiplatelet agents was discontinued 5 days prior to surgery. CRP, C-reactive protein; INR, International normalized ratio.

Surgical procedures and operative data

On comparison with the CPPF group patients in the non-CPPF group had undergone significantly more procedures for left-sided lesions (*P*=0.030), aortic root surgery (*P*<0.001), aortic valve replacement (*P*=0.008) and Bentall procedure (*P*=0.008). Patients in the CPPF group underwent significantly more procedures for right-sided lesions (*P*<0.001). The complexity of surgical procedures is shown in Table 2 . Intraoperative variables such as the duration of CPB and aortic cross-clamping were similar in both groups and are presented in Table 3.

CPPF safety and feasibility

Postoperative safety aspects of the CPPF group and historic group of patients are summarized in Table 4. CPPF was successfully completed in 20 (95.2%) patients. In 1 (4.8%) patient, CPPF was stopped as a precaution 3.5 h postoperatively as there was a >200 ml lag in MCTD volume as fluid had accumulated in the right pleural cavity. On extubation the patient spontaneously evacuated all accumulated fluid, which was confirmed by chest radiography. All irrigation solution was successfully evacuated from the pericardial and/or pleural cavities in all patients before the chest tubes were removed. There were no significant differences between groups in pleural effusions (*P*=0.272) or pericardial effusions (*P*=0.486) at discharge. Postoperative inflammatory markers were not significantly different between groups.

Table 2. Surgical procedures of the CPPF and non-CPPF group.

	CPPF	non-CPPF	P-value
	n=21 (%)	n=126 (%)	
Aortic surgery:			
AVP	0 (0.0)	1 (0.8)	0.685
AVR +Asc. Repl. (0|2); +PVR (0|1); +MVP,PVI (0|1); +VSD repair (0|1)	0 (0.0)	7 (5.6)	0.008 *
Bentall +PVR (1|4); +Hemiarch Repl. (0|1); +MVP,TVP (0|3); +VSD repair (0|2)	1 (4.8)	27 (21.4)	0.008 *
VSRR +AVP (0|3); +MVP (0|2)	1 (4.8)	20 (15.9)	0.061
Ascending aorta replacement +MVP (0|1)	0 (0.0)	4 (3.2)	0.411
Hemiarch replacement + Ductus closure	0 (0.0)	1 (0.8)	0.685
Atrioventricular valve surgery:			
MVP +PVI (1|3); +TVP (0|6); +VSD repair (0|2)	1 (4.8)	12 (9.5)	0.480
MVR	0 (0.0)	5 (4.0)	0.356
TVP	0 (0.0)	4 (3.2)	0.411
Septal defects:			
ASD I,MVP,TVP	2 (9.5)	1 (0.8)	0.201
ASD II +MVP,TVP (2|1); +TVP (1|3); +PVP (0|1)	2 (9.5)	6 (4.8)	0.377
VSD +TVP (1|1); +DCRV (1|0)	2 (9.5)	3 (2.4)	0.298
Pulmonary venous anomalies:			
PAPVC repair +ASD (1|4); +TVP (1|1)	2 (9.5)	5 (4.0)	0.422
Coronary artery anomalies:			
ALCAPA / ARCAPA repair +PA plasty (0|2); +CABG (0|1)	2 (9.5)	3 (2.4)	0.298
Other:			
PVR +PA plasty (5|16); +TVP (1|4); +MVP (1|0)	7 (33.3)	20 (15.9)	0.127
AP plasty + Hybrid stent placement	0 (0.0)	1 (0.8)	0.685
DSAS repair / Morrow	0 (0.0)	1 (0.8)	0.685
Atrial baffle + TVP	1 (4.8)	2 (1.6)	0.344
TCPC	0 (0.0)	3 (2.4)	0.478

ALCAPA, anomalous left coronary artery from the pulmonary artery; ARCAPA, anomalous right coronary artery from the pulmonary artery; Asc, ascending aorta; ASD, atrial septal defect; AVP, aortic valve plasty; DCRV, double-chambered right ventricle; DSAS, discrete subaortic stenosis; MVP, mitral valve plasty; MVR, mitral valve replacement; PA, pulmonary artery; PAPVC, partial anomalous pulmonary venous connection; PVI, pulmonary vein isolation; PVP, pulmonary valve plasty; PVR, pulmonary valve replacement; Repl, replacement; TCPC, total cavopulmonary connection; TVP, tricuspid valve plasty; VSD, ventricular septal defect.

Table 3. Operative data of the CPPF and non-CPPF group.

	CPPF	non-CPPF	P-value
	n=21 (%)	n=126 (%)	
Reoperation	10 (47.6)	57 (45.2)	0.841
Left sided lesions	9 (42.9)	85 (67.5)	0.030 *
Right sided lesions	17 (81.0)	56 (44.4)	< 0.001 *
Aortic root surgery	2 (9.5)	65 (51.6)	< 0.001 *
Septal defects	6 (28.6)	21 (16.7)	0.274
Pulmonary venous anomalies	2 (9.5)	5 (4.0)	0.422
Coronary artery anomalies	2 (9.5)	3 (2.4)	0.298
Single procedure	8 (38.1)	63 (50.0)	0.320
Double procedure	11 (52.4)	43 (34.1)	0.110
Triple procedure	1 (4.8)	18 (14.3)	0.103
Quadruple procedure	1 (4.8)	2 (1.6)	0.344
Mean surgical procedures per patient	1.76	1.67	0.230
Mean CPB time (min) ± SD	140 ± 72	151 ± 63	0.443
Mean cross-clamp time (min) ± SD	86 ± 41	101 ± 46	0.186

No system-related or other serious problems were encountered. Monitoring of inflow and outflow volumes was considered time-consuming but feasible in this experimental setting where the monitoring was safeguarded and controlled by a research assistant. However, it was considered to be a major drawback for normal clinical setting as it would be a significant increase in workload for the ICU nurse and repeated calculation will be error prone. Especially in case of bleeding problems when a more frequent monitoring and calculation of actual blood loss is required. Also, the normal clinical assessment of blood content of MCTD is disturbed as the blood is constantly diluted in varying degrees.

Table 4. Postoperative safety aspects of the CPPF and non-CPPF group.

	CPPF	non-CPPF	P-value
	n=21 (%)	n=126 (%)	
In-hospital adverse events:			
Cardiac tamponade	0 (0.0)	4 (3.2)	0.411
Reexploration for bleeding	0 (0.0)	9 (7.1)	0.002 *
Subxyphoidal drainage	0 (0.0)	3 (2.4)	0.478
Mortality	0 (0.0)	2 (1.6)	0.564
In-hospital infection:			
Sternal wound infection	0 (0.0)	3 (2.4)	0.478
Mediastinitis	0 (0.0)	2 (1.6)	0.564
Pneumonia	1 (4.8)	3 (2.4)	0.538
Urine tract infection	1 (4.8)	1 (0.8)	0.420
Fever > 38,5°C	2 (9.5)	9 (7.1)	0.703
max. CRP (mg/l, median;Q1;Q3;IQR)	138 (95;200;105)	182 (136;231;95)	0.057
max. Leukocytes (×109/l, median;Q1;Q3;IQR)	13.5 (11.1;15.9;4.8)	12.6 (10.7;15.8;5.1)	0.867
In-hospital data: [a]			
Time until extubation (h ± SD)	7.6 ± 6.1	6.8 ± 5.2	0.535
Time until chest tube removal (h ± SD)	21 ± 8	22 ± 14	0.762
ICU stay (d ± SD)	1.4 ± 0.9	1.7 ± 3.8	0.746
Total hospitalization (d ± SD)	7.7 ± 2.4	9.0 ± 8.6	0.472
Fluid accumulation at discharge:			
Pleural effusion (trace to mild)	10 (47.6)	77 (61.1)	0.272
In a surgically opened pleural cavity	5 (23.8)	23 (18.3)	0.563
Pericardial effusion (trace to mild)	7 (33.3)	38 (30.2)	0.486
Circular (≥50% / ≥6mm)	1 (4.8)	10 (7.9)	0.606
Adverse events after discharge:			
Late cardiac tamponade	1 (4.8)	3 (2.4)	0.477
for which subxyphoidal drainage	1 (4.8)	2 (1.6)	0.344
for which re-sternotomy	0 (0.0)	1 (0.8)	0.685
Reoperation	1 (4.8)	4 (3.2)	0.718
3-year mortality	0 (0.0)	6 (4.8)	0.014 *
Mean follow-up (y ± SD)	2.9 ± 0.1	4.1 ± 1.0	< 0.001 *

[a] Time until extubation and chest tube removal were defined as the mean number of hours between surgery and the time of removal. Length of ICU and hospital stay were defined as the mean number of days between the date of surgery and the date of ICU and hospital discharge, respectively.

Effect on mean actual blood loss

Differences in mean actual blood loss on arrival at ICU, at 6 and 12-h postoperatively, and the total postoperative blood loss between the CPPF group and non-CPPF group are presented in Table 5 . The magnitudes of the significant decrease in means were 41% (mean difference = 57 ml, 95% CI: 12 to 102) on arrival at ICU and 30% (mean difference = 162 ml, 95% CI: 9 to 315) at 12 h postoperatively; both had a moderate effect (eta squared = 0.04 and 0.03 respectively). Within the non-CPPF group, those who underwent aortic root surgery had 65 ml (*P*=0.286) less blood loss at 12 h postoperatively compared to the non-aortic root surgery group. Postoperative mean actual blood loss over hourly intervals and the cumulative total over the first 12 postoperative hours is shown in Figure 1.

Table 5. Blood loss and PRBC transfusion requirements of the CPPF and non-CPPF group.

	CPPF	non-CPPF	P-value
	n=21 (%)	n=126 (%)	
Postoperative blood loss: (mean ± SD)			
T0: ICU arrival	83 ± 74	140 ± 100	0.014 *
T6: six hours postoperative	310 ± 195	415 ± 297	0.131
T12: twelve hours postoperative	376 ± 249	538 ± 330	0.038 *
Total postoperative	608 ± 422	768 ± 630	0.263
PRBC transfusion requirements:			
Patients transfused PRBC intraoperatively	7 (33.3)	38 (30.2)	0.772
Patients transfused PRBC postoperatively	1 (4.8)	19 (15.0)	0.080

Secondary end-points

Differences in allogeneic transfusion requirements between the two groups was not statistically significant as shown in Table 5 . No significant differences in means between the two groups were found with respect to the time to extubation (*P*=0.535), time to chest tube removal (*P*=0.762), length of ICU stay (*P*=0.746), and total hospitalization (*P*=0.472).

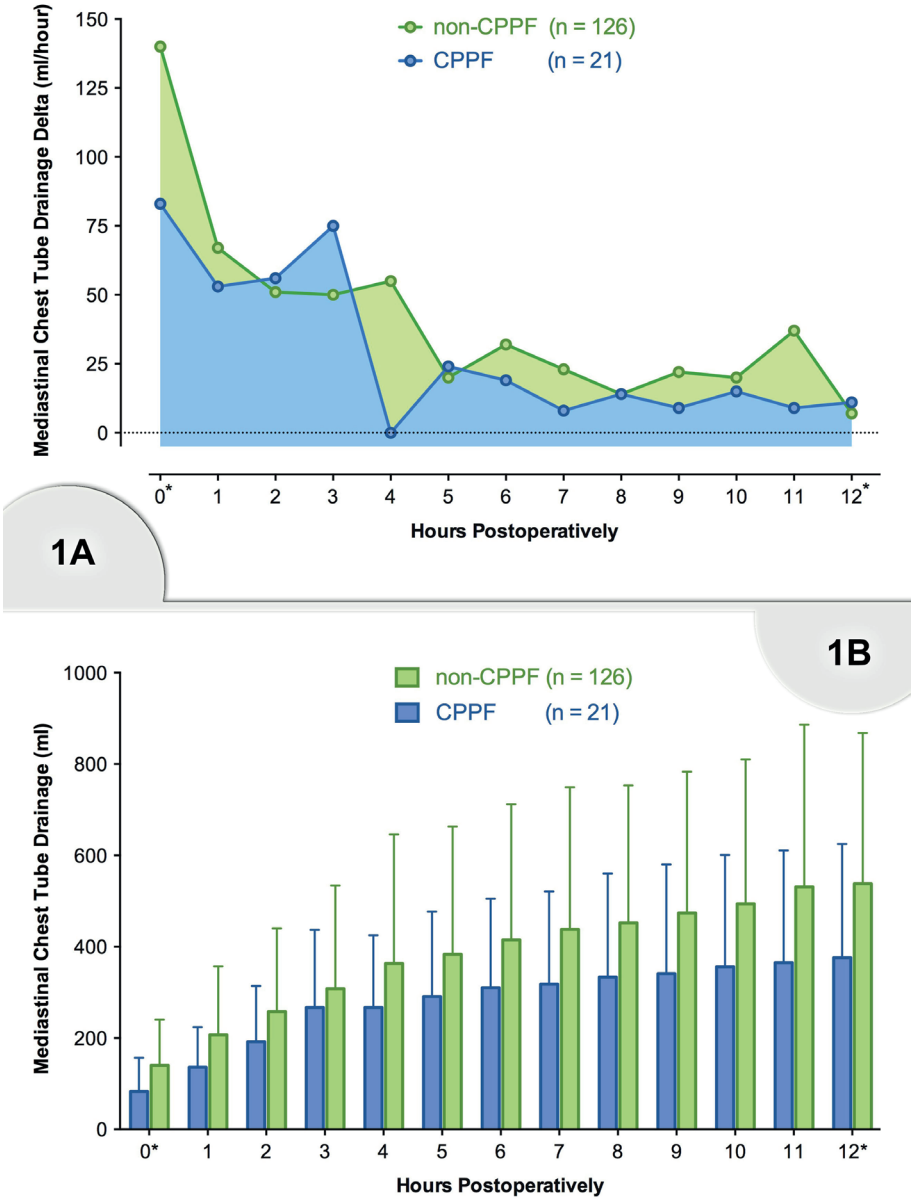

Figure 1. Postoperative actual blood loos over hourly intervals (A) and total cumulatively (B) during the first 12 postoperative hours.

DISCUSSION

There are several techniques available that aim to reduce blood loss and associated exposure to allogeneic blood after surgery. These include minimally invasive surgical techniques, blood conservation strategies (9) extracorporeal circulation systems (10) and, the systemic (11,12) and topical (13) pharmacological correction of hemostasis. Despite the improvements achieved by these techniques we still tend to accept a considerable amount of blood loss as being inherent to cardiac surgery. Moreover, all techniques available have different targets and/or time of action than the CPPF. Therefore, regardless of their effects, the result of CPPF is always an additional improvement to the current state of the art.

Review of literature revealed only a few studies that were focused on chest tube functionality and improvement of the postoperative drainage system (14-16). None of the studies reported a technique similar to CPPF as described in our paper. In addition, none of the postoperative drainage systems initially aimed for the complete cleaning of the pericardial space of blood and clots and, also, did not have reduction of blood loss as a primary study end point. Thus, to our knowledge, a flushing system that has specifically been designed to promote the evacuation of contaminated blood and clots out of the pericardial cavity in order to reduce postoperative blood loss has not been used or described before.

CPPF showed a 30% reduction in 12-h postoperative blood loss and there were no re-explorations for bleeding complications in the CPPF group. The non-CPPF group included significantly more aortic root surgery but within the non-CPPF group, procedures on the aortic root showed even less blood loss when compared to those that had non-aortic root surgery. With respect to important bleeding parameters (percentage of redo surgery, cross-clamp and CPB time, single and multiple procedures, use of anticoagulants, EUROSCORES) no significant differences were found between the groups. None of the other significant differences between the two groups, as depicted in tables 1 – 3, are known to have implications with respect to more or less bleeding tendency. Overall, the mean postoperative blood loss in both the CPPF and non-CPPF group can be considered to be low given the surgical complexity and high percentage of reoperations; this makes the difference

that we found stronger. However, the evidence from this pilot study (prospective cohort in comparison with a historic group of patients) is not strong enough to draw definitive conclusions with respect to blood loss and bleeding complications. Currently, two randomized clinical trials are ongoing at our institution that need to provide proof of concept by confirmation of these pilot findings.

On the contrary, one can also put forward that flushing a fresh wound carries the risk of disturbing the normal coagulation process, which therefore may lead to increased blood loss. Considering the little amount of blood loss that we observed in the CPPF group (mean 376 ml) and the fact that no continued bleeding was observed, we may abstract that CPPF patients did not tend to have more blood loss when compared to the "normal" non-CPPF patients. It is likely that this phenomenon did not occur is because our flushing method includes a slow continuous irrigation of flushing fluid rather than hosing the wound using strong mechanical forces. CPPF works mainly by dilution and lowering the viscosity of the blood and clot mixture in the wound, which enhances evacuation through the chest tubes.

Surgical re-exploration bleeding or suspected or acute cardiac tamponade is associated with increased mortality and morbidity (17) and is still needed in 2-6% of patients (18,19). As well as its effect on blood loss, CPPF may have an important impact on the prevention of acute cardiac tamponade, which was not seen in the CPPF group. As stated above, CPPF lowers the viscosity of drainage fluid and prevents abundant formation of clots, thereby preventing the chest tubes from blocking and promoting chest tube patency. Even the use of multiple rather than single mediastinal chest tubes is known not to solve this problem (20).

In theory, partial or complete chest tube blockage can never be completely eliminated, which emphasizes the need for careful monitoring of inflow and outflow volumes. Intrapericardial pressure monitoring could serve as an extra safety assurance. CPPF requires real-time accurate quantification of MCTD volume within the CPPF window in order to monitor actual blood loss and to trace possible fluid accumulation in the pericardial and/or pleural cavity. The manual monitoring procedure at time intervals that was used in this study was considered labor intensive and should ideally be automated for future clinical use. For the clinical assessment of actual blood loss and the detection of surgical hemorrhage, the blood content of

MCTD must be known at any time. Therefore, real time monitoring of hematocrit values of MCTD seems indispensable.

On transthoracic echocardiograms on discharge no significant differences in pericardial effusion were found between the CPPF group and non-CPPF group of patients, and no clinically significant pericardial effusions were encountered in the CPPF group. CPPF was stopped in one patient 3.5 h postoperatively as a precautionary measure due to a lag of >200 ml of fluid drainage from the right pleural cavity. As long as the protocol is followed strictly, i.e. the maximum accumulation volume does not exceed 200 ml, these pericardial or pleural effusions may be considered as clinically insignificant.

CPPF requires insertion of an extra drain and infusion of a saline solution into a fresh wound area, thereby theoretically increasing the risk of infection. However, since both the infusion solution and drain are sterilized and the same incision is used as for the standard chest tubes, this risk is considered to be negligible. No manifest clinical infections were seen in the CPPF group. Continuous irrigation of the wound area may reduce bacterial load and in theory decrease the risk of infection. In addition, by evacuating all blood and clots an important nutrient medium for potential bacterial infections is eliminated. Besides this, blood outside the vascular system i.e. in the pericardial cavity, may itself induce a serious local inflammatory reaction. Aiming for a cleaner pericardial space, CPPF may reduce postoperative complications related to this inflammatory process. In this context, the effect of CPPF on atrial fibrillation (22), the development of adhesions (23,24) and impaired postoperative right ventricular function (25) will be the subject of future studies.

In summary, so far CPPF can be regarded as safe since no postoperative adverse events that could be related to the CPPF were encountered in this study. In this experimental setting the CPPF method was considered feasible. However, in our judgement the CPPF method must be automated and the system should be equipped with an intra-pericardial pressure sensor and real-time hematocrit analysis of MCTD to provide the required high level of safety and feasibility in the clinical setting.

A limitation of this study is the retrospective group comparison. Results regarding the clinical impact on blood loss reduction are not yet conclusive at this stage and therefore randomized clinical trials are mandatory for a final conclusion. For instance, the surgical procedures in the CPPF group may have been biased towards "dryer surgery". Also, the difference between inflow and outflow volumes may not be an accurate measure for actual blood loss while the composition of MCTD fluid is both variable in time and patient-dependent.

Conclusion

From our study findings we conclude that CPPF after cardiac surgery is safe and feasible in this experimental setting. A positive effect on blood loss and related complications may be anticipated, but standardized randomized clinical trials are necessary to draw definitive conclusions.

AUTHOR CONTRIBUTIONS

CG and DK wrote the study protocol. RK and BdM provided expert clinical perspective to content of study protocol and manuscript. CdB provided expert methodological and clinical feedback to protocol and manuscript, providing methodological perspective and data analysis. JM was responsible for inclusion of study participants, execution of study and data collection. All authors were involved in the data analyses and discussion. JM, CG and DK wrote the manuscript.

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CHAPTER 8

Summary and General Discussion

Postoperative blood loss requiring transfusions either or not combined with surgical re-exploration is relatively common after cardiac surgery (1-3). There are several factors associated with this blood loss, that can be separated into bleeding complications with a surgical origin (bleeding vessel, anastomosis or other suture line) and those related to ineffective hemostasis. The main focus in this thesis is on the latter. Ineffective hemostasis can be due to pre-existing coagulation factor deficiencies, drug-induced inhibition of hemostasis or surgery related, acquired hemostatic defects. Suggested causes of acquired hemostatic defects in cardiac surgery include hemodilution due to priming fluids of CPB, direct hemostatic disturbance due to the CPB circuit, tissue trauma, consumption that ultimately may result into disseminated intravascular coagulation, platelet dysfunction and degradation due to excessive fibrinolysis (4-8). Furthermore, factors like hypothermia, hypocalcemia, and acidosis hinder adequate hemostasis.

In order to support the process of decision making concerning medication and blood product substitution, the aim of this thesis is to improve our understanding of the development of (excessive) blood loss due to ineffective hemostasis after coronary artery bypass graft surgery. Furthermore, several preventative measures to reduce the risk of bleeding are explored.

PART I ETIOLOGY AND DIAGNOSIS OF BLOOD LOSS AFTER CABG SURGERY

In part I of the thesis the etiological mechanisms for blood loss after CABG procedures are investigated. Fibrinogen is a protein that plays an essential role in coagulation. Due to the development of e.g. blood loss, hemodilution, platelet activation by cardiopulmonary bypass (CPB), a large wound area for clot formation, hypothermia and acidosis, cardiac surgery can result in a significant reduction of fibrinogen concentration and function (9,10). An inverse association between pre- and postoperative fibrinogen levels, and bleeding risk, even for levels within the normal reference range (1.5-4.0 g/l), has been suggested in several previous studies (10,11). However, most of those studies are underpowered to give an

unambiguous conclusion. Therefore, we performed a systematic review and meta-analysis to further clarify this effect of fibrinogen, as described in **Chapter 2**. Our meta-analysis indicated a significant, but weak to moderate correlation between pre- and postoperative fibrinogen concentrations and excessive postoperative blood loss in cardiac surgery. This confirms the important role of fibrinogen in the coagulation process. However, since the correlation is not very strong, it is likely that other factors like hematocrit, thrombin formation, platelet number and function and clotting factor activities are at least as important in the development of (excessive) blood loss. Nevertheless, administration of fibrinogen concentrate before and during operation to prevent this (excessive) blood loss, is increasingly applied (10,12-14). Although no thrombosis was reported, it is important to keep in mind that fibrinogen is an acute-phase protein which level gradually increases after surgical procedures (10,15). This might ultimately lead to an increased risk of thromboembolic complications in the postoperative period.

Now that we have established an association, although weak, between fibrinogen concentrations and the development of (excessive) blood loss after cardiac surgery in chapter 2, we studied several factors that might contribute to a reduction in fibrinogen concentrations immediately after surgery in **Chapter 3** . Three main mechanisms involved in a reduction of fibrinogen concentration or function are; hemodilution, consumption and degradation (9,16,17). Our results showed that hemodilution is likely to be the most important mechanism to reduce fibrinogen concentration and function during CABG procedures with the usage of CPB and tranexamic acid. To determine if indeed the reduction in fibrinogen concentration is causally related to the observed coagulopathy as measured by ROTEM^{TM} (with prolonged CT and reduced MCF), and not the dilution of other coagulation factors, we performed an ex vivo supplementation study in which we added purified fibrinogen (Haemocomplettan, CSL) to blood of the studied CABG patients. Normalization of the coagulation profile after addition of purified fibrinogen confirmed that fibrinogen is the first clotting factor to drop below a critical level during hemodilution and a major contributor to the onset of dilutional coagulopathy.

The important role of hemodilution in the development of coagulopathy immediately after CABG surgery is confirmed in **Chapter 4** in which the hemostatic profile

throughout the perioperative period up to 5 days after CABG surgery is further revealed. In this chapter the effects of surgical trauma and CPB on coagulation and fibrinolysis were compared between three groups: 1) isolated CABG without use of CPB (OPCAB), 2) isolated CABG with the use of CPB (CABG) and 3) CABG procedures combined with aortic valve replacement (CABG+AVR). By comparing these groups we could determine the effects of CPB usage on coagulation and fibrinolysis and differentiate between the effects of short (CABG) and long (CABG+AVR) CPB time. Overall, patients undergoing CABG surgery without the use of CPB revealed higher fibrinogen, D-dimer, and prothrombin fragment 1 and 2 concentrations in the preoperative phase relative to patients operated on CPB (Figure 1). This elevation cannot be explained by older age or the presence of comorbidities among patients in the OPCAB group, relative to patients operated on CBP in our study population (18).

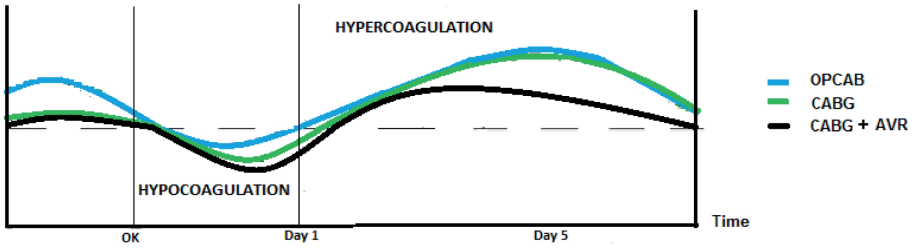

Figure 1. Hemostatic curve in CABG surgery.
OPCAB, off-pump coronary artery bypass surgery, without CPB; CABG, CABG with use of CPB; CABG+AVR, CABG with use of CPB combined with aortic valve replacement.

During CABG surgery and in the first hours after operation hypocoagulability develops, most pronounced seen in the CABG and CABG+AVR groups. This bleeding tendency in 'normal bleeding patients' (a blood loss of less than 2 liters in total) appears to be mainly influenced by hemodilution. No perioperative platelet activation was observed and functional (collagen induced) platelet aggregation was transiently impaired, but recovered after surgery in all groups. Furthermore, plasma prothrombin fragment 1+2 and D-dimer levels remained quite stable throughout operation and the changes demonstrated in thrombin generation were

not above standard errors. With hemodilution as the most critical factor impairing hemostasis, minimizing the volume of priming fluids used in the CPB circuit and amount of colloids administered during cardiac surgery, may reduce postoperative blood loss. A critical evaluation should be made of all vital signs indicating tissue saturation and the actual need for volume administration before providing so. The use of minimized extracorporeal systems might further reduce hemodilution (19), although magnitude of this effect is debated (20). Another useful technique is priming of the CPB circuit with patients' own blood (retrograde autologous priming), in which the priming fluid of the CPB circuit is retrograde, through the aortic cannula. There is no evidence for a clinically relevant fibrinolysis during or after operation when tranexamic acid is administrated, since the t-PA burst seen after reperfusion in patients on CPB, probably partly resulting from surgical tissue damage during surgery (21), is without clinical consequences. The Nijmegen Hemostasis Assay (NHA) even revealed an impaired fibrinolysis 5 days after operation in all groups. Probably, without the use of tranexamic acid (which is administered accordingly to standard procedure in most hospitals in the Netherlands), the role of fibrinolysis in bleeding might be more pronounced (22). This is confirmed in chapter 3 in which the effects of the fibrinogen degradation products, originating after e.g. plasmin mediated proteolysis, were analyzed *in vitro* using ROTEM^{TM}. We observed that early fibrinogen degradation products have a pronounced effect on blood clot formation *in vitro* and therefore may induce or enhance *in vivo* coagulopathy. However, when tranexamic acid is used during surgery no fibrinogen degradation products were observed in the plasma of patients, indicating that *in vivo* the relatively low tranexamic acid levels are sufficient to prevent fibrinogenolysis.

Although, during and immediately after cardiac surgery patients have a bleeding tendency, coagulation restores in the postoperative period. This process develops from day 1 until at least day 5 postoperative, in which a rebound above baseline levels of fibrinogen, D-dimer, prothrombin fragments 1 and 2 concentrations and platelet aggregation, combined with an increased thrombin generation was seen in all patients, suggesting a hypercoagulable state. This rebound is probably attributed to an acute phase response to surgical trauma and CPB usage (23,24). It was most pronounced among patients undergoing isolated CABG (OPCAB and CABG) who do

not receive vitamin K antagonists as prescribed in patients undergoing aortic valve replacement. The presence of a hypercoagulable state combined with postoperative impaired fibrinolysis, may promote the risk of thromboembolic complications (23,25,26). These results emphasize the need for adequate anticoagulant therapy to prevent thromboembolic complications in the postoperative phase, especially in patients who do not receive vitamin K antagonists by protocol, even in those who were bleeding before. A delicate balance between a bleeding tendency and a hypercoagulable state has to be maintained. Probably, point-of-care (POC) testing might provide a more precise and individual patient orientated diagnostic tool to do so in the future (27).

PART II MANAGEMENT AND PREVENTION OF BLOOD LOSS AFTER CABG SURGERY

In part II of the thesis the management options to prevent and treat bleeding in cardiac surgery are described. In order to reduce the amount of blood loss and blood transfusions required during and after cardiac surgery it is important that antiplatelet medications like, e.g. acetylsalicylic acid and clopidogrel, prescribed routinely in patients prior to cardiac surgery, especially CABG, are discontinued timely before operation. Solid data on the optimal timing of discontinuation of both medications is lacking (28-31). In **Chapter 5** we tried to determine the day before surgery at which acetylsalicylic acid, either or not combined with clopidogrel, should be used the latest to establish the lowest amount of blood loss 48 h after surgery. Our results showed that there is no clinically relevant optimal stop day (defined as the last day before surgery on which antiplatelet medication was used) for acetylsalicylic acid alone or in combination with clopidogrel in relation to 48 h blood loss. This is in agreement with findings of Mannacio (32) on the large variability in platelet function restoration among individuals. However, last use on day -2 resulted in a reduction of the percentage of patients receiving platelet transfusions, especially with combined preoperative ASA and clopidogrel use, and should be considered as the best compromise in the vast majority of these patients. So, in

order to reduce the amount of platelet transfusions a combination of acetylsalicylic acid and clopidogrel should not be used the day before surgery. There was no association between any stop day and major adverse cardiovascular and cerebral events (MACCE), however, the termination date of the antiplatelet medication was left to the discretion of the physician and influenced by expected risk for thromboembolic complications. Due to this large variation in patient responsiveness to clopidogrel administration, use of antiplatelet medication might possibly be better monitored by POC platelet function measurement, in order to reduce peri- and postoperative blood loss and transfusion replacement to a minimum. However, before the incorporation of POC platelet function tests as standard care or diagnostic tool can be realized, it needs further validation in clinical practice. Our recommendation should, therefore, be considered as a practical alternative.

Other preventative measures to reduce blood loss and consecutive blood transfusion requirements in cardiac surgery include minimally invasive surgical techniques, miniaturized extracorporeal cardiopulmonary circuit, ultrafiltration, the use of blood conservation strategies, and the systemic and topical (33) pharmacological correction of hemostasis (34-36). An example of a topical pharmacological correction is the use of fibrin sealants during operation. Fibrin sealants have gained increasing popularity, but have been shown to be less effective than antifibrinolytics (37). However, since one of the most effective antifibrinolytics, aprotinin, had been removed from the market for safety reasons, using fibrin sealants became more significant. A recently developed fibrin sealant produced from allogeneic single donor plasma, without the addition of fibrinolysis inhibitors or bovine proteins, called CryoSeal™ was studied to evaluate its safety and cost-effectiveness in patients undergoing elective isolated CABG surgery, using at least one internal thoracic artery, as outlined in **Chapter 6** . Results show that the use of the fibrin sealant CryoSeal™ did not result in health benefits. Therefore, implementation in elective isolated CABG is not recommended.

Despite the improvements achieved by the existing techniques we still tend to accept a considerable amount of blood loss as being inherent to cardiac surgery. In the final chapter of this thesis, **Chapter 7** , the safety, feasibility, and effect on blood loss of continuous postoperative pericardial flushing (CPPF) after cardiac

surgery was evaluated. We developed a device to remove contaminated pericardial blood (with high fibrinolytic activity and tissue factor concentration (6,21)) and clots via dilution and a continuous circulation of infusion liquid through the pericardial cavity. Removing this blood and clots probably not only reduces the chance of excessive blood loss by preventing a systemic coagulopathy, but also might have beneficial effects on several other factors associated with surgery like, e.g. inflammation, atrial fibrillation, pericardial effusions (tamponade), and the development of adhesions. Our findings support technical safety and feasibility regarding infections and (pleural or pericardial) fluid accumulation of the device in 20 adult patients undergoing surgical correction for congenital heart disease. In one patient CPPF was stopped as a precaution 3.5 h postoperatively as there was a >200 ml lag in total mediastinal chest tube drainage volume due to fluid accumulation in the right pleural cavity. The accumulated fluid had spontaneously resolved on extubation, confirmed by chest radiography. No system-related or other serious problems were encountered. Although, this study was primarily aimed to evaluate safety and feasibility of the device, we demonstrated a 30% reduction in 12 h postoperative blood loss and there were no re-explorations for bleeding complications in the CPPF group. These observations suggest a possible role for the device in the reduction of postoperative blood loss.

CONCLUSIONS AND FUTURE RECOMMENDATIONS

After careful evaluation, hemodilution seems to be the most pronounced factor associated with the development of the coagulopathy after cardiac surgery, and, probably plays an important role in the origination of blood loss after cardiac surgery. Although fibrinogen is (one of the first) clotting factors to drop below a critical level during hemodilution, care should be taken with administration of fibrinogen concentrate. Fibrinogen is an acute phase protein which levels rise gradually during and after operation as response to surgical trauma and CPB usage. The increased levels of D-dimer and prothrombin fragments 1 + 2, together with the increased thrombin generation, indicate that a hypercoagulable state develops up to at least

5 days after cardiac surgery. The combination of both this 'natural' rebound towards a hypercoagulable state and the administered fibrinogen concentrate, might enhance the risk of thromboembolic complications in the postoperative period. Therefore, adequate anticoagulant therapy to prevent thromboembolic complications in the postoperative phase is needed, especially in patients who do not receive vitamin K antagonists by protocol, even in the patients previously bleeding. A delicate balance between a bleeding tendency and a hypercoagulable state has to be maintained. The use of point of care (POC) evaluation (e.g. TEG or ROTEM) might provide faster and a more complete insight in this delicate balance, creating a more individual patient orientated treatment. The large variation in patient responsiveness to clopidogrel administration, often administered before operation, further advocates the usage of POC platelet function measurement before, during, and after cardiac surgery. A more individual patient orientated treatment might contribute to reducing peri- and postoperative blood loss and transfusion replacement to a minimum.

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Nederlandse Samenvatting

Bloedverlies na een hartoperatie is een veelvoorkomende complicatie. Veelal zijn als gevolg van dit bloedverlies bloedtransfusies vereist, al dan niet gepaard gaande met nog een tweede operatie om de bloeding te stoppen. Er zijn verschillende uitlokkende factoren voor dit bloedverlies, te verdelen in bloedingen met een chirurgische oorzaak (bijvoorbeeld door een naadlekkage) en bloedingen die gerelateerd zijn aan een stollingsprobleem. Dit proefschrift richt zich met name op de laatste vorm. Stollingsproblemen bij hartoperaties kunnen ontstaan door een reeds bestaand tekort aan stollingsfactoren (bijvoorbeeld door een genetische afwijking), het gebruik van bepaalde medicamenten voorgaande aan de operatie of kunnen veroorzaakt worden door aan de operatie gerelateerde factoren. Bij deze laatste groep kan men denken aan stollingsproblemen die optreden als gevolg van verdunning door het toedienen van infusievloeistof ten behoeve van het gebruik van de hartlongmachine of om de bloeddruk te stabiliseren. Ook contact van bloed met het oppervlak van de hartlongmachine, weefselschade, overmatige activatie van stolling en een verhoogde afbraak van stolsels, als gevolg van de operatie, kunnen een stollingsprobleem veroorzaken. Andere factoren die een adequate stolling in de weg staan, zijn onderkoeling, een laag calciumgehalte in het bloed en een hoge zuurgraad (lage pH).

Het verkrijgen van een beter inzicht in de processen die leiden tot het ontstaan van (overmatig) bloedverlies kan ondersteunend zijn in de besluitvorming rondom het toedienen van medicatie en bloedproducten bij deze patiënten. In dit proefschrift wordt het ontstaan van bloedverlies na hartoperaties bestudeerd en worden mogelijke therapieën en preventieve strategieën geëxploreerd.

In het eerste gedeelte van het proefschrift worden de ontstaansmechanismen van bloedverlies na hartchirurgie, specifiek na bypass coronaire operaties (CABG), bestudeerd. In **hoofdstuk 1** wordt een overzicht gegeven van de bestaande literatuur over oorzaken van stollingsproblemen bij hartoperaties. Vervolgens wordt in **hoofdstuk 2** de rol van fibrinogeen, een essentieel eiwit in het stollingsproces, bij het ontstaan van overmatig bloedverlies (gedefinieerd als meer dan 2 liter per 24 uur of meer dan 200 ml per uur) na hartoperaties bestudeerd. In een meta-analyse

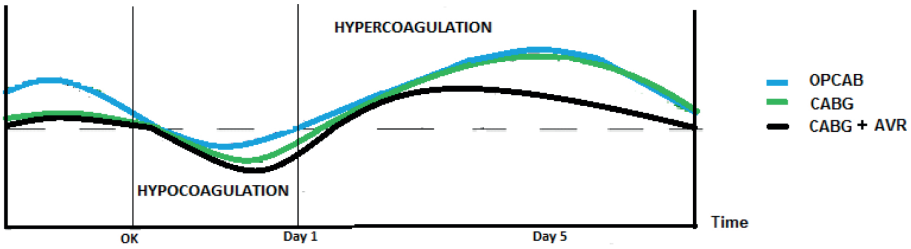
wordt een zwakke, significante correlatie gevonden tussen fibrinogeen concentratie (voor en na operatie) en overmatig bloedverlies na hartoperaties. Dit betekent dat, hoewel de rol van fibrinogeen belangrijk is in de ontwikkeling van overmatig bloedverlies, andere factoren zoals een laag hemoglobine gehalte, de dikte van het bloed (hematocriet), de hoeveelheid bloedplaatjes en de functie en activiteit van stollingsfactoren op zijn minst net zo belangrijk zijn in dit proces. In de dagelijkse praktijk wordt echter, ter preventie van overmatige bloedverlies, in toenemende mate fibrinogeen concentraat toegediend voor en tijdens hartoperaties. Van belang hierbij is dat de behandelaar zich realiseert dat fibrinogeen een acute fase eiwit is waarvan, als reactie op de operatie, de concentratie geleidelijk toeneemt na de operatie. Dit heeft niet geleid tot een toegenomen, gerapporteerd, aantal stollingscomplicaties, maar zou uiteindelijk wel degelijk kunnen leiden tot een verhoogd risico op trombose na hartoperaties.

In **hoofdstuk 3** worden factoren bestudeerd die zouden kunnen bijdragen aan een afname van de fibrinogeen concentratie direct na operatie. Er zijn 3 hoofdmechanismen betrokken bij deze afname: 1) verdunning (hemodilutie), 2) verbruik van stollingsfactoren (consumptie) en 3) verhoogde afbraak van een stolsel (fibrinolyse of degradatie). Hemodilutie leek de belangrijkste factor te zijn die betrokken was bij de afname van fibrinogeen concentratie en functie na CABG operaties, waarbij gebruik wordt gemaakt van de hartlongmachine en tranexaminezuur, een remmer van fibrinolyse, wordt toegediend (standaard protocol in Nederland). Om te bevestigen dat de geobserveerde stollingsproblemen daadwerkelijk werden veroorzaakt door een afname in fibrinogeen concentratie en niet door afname van andere stollingsfactoren, werd (puur) fibrinogeen concentraat (Haemocomplettan, CSL) *ex vivo* toegevoegd aan het bloed van CABG patiënten. Normalisatie van het stollingsprofiel na deze toevoeging bevestigde dat fibrinogeen de eerste stollingsfactor is die beneden een kritisch niveau zakt bij hemodilutie.

De belangrijke rol van hemodilutie in de ontwikkeling van een stollingsprobleem werd bevestigd in **hoofdstuk 4**. In dit hoofdstuk wordt de hemostase, oftewel het stollingsevenwicht, gedurende de operatie tot en met 5 dagen na CABG

operatie bestudeerd. De effecten van weefselschade (ontstaan door de operatie zelf) en het gebruik van de hartlongmachine, op zowel de stolling als fibrinolyse, werden vergeleken tussen 3 groepen: 1) CABG operaties zonder gebruikmaking van de hartlongmachine (OPCAB), 2) CABG operaties met gebruikmaking van de hartlongmachine (CABG) en 3) CABG operaties gecombineerd met een aorta(hart) klepvervanging (CABG +AVR). Door deze groepen onderling te vergelijken kon gedifferentieerd worden tussen de effecten van kort (CABG), lang (CABG + AVR) of helemaal geen gebruik (OPCAB) van de hartlongmachine. Voor hun operatie hadden patiënten die zonder hartlongmachine geopereerd zouden worden een meer procoagulant stollingsprofiel, dan patiënten die geopereerd zouden worden met gebruikmaking van de hartlongmachine. Tijdens en in de eerste uren na CABG operatie, neemt de sterkte van de stolling in alle groepen af. Dit is het meest uitgesproken in de CABG en CABG + AVR groepen, beiden geopereerd met gebruikmaking van de hartlongmachine. Deze bloedingstendens bij een normale hoeveelheid bloedverlies (gedefinieerd als minder dan 2 liter in totaal na operatie), wordt met name veroorzaakt door hemodilutie. Er werd geen bloedplaatjes activatie geobserveerd tijdens operatie en functionele plaatjes aggregatie (samenklontering) was weliswaar verminderd, maar herstelde (tot normaal) direct na de operatie in alle groepen. Dit maakt de rol van een verminderde werking van bloedplaatjes in het ontstaan van bloedverlies in het eerste uur na operatie minder waarschijnlijk. Ook de plasma concentraties van protrombine fragmenten 1+2 en D-dimeer, welke beiden verhoogd zouden zijn bij overmatige consumptie, bleven redelijk stabiel gedurende de operatie. De veranderingen in trombine generatie kwamen niet boven de standaard fout. Tevens was er geen bewijs voor klinisch relevante fibrinolyse, tijdens of na operatie, onder toediening van tranexaminezuur. Mogelijk zou de rol van fibrinolyse meer uitgesproken zijn zonder toediening van tranexaminezuur. Dit wordt bevestigd in hoofdstuk 3 waarin fibrinogeen afbraakproducten een duidelijk remmend effect op de stolselvorming *in vitro* veroorzaakten en mogelijk dus *in vivo* een stollingsprobleem (kunnen) verergeren. Echter wanneer tranexaminezuur werd toegediend tijdens de CABG operatie, werden geen fibrinogeen afbraakproducten geobjectiveerd in het plasma van patiënten, wat suggereert dat *in vivo* de

relatief lage tranexaminezuur niveaus voldoende zijn om (overmatige) afbraak van fibrinogeen te voorkomen.



Figuur 1. Stollingsevenwicht tijdens CABG operatie.
OPCAB, CABG operaties zonder gebruik making van de hartlongmachine; CABG, operaties met gebruik making van de hartlongmachine en CABG +AVR, CABG operaties gecombineerd met een aorta(hart)klepvervanging.

Ondanks dat patiënten tijdens en direct na een hartoperatie voornamelijk een bloedingstendens laten zien, herstelt de stolling zich geleidelijk tussen de 1e en de 5e dag na operatie. Gedurende deze periode wordt een 'rebound' boven de uitgangswaarden gezien van fibrinogeen, D-dimeer, protrombine fragment 1+2 concentraties en plaatjes aggregatie, gecombineerd met een verhoogde trombine generatie. In deze postoperatieve periode lijkt er sprake van een 'hypercoagulable state', oftewel een staat van overmatige stolling. Deze overmatige stolling is hoogstwaarschijnlijk het gevolg van een acute fase respons op weefselschade en het gebruik van de hartlongmachine. Het proces is het meest uitgesproken bij patiënten die geen vitamine K antagonisten gebruiken (OPCAB en CABG, oftewel CABG operaties zonder klepvervanging). Uiteindelijk kan dit proces leiden tot een verhoogd risico op stollingscomplicaties, oftewel trombose, na operatie. Adequate instelling op antistollingsmedicatie is daarom van belang, ook bij mensen die eerder een bloedingsprobleem hadden. Dit betekent dat een delicaat evenwicht tussen een bloedingstendens en overmatige stolling zal moeten worden gehandhaafd. Mogelijk kan 'point of care testing', een methode om een laboratoriumtest naast of in de buurt van de patiënt uit te voeren, hier in de toekomst, als preciezer en individueel georiënteerd diagnosticum, een belangrijke rol in spelen.

In het tweede deel van het proefschrift wordt ingegaan op de behandeling en preventie van (overmatig) bloedverlies. Het doel hiervan is om bloedverlies en de benodigde bloedtransfusies tijdens en na hartoperaties te verminderen. Om dit doel te bereiken is het onder andere van belang dat de gebruikte antistollingsmedicatie tijdig wordt gestopt (**hoofdstuk 5**). Er blijkt voor acetylsalicylzuur, al dan niet gecombineerd met clopidogrel, geen optimale stop dag te zijn voor een hartoperatie om het bloedverlies binnen 48 uur na operatie zo laag mogelijk te houden. Het niet gebruiken van deze medicatie op de dag voor operatie leidde wel tot een lager percentage patiënten dat bloedtransfusies toegediend kreeg, vooral bij mensen die een combinatie van beide medicamenten gebruikten voor operatie. Door de hoge variantie in 'responsiveness', oftewel de effectiviteit, van clopidogrel kan de werking ervan mogelijk het beste geëvalueerd worden middels 'point of care testing' om zo het bloedverlies en de transfusiebehoefte gedurende en na operatie te minimaliseren.

Andere preventieve methoden om bloedverlies en transfusies te verminderen zijn minimaal invasieve technieken, ultrafiltratie, bloedconservatie strategieën en het gebruik van systemische of plaatselijke farmacologische middelen. Een voorbeeld van een plaatselijk farmacologisch middel is het gebruik van fibrinelijm tijdens operatie. CryoSeal, een fibrinelijm geproduceerd uit het plasma van een enkele donor, blijkt bij CABG operaties geen vermindering van het bloedverlies of andere gezondheidswinst te geven (**hoofdstuk 6**) en daarom wordt het gebruik ervan niet aangeraden.

In **hoofdstuk 7** wordt de toepassing van 'continuous postoperative pericardial flushing (CPPF)', oftewel het continue spoelen van het hartzakje na operatie met een ontwikkeld spoelsysteem, geëvalueerd. Onderzoek onder 20 patiënten die een chirurgische correctie ondergingen vanwege aangeboren hartaandoeningen, bevestigde de veiligheid en toepasbaarheid van dit systeem. Daarnaast werd een reductie van 30% in het bloedverlies 12 uur na operatie vastgesteld. Het verwijderen van 'vervuild' bloed (met hoge fibrinolyse activiteit) en stolsels uit het hartzakje middels verdunning kan waarschijnlijk de kans op overmatig bloedverlies

verkleinen (door het voorkomen van een systemische reactie), maar heeft mogelijk ook een positief effect op andere factoren zoals ontsteking, atriumfibrilleren, tamponade en de ontwikkeling van verklevingen na operatie.

In het laatste hoofdstuk (**hoofdstuk 8**) worden de resultaten in het licht geplaatst van de huidige literatuur en de klinische implicaties voor toekomstige therapie bediscussieerd.

‘First and foremost is the need for those who order transfusions to do so mindfully, rather than automatically, in response to a given pathophysiologic trigger’

(Hardy JF . Ann Thorac Surg 1996;62:1935-43)

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Co-auteurs

Professor Theo Stijnen, drs. Jan Schoones, Professor Olaf Dekkers, Jos Grimbergen, dr. Jaap Koopman, dr. Waander van Heerde, dr. Giuseppe Tavilla, dr. Wilbert van den Hout, drs. Johan Manshanden, dr. Corianne de Borgie en dr. Dave Koolbergen, bedankt voor jullie bijdrage aan dit proefschrift.

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Onderzoeksassistente

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Casper, mijn liefste, mijn verloofde, mijn maatje, mijn alles. Samen lachen, samen huilen. Dank dat jij er altijd bent.

CURRICULUM VITAE

Chantal Laura Ingrid Gielen werd geboren op 2 juni 1984 in het Bronovo Ziekenhuis te Den Haag. Zij groeide op in Zoetermeer en Miri (Maleisië), en startte daarna in 1996 met haar VWO opleiding aan het Alfrink College te Zoetermeer. Tijdens haar middelbareschooltijd maakte ze gedurende twee jaar onderdeel uit van de medezeggenschapsraad en droeg actief bij aan de invoering van tweetalig onderwijs. Na het behalen van haar VWO diploma in 2002 startte zij, na plaatsing middels decentrale selectie, met de opleiding Geneeskunde aan de Universiteit van Leiden. Tijdens haar studie was ze bestuurslid bij de International Federation of Medical Student's Associations (IFMSA). Binnen dit bestuur was ze verantwoordelijk voor het organiseren van stages voor medische studenten naar ontwikkelingslanden, en ontwikkelde en nam deel aan verschillende 'public health' projecten. Na het behalen van haar artsexamen in oktober 2008 startte zij als promovenda op de afdeling Thoraxchirurgie, onder begeleiding van professoren dr. R.J.M. Klautz en dr. J. Eikenboom. Binnen het proefschrift onderzocht zij verschillende ontstaansmechanismen voor bloedverlies na CABG procedures. Verder bestudeerde ze diagnostische methoden om dit vast te stellen en onderzocht preventieve maatregelen om de hoeveelheid bloedverlies na CABG procedures te reduceren. De resultaten van dit onderzoek staan beschreven in dit proefschrift. Tijdens haar promotie is zij gestart als assistent op de afdeling Thoraxchirurgie, maar besloot begin 2014 een overstap te maken naar de Verzekeringsgeneeskunde. Per 3 november 2015 is zij gestart met de opleiding tot verzekeringsarts aan de NSPOH te Utrecht en werkt bij het UWV te Den Haag. Daarnaast is zij sinds oktober 2015 werkzaam als senior onderzoeker bij het Kennis Centrum Verzekeringsgeneeskunde (KCVG) aan de Vrije Universiteit in Amsterdam, alwaar ze zich actief inzet voor de landelijke academisering en implementatie van verzekeringsgeneeskundig onderzoek.

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