

# Effects of Fibrinogen Concentrate as First-line Therapy during Major Aortic Replacement Surgery

## A Randomized, Placebo-controlled Trial

Niels Rahe-Meyer, M.D., Ph.D.,\* Cristina Solomon, M.D.,† Alexander Hanke, M.D.,‡ Dirk S. Schmidt, Ph.D.,§ Dietrich Knoerzer, Ph.D.,§ Gerald Hochleitner,|| Benny Sørensen, M.D., Ph.D.,# Christian Hagl, M.D.,\*\* Maximilian Pichlmaier, M.D.\*\*

### ABSTRACT

**Background:** Fibrinogen is suggested to play an important role in managing major bleeding. However, clinical evidence regarding the effect of fibrinogen concentrate (derived from human plasma) on transfusion is limited. The authors assessed whether fibrinogen concentrate can reduce blood transfusion when given as intraoperative, targeted, first-line

\* Professor, Clinic for Anesthesiology and Intensive Care Medicine, Franziskus Hospital, Bielefeld, and Clinic for Anesthesiology and Intensive Care Medicine, Hannover Medical School, Hannover, Germany. † Associate Professor, Department of Anaesthesiology and Intensive Care, Salzburger Landeskliniken SALK, Salzburg, Austria, and Clinic for Anesthesiology and Intensive Care Medicine, Hannover Medical School. ‡ Clinic for Anesthesiology and Intensive Care Medicine, Hannover Medical School. § CSL Behring, Marburg, Germany. || CSL Behring, Vienna, Austria. # Associate Professor, Haemostasis Research Unit, Centre for Haemostasis and Thrombosis, Guy's and St Thomas NHS Foundation Trust and King's College London School of Medicine, London, United Kingdom, and Centre for Haemophilia and Thrombosis, Aarhus University Hospital, Aarhus, Denmark. \*\* Professor, Clinic for Cardiothoracic, Transplant and Vascular Surgery, Hannover Medical School.

Received from the Clinic for Anesthesiology and Intensive Care Medicine, Hannover Medical School, Hannover, Germany. Submitted for publication January 23, 2012. Accepted for publication August 10, 2012. Niels Rahe-Meyer, Cristina Solomon, Alexander Hanke, Benny Sørensen, Christian Hagl, and Maximilian Pichlmaier received research funding from CSL Behring (Marburg, Germany) to perform this study. Niels Rahe-Meyer has participated in advisory boards and received speaker honoraria and research support from CSL Behring and Tem International (Munich, Germany). Cristina Solomon has received speaker honoraria and research support from Tem International and CSL Behring. Benny Sørensen has participated in advisory boards and/or received speaker honoraria from Novo Nordisk (Bagsværd, Denmark), Baxter (Deerfield, Illinois), CSL Behring, Bayer (Leverkusen, Germany), Tem International, and Biovitrum (Stockholm, Sweden). Dirk Schmidt and Gerald Hochleitner are employees of CSL Behring. Dietrich Knoerzer was an employee of CSL Behring throughout the period of the study and during the data analysis. The study was sponsored (funded) by CSL Behring. Statistical support was provided by ClinResearch GmbH (Aesch, Switzerland) and Accovion GmbH (Eschborn, Germany). Hannover Clinical Trial Center, Hannover, Germany, provided the monitoring as a contract research organization.

Address correspondence to Dr. Rahe-Meyer: Clinic for Anesthesiology and Intensive Care Medicine, Franziskus Hospital, Kiskerstr. 26, D-33615 Bielefeld, Germany. niels.rahe-meyer@franziskus.de. This article may be accessed for personal use at no charge through the Journal Web site, [www.anesthesiology.org](http://www.anesthesiology.org).

Copyright © 2012, the American Society of Anesthesiologists, Inc. Lippincott Williams & Wilkins. Anesthesiology 2013; 118:40-50

### What We Already Know about This Topic

- Although fibrinogen plays an important role in hemostasis, clinical evidence regarding the effect of administering fibrinogen concentrate remains limited

### What This Article Tells Us That Is New

- In a placebo-controlled randomized trial including patients (n = 61) undergoing aortic replacement surgery under cardiopulmonary bypass, the transfusion of allogeneic blood components was significantly reduced in the fibrinogen concentrate group (median 2 vs. 13 U,  $P < 0.001$ )

hemostatic therapy in bleeding patients undergoing aortic replacement surgery.

**Methods:** In this single-center, prospective, placebo-controlled, double-blind study, patients aged 18 yr or older undergoing elective thoracic or thoracoabdominal aortic replacement surgery involving cardiopulmonary bypass were randomized to fibrinogen concentrate or placebo, administered intraoperatively. Study medication was given if patients had clinically relevant coagulopathic bleeding immediately after removal from cardiopulmonary bypass and completion of surgical hemostasis. Dosing was individualized using the fibrin-based thromboelastometry test. If bleeding continued, a standardized transfusion protocol was followed. **Results:** Twenty-nine patients in the fibrinogen concentrate group and 32 patients in the placebo group were eligible for the efficacy analysis. During the first 24 h after the administration of study medication, patients in the fibrinogen concentrate group received fewer allogeneic blood components than did patients in the placebo group (median, 2 vs. 13 U;  $P < 0.001$ ; primary endpoint). Total avoidance of transfusion was achieved in 13 (45%) of 29 patients in the fibrinogen concentrate group, whereas 32 (100%) of 32 patients in the placebo group received transfusion ( $P < 0.001$ ). There was no observed safety concern with using fibrinogen concentrate during aortic surgery.

◇ This article is featured in "This Month in Anesthesiology." Please see this issue of ANESTHESIOLOGY, page 9A.

◆ This article is accompanied by an Editorial View. Please see: Faraday N: Fibrinogen concentrate and allogeneic blood transfusion in high risk surgery. ANESTHESIOLOGY 2013; 118:7-9.

**Conclusions:** Hemostatic therapy with fibrinogen concentrate in patients undergoing aortic surgery significantly reduced the transfusion of allogeneic blood products. Larger multicenter studies are necessary to confirm the role of fibrinogen concentrate in the management of perioperative bleeding in patients with life-threatening coagulopathy.

**M**AJOR bleeding during complex surgery increases the need for blood transfusions, prolongs the patient's stay in the intensive care unit (ICU), and is associated with increased morbidity and mortality.<sup>1,2</sup> The standard treatment of perioperative bleeding involves the transfusion of allogeneic blood components (erythrocytes, fresh frozen plasma [FFP], platelet concentrate, or cryoprecipitate). Although these components have become safer, there is evidence that the transfusion of allogeneic blood components may be associated with a risk of serious adverse events.<sup>3-5</sup> Thus, treatment approaches that can reduce transfusion appear desirable.

Clot strength increases with increasing concentrations of fibrinogen, and fibrinogen levels higher than 200 mg/dl have been suggested for optimal clot formation.<sup>6-8</sup> Immediately after cardiopulmonary bypass (CPB), fibrin formation is impaired to a greater extent than either thrombin generation or the platelet component of clot strength.<sup>9</sup> The plasma concentration of fibrinogen<sup>10,11</sup> and the firmness (elasticity/strength) of the fibrin-based clot<sup>9</sup> have been reported to decrease by an average of 34–42% in response to CPB. Furthermore, low levels of fibrinogen are associated with an increased risk of postoperative bleeding.<sup>10,12,13</sup> Overall, there is considerable evidence to support fibrinogen supplementation as a first-line treatment for coagulopathic bleeding among patients undergoing CPB. Several studies have suggested that fibrinogen concentrate therapy may be effective in controlling perioperative bleeding, reducing transfusion requirements as well as blood loss.<sup>11,14-19</sup>

Fibrinogen concentrate is derived from human plasma and does not contain relevant levels of other coagulation factors.<sup>20</sup> Our group has developed and validated a model for individualized dosing of fibrinogen concentrate,<sup>17,18</sup> by measuring firmness of the fibrin-based clot, which is mainly dependent on plasma fibrinogen levels.<sup>13,21,22</sup> Maximum clot firmness (MCF) of the fibrin-based clot can be monitored using a commercially available fibrin-based thromboelastometry test (FIBTEM).<sup>11,23</sup>

A clinical study is needed to investigate the efficacy and safety of fibrinogen concentrate in managing severe perioperative bleeding. We hypothesized that fibrinogen concentrate can reduce the need for blood transfusions without increasing the rate of serious adverse events when given intraoperatively as individualized first-line hemostatic therapy in bleeding patients undergoing aortic replacement surgery.

## Materials and Methods

### Study Design and Population

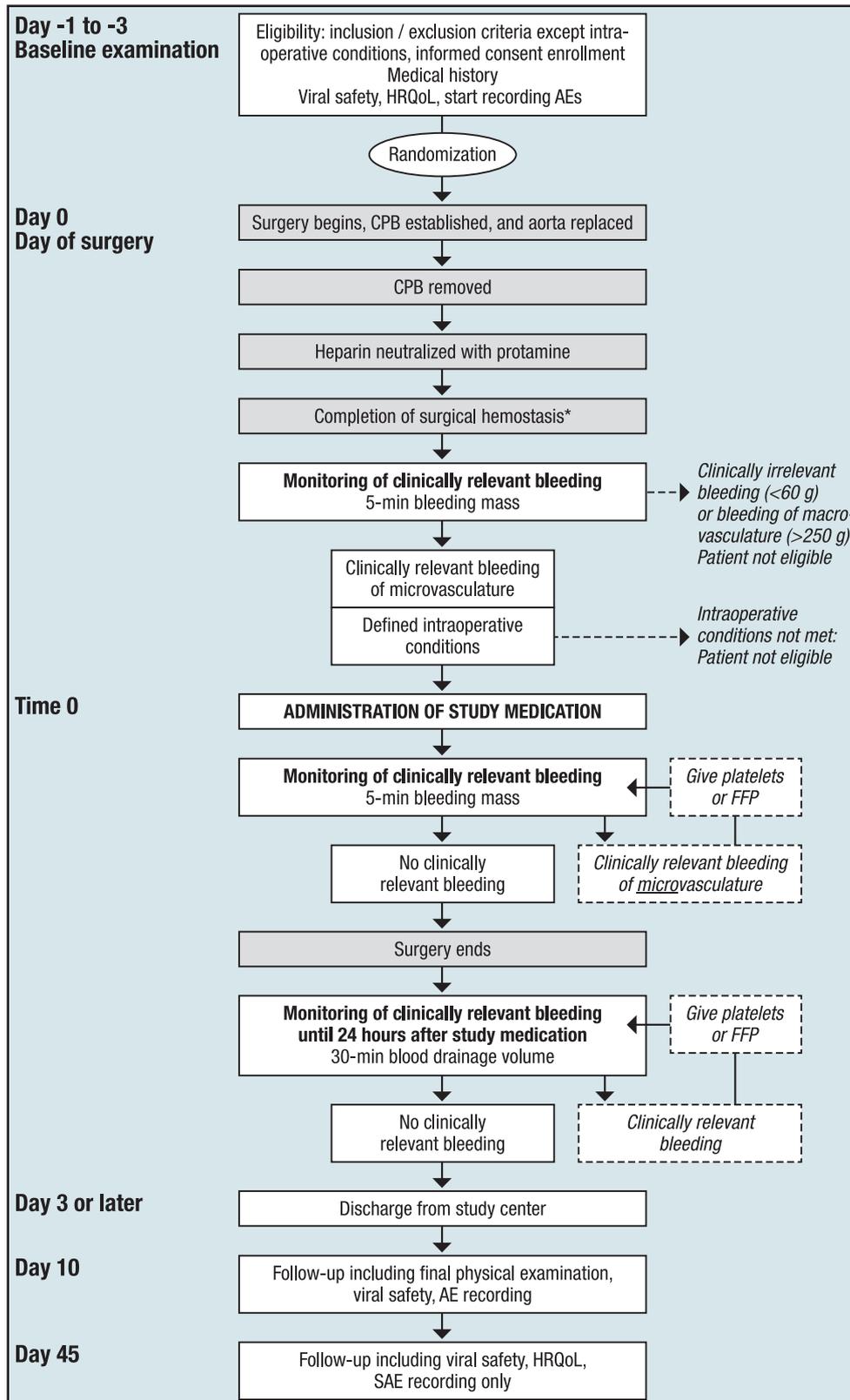
This phase 2, prospective, randomized, double-blind, placebo-controlled, parallel-group, stratified, clinical study was

conducted at a single center (Hannover Medical School, Hannover, Germany). It was approved by the Local Ethics Committee in Hannover, Germany, and by the German Regulatory Authorities; it was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice. The study was assigned Local Ethics Committee reference code no. 4891M-mono, EudraCT trial no. 2007-004612-31, and ClinicalTrials.gov identifier no. NCT00701142.

Patients aged 18 yr or older for whom elective aortic replacement surgery involving CPB was planned and who provided signed informed consent were enrolled between June 2008 and April 2010. All aortic valve operations with root/ascending aorta replacement (thoracic aortic aneurysm [TAA]), with or without aortic arch replacement, and thoracoabdominal replacements were eligible. Patients were excluded from the study if they had undergone previous surgery at the same aortic site, if they had a congenital or acquired coagulation disorder, if they had a myocardial infarction or stroke in the previous 2 months, or if they used aspirin, clopidogrel, or vitamin K antagonists in the previous 2–5 days. All patients received antifibrinolytic prophylaxis with tranexamic acid (30 mg/kg bodyweight as a preoperative loading dose, followed by 1 mg/kg bodyweight/h throughout surgery). CPB was established after aortic cannulation and administration of 400 IU/kg heparin (Heparin-Natrium-25000-ratiopharm®; Merckle GmbH, Blaubeuren, Germany).

### Procedures

Before surgery, patients were randomized to receive either fibrinogen concentrate or placebo; study medication was administered if clinically relevant bleeding occurred. This was defined as a 5-min bleeding mass of 60–250 g immediately after removal from CPB, neutralization of heparin with protamine sulfate (Protamin Valeant, Valeant Pharmaceuticals GmbH, Eschborn, Germany; protamine–heparin ratio of 1:1), and completion of surgical hemostasis, which includes suture placement and electrocautery (or diathermy) (fig. 1). Completion of surgical hemostasis consisted of surgical control of focal bleeding. Surgical hemostasis was deemed to be complete if no obvious sources of bleeding were identified. A 5-min bleeding mass was subsequently determined by weighing dry surgical cloths and compresses, applying them to the surgical area for 5 min and weighing them again. No irrigation of the operative field was performed during the measurement of bleeding mass. Bleeding mass in the range 60–250 g was considered to be clinically relevant and coagulopathic in nature; an upper limit was included because of the life-threatening nature of massive bleeding. The thresholds of 60 and 250 g were chosen based on clinical experience at the authors' center.<sup>17,18</sup> Randomized patients not eligible for treatment or withdrawn from the study before 24 h after the administration of study medication were replaced until there were sufficient patients to provide primary efficacy endpoint data for each study arm. Stratification was done according to surgery type (thoracoabdominal aortic aneurysm or TAA).



**Fig. 1.** Treatment algorithm and study design. Flowchart showing details of clinical steps, assessments performed, and treatments administered throughout the study. AE = adverse event; CPB = cardiopulmonary bypass; FFP = fresh frozen plasma; HRQoL = health-related quality of life; SAE = serious adverse event. \* Standard control step to ensure no obvious sources of surgical bleeding.

Study medication was prepared in 50-ml opaque syringes by the pharmacist, according to a randomization code generated by the contract research organization (Accovion GmbH, Eschborn, Germany) using SAS (SAS Institute, Cary, NC) (the investigator initially assigned a randomization number to each patient). The randomization ratio of the treatment arms in each stratum (thoracoabdominal aortic aneurysm and TAA) was 1:1, with a block size of 4. Each 50-ml syringe contained either 1 g fibrinogen concentrate (Haemocomplettan P<sup>®</sup>, Ria-STAP<sup>™</sup>; CSL Behring, Marburg, Germany) diluted in 50 ml sterile water, or an equivalent volume of 0.9% saline as placebo. The medication was delivered to the operation room upon request of the study investigator (anesthesiologist) and administered intravenously within 5 min of bleeding measurement. In order for study medication to be administered, patients had to fulfill the following conditions: activated clotting time less than 150 s, body temperature higher than 36°C, pH more than 7.3, and hemoglobin level higher than 8.5 g/dl. To ensure blinding, only the pharmacist had access to the randomization code; the use of opaque syringes ensured that fibrinogen concentrate and placebo were visually identical.

Doses were determined from the MCF of the FIBTEM test, using a model developed in previous studies for individualizing fibrinogen concentrate dosing.<sup>17,18</sup> The FIBTEM test was performed by point-of-care thromboelastometry (ROTEM<sup>®</sup> device; TEM International, Munich, Germany), using blood samples taken 20 min before the end of CPB. The time taken to obtain the MCF value was 15 min. The fibrinogen concentrate dose was calculated by the unblinded point-of-care laboratory staff as follows: Fibrinogen concentrate dose (g) = (target FIBTEM MCF – actual FIBTEM MCF) (mm) × (bodyweight [kg] / 70) × 0.5 g/mm.<sup>11,17,18</sup> The target FIBTEM MCF was 22 mm. None of the blinded personnel (investigator and staff of the operating room and ICU) had access to FIBTEM or fibrinogen concentration values after the administration of study medication.

Approximately 5 min after the administration of study medication, bleeding mass was measured again. A transfusion algorithm was initiated in patients with a bleeding mass of 60–250 g as follows: if, upon removal of the aortic clamp, platelet count was lower than 100,000/μl, 2 U of apheresis platelet concentrate was administered; if platelet count was 100,000/μl or higher, 4 U FFP was administered in approximately 10 min. Subsequently, 5-min bleeding mass was reevaluated. If clinically relevant bleeding continued, the patient was given the blood component (platelet concentrate/FFP) not administered after the first bleed, and 5-min bleeding mass was again measured. Transfusion packages of 1 U platelet concentrate and 2 U FFP were administered until the 5-min bleeding mass was less than 60 g. Upon completion of hemostatic therapy, the thorax was closed (last suture = end of surgery), and the patient was transferred to the ICU. In the event of blood drainage higher than 400 ml during 1 h in the ICU, 1 U platelet concentrate and 2 U FFP were administered. Erythrocytes were also administered after

CPB to maintain a hemoglobin level higher than 8.5 g/dl. Fibrinogen levels were measured using the Clauss assay, before and after the administration of study medication.

### Study Endpoints

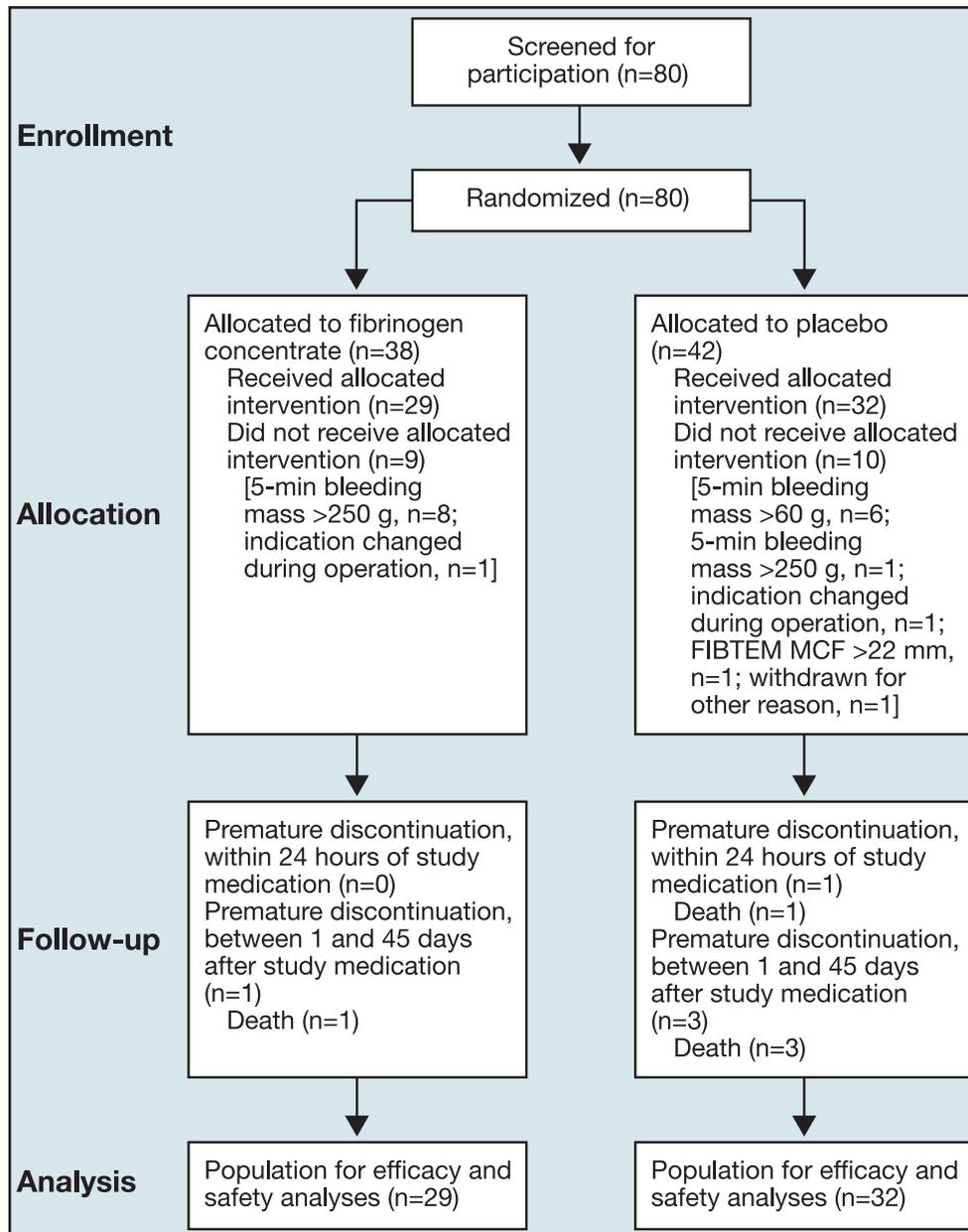
The primary endpoint was the total number of units of allogeneic blood components (erythrocytes plus FFP plus platelet concentrate) given to patients between the administration of study medication and 24 h thereafter. Secondary endpoints included the number of units of each individual allogeneic blood component given (erythrocytes, FFP, and platelet concentrate), the proportion of patients who received no allogeneic blood components (total avoidance), the number of days not in the ICU or hospital during the 45 days after surgery (assumed to be zero for patients dying within that period), and mortality at 45 days after surgery.

Safety was assessed principally by treatment-emergent adverse events occurring within 10 days of treatment. The follow-up period for serious adverse events was 45 days. Patients were monitored for viral seroconversion by hepatitis A virus, hepatitis B virus, hepatitis B core, hepatitis B surface antigen, hepatitis C virus, human immunodeficiency virus 1 and 2, and parvovirus B19 testing (enzyme immunoassays and polymerase chain reaction were used). In accordance with Good Clinical Practice, the study sponsor evaluated safety data throughout the study.

### Statistical Analysis

Sixty patients were planned for the primary endpoint based on an assumed difference between the treatment group means of  $4.25 \pm 5.3$  U (where 5.3 is the SD) with a two-sided type I error rate of 5% and more than 80% power for the nonparametric Wilcoxon rank sum test. These assumptions were derived from a previously reported average transfusion of  $8.5 \pm 5.3$  U during TAA surgery.<sup>17</sup> We assumed that the use of fibrinogen concentrate would reduce transfusion of allogeneic blood products by 50% compared with the standard-of-care group. The efficacy and safety analyses included all patients who were randomized and received study treatment. This was not strictly an intention-to-treat analysis because of the need to randomize patients before ascertaining whether they met the study inclusion criteria (if randomization had been performed after establishing that patients met the study inclusion criteria, hemostatic therapy would have been delayed unethically).

Inferential testing of the primary efficacy endpoint was based on a nonparametric Wilcoxon rank sum test with a two-sided type I error rate of 5%, testing the null hypothesis of no difference between the treatment groups. An unstratified Hodges–Lehman point estimate and the corresponding two-sided 95% CI for the treatment difference were also calculated. The primary endpoint was found to have a positively skewed nonnormal distribution. The proportion of patients receiving no allogeneic blood components within the first 24 h after start of infusion of study medication was analyzed in an exploratory manner applying a chi-square test.



**Fig. 2.** Patient disposition. Flowchart showing patient numbers throughout the study, including details of withdrawals and the analysis population. FIBTEM = fibrin-based thromboelastometry test; MCF = maximum clot firmness.

Exploratory analysis was performed for selected secondary endpoints (24-h transfusion of erythrocytes, FFP, and platelet concentrate [each component analyzed separately]). Additional analyses were performed for fibrinogen and hemoglobin using the two-sample *t* test for normally distributed outcomes and the Wilcoxon rank sum test for outcomes with a skewed distribution.

All other secondary efficacy endpoints were analyzed by descriptive statistics, with values typically presented as mean  $\pm$  SD. For the between-group comparison of safety outcomes, risk ratios with 95% CIs were calculated where possible (nonzero incidence).

Data for the main efficacy endpoints are presented as median (with interquartile range, between the 25th and the

75th percentiles) values because the data were not normally distributed. SAS version 9.1.3 (SAS Institute) was used for all of the statistical analyses, except the Wilcoxon rank sum test, which was performed using StatXact (version 8.1; Cytel, Cambridge, MA).

## Results

### Patient Disposition

Of 80 patients screened, 61 were randomized and treated (fig. 2). Of these, 18 patients underwent thoracoabdominal aortic aneurysm surgery, 22 underwent TAA with arch surgery, and 21 underwent TAA without arch surgery (table 1). The two treatment groups had similar perioperative

characteristics, and the population was typical for patients undergoing aortic surgery (table 1). Preoperative mean  $\pm$  SD FIBTEM MCF was  $17.9 \pm 6.1$  mm in the fibrinogen concentrate group and  $16.4 \pm 3.8$  mm in the placebo group.

### Dose of Study Medication

On the basis of MCF values from the FIBTEM test performed during CPB, a median dose of 8 g (interquartile range [IQR], 6–9 g) of fibrinogen concentrate was administered to patients in the fibrinogen concentrate group (median volume, 400 ml; IQR, 300–450 ml). The median

volume of study medication administered to the placebo group was also 400 ml (IQR, 300–450 ml). These doses were calculated from the FIBTEM MCF of samples taken approximately 20 min before removal from CPB; the mean MCF was  $9.7 \pm 3.1$  mm in the fibrinogen concentrate group and  $9.5 \pm 2.7$  mm in the placebo group. As shown in table 1, the two study groups were also comparable immediately before the administration of study medication (after removal from CPB, administration of protamine, and completion of surgical hemostasis), in terms of 5-min bleeding mass and activated clotting time.

**Table 1.** Patients' Characteristics and Perioperative Data (Fibrinogen Concentrate, N = 29; Placebo, N = 32)

	Fibrinogen Concentrate	Placebo
Males, n (%)	19 (66)	25 (78)
Females, n (%)	10 (34)	7 (22)
Age, mean $\pm$ SD, yr	59 $\pm$ 14	61 $\pm$ 12
Weight, mean $\pm$ SD, kg	88 $\pm$ 18	87 $\pm$ 20
Body mass index, mean $\pm$ SD, kg/m <sup>2</sup>	28 $\pm$ 5	28 $\pm$ 5
Obesity (body mass index >30 kg/m <sup>2</sup> ), n (%)	7 (24)	7 (22)
Diabetes mellitus, n (%)	2 (7)	1 (3)
Hypertension, n (%)	21 (72)	25 (78)
Hyperlipidemia, n (%)	7 (24)	7 (22)
Previous myocardial infarction, n (%)	9 (31)	6 (19)
Previous heart operation, n (%)	5 (17)	2 (6)
Smoking, n (%)	8 (28)	8 (25)
Preoperative data		
FIBTEM MCF, mean $\pm$ SD, mm	18 $\pm$ 6.1	16 $\pm$ 3.8
Plasma fibrinogen concentration, mean $\pm$ SD, mg/dl	304 $\pm$ 99	289 $\pm$ 63
Hemoglobin concentration, mean $\pm$ SD, g/dl	12.5 $\pm$ 1.3	13.2 $\pm$ 1.3
Platelet count, median (IQR), $\times 10^9/l$	208 (182–226)	185 (166–223)
aPTT, median (IQR), s	31 (29–32)	32 (30–34)
PT, median (IQR), s	14.2 (13.8–14.8)	14.4 (13.7–14.9)
Antithrombin, median (IQR), %	82 (76–90)	81 (74–90)
Operation data		
CPB time, mean $\pm$ SD, min	48 $\pm$ 7	51 $\pm$ 5
Operation type TAAA, n (%)	8 (28)	10 (31)
Operation type TAA with arch, n (%)	12 (41)	10 (31)
Operation type TAA without arch, n (%)	9 (31)	12 (38)
5-min bleeding mass before administration of study medication, mean $\pm$ SD, g	121 $\pm$ 50	116 $\pm$ 46
Activated clotting time before administration of study medication, mean $\pm$ SD, s	134 $\pm$ 12	130 $\pm$ 14
Postoperative outcome data		
ICU-free time during first 45 days postoperatively, median (IQR), days	43.1 (40.2–44.1)*	42.7 (41.1–44.1)†
Hospitalization-free time during first 45 days postoperatively, median (IQR), days	31 (24–35)*	33 (24–36)†
Blood drainage volume between last suture and 24 h after study medication, median (IQR), ml	650 (480–1,305)	850 (655–1,565)

Data are presented as mean  $\pm$  SD, median (IQR), or absolute and percentage frequency.

\* One patient died during the 45-day period; a value of zero was attributed to this patient for ICU- and hospitalization-free time.

† Four patients died during the 45-day period; a value of zero was attributed to these patients for ICU- and hospitalization-free time. aPTT = activated partial thromboplastin time; CPB = cardiopulmonary bypass; FIBTEM = fibrin-based thromboelastometry test; ICU = intensive care unit; IQR = interquartile range; MCF = maximum clot firmness; N = total number of patients; n = number of patients in given category; PT = prothrombin time; TAA = thoracic aortic aneurysm; TAAA = thoracoabdominal aortic aneurysm.

**Table 2.** Efficacy Endpoints

Endpoint	Fibrinogen Concentrate		Placebo		Point Estimate*	P Value
	N	Value	N	Value		
<b>Primary endpoint</b>						
No. units of allogeneic blood components within 24 h after infusion of study medication, median (IQR)						
All patients	29	2 (0–8)	32	13 (8–21)	–9 (–13 to –6)	<0.001
TAAA	8	2 (0–5)	10	17 (10–26)	NC†	NC†
TAA with arch surgery	12	2 (0–13)	10	14 (9–21)	NC†	NC†
TAA without arch surgery	9	0 (0–7)	12	9 (7–14)	NC†	NC†
<b>Secondary endpoints</b>						
No. patients with total avoidance of allogeneic blood components, n (%)	29	13 (45%)	32	0 (0%)	NC	<0.001
Units of packed red blood cells (erythrocytes), median (IQR)	29	0 (0–3)	32	2 (2–5)	–2 (–2 to 0)	0.007
Units of FFP, median (IQR)	29	0 (0–4)	32	8 (4–10)	–5 (–8 to –4)	<0.001
Units of platelet concentrate, median (IQR)‡	29	0 (0–2)	32	4 (2–5)	–2 (–3 to –2)	<0.001

\* Unstratified Hodges–Lehmann point estimate and corresponding nonparametric 95% CI calculated for determination of the reduction. † Point estimates and P values were not calculated for these subgroups because of assumed small sample size and between-group imbalances. ‡ One unit of apheresis platelet concentrate = 6–8 U of single donor platelets.

FFP = fresh frozen plasma; IQR = interquartile range; N = total number of patients; NC = not calculated; TAA = thoracic aortic aneurysm; TAAA = thoracoabdominal aortic aneurysm.

### Primary and Secondary Efficacy Endpoints

During the 24-h period after start of study medication, patients treated with fibrinogen concentrate received fewer units of allogeneic blood components (median, 2 U; IQR, 0–8 U) than patients treated with placebo (median, 13 U; IQR, 8–21 U); the treatment difference (–9 U; 95% CI, –13 to –6 U) was statistically significant ( $P < 0.001$ , table 2). A sensitivity analysis was performed, excluding patients who dropped out within 24 h of the administration of study medication (one patient in the placebo group). This showed a similar treatment difference (–8 U; 95% CI, –13 to –6 U). The treatment difference was also generally similar for each of the aortic replacement procedures included in the study. The number of units of erythrocytes, FFP, and platelet concentrate administered during the 24 h after start of study medication was lower in the fibrinogen concentrate group than that in the placebo group (table 2). These treatment differences were statistically significant for erythrocytes (reduction, –2 U;  $P = 0.007$ ), FFP (reduction, –5 U;  $P < 0.001$ ) and platelet concentrate (reduction, –2 U;  $P < 0.001$ ).

Total avoidance of transfusion was achieved in 13 of 29 patients (45%) in the fibrinogen concentrate group; in contrast, 32 of 32 patients (100%) in the placebo group received treatment with allogeneic blood components to control their coagulopathic bleeding ( $P < 0.001$ ). The mean fibrinogen concentration and the mean FIBTEM MCF were similar in the two study groups at all time points except for

the first measurement after the administration of study drug (at the “last suture” time point, *i.e.*, end of surgery), when higher values were observed in the fibrinogen concentrate group (fibrinogen level, 260 *vs.* 189 mg/dl;  $P < 0.001$ ; table 3; FIBTEM MCF, 16.2 ± 2.8 *vs.* 11.5 ± 3.3 mm;  $P < 0.001$ ). There was no between-group difference in hemoglobin at any time point (table 3). Numbers of ICU-free days and hospital-free days were also similar in the two study groups (table 1).

### Safety

Similar proportions of patients in each treatment group reported treatment-emergent adverse events (relative risk, 1.0; table 4). The majority of treatment-emergent adverse events reported were typical for patients undergoing cardiac surgery, the most common ones being pleural effusion and atrial fibrillation. None of the treatment-emergent adverse events reported in the study was considered to be related to study medication, and none led to discontinuation from the study. Reoperation because of persistent bleeding was undertaken in four patients in the fibrinogen concentrate group and four patients in the placebo group. Although this constituted a relative risk of 4.4, there was no significant between-group difference. In all five patients, a surgical source of bleeding was identified during reoperation.

The incidence of serious adverse events was similar in both study groups (relative risk, 1.1). More serious adverse events led to death in the placebo group *versus*

**Table 3.** Changes in Fibrinogen and Hemoglobin Levels over Time (Fibrinogen Concentrate, N = 29; Placebo, N = 32)

Time Point	Fibrinogen Concentrate	Placebo	Difference
Fibrinogen, mg/dl, plasma			
Before surgery	304 ± 99	289 ± 63	16 (–26 to 58)
20 min before removal of CPB	166 ± 38	161 ± 36	5 (–14 to 24)
Immediately after removal from CPB (before administration of study medication)	157 ± 40	155 ± 40	–2 (–18 to 23)
At time of last suture ( <i>i.e.</i> , end of surgery, after administration of study medication)	260 ± 48	189 ± 34	70 (49 to 91)*
1 day after surgery	343 ± 74	333 ± 54†	10 (–24 to 43)
Hemoglobin, g/dl			
Before surgery	12.5 ± 1.3	13.2 ± 1.3	–0.6 (–1.3 to 0.0)
20 min before removal of CPB	9.4 ± 1.0	9.6 ± 1.1	–0.2 (–0.8 to 0.3)
Immediately after removal from CPB	9.7 ± 0.9	10.0 ± 0.9	–0.3 (–0.8 to 0.2)
At time of last suture	10.0 ± 1.1	9.7 ± 1.6	0.3 (–0.4 to 1.0)
1 day after surgery	10.4 ± 1.1	10.1 ± 0.9‡	0.3 (–0.2 to 0.9)

Data are presented as mean ± SD, except “difference,” which is presented as mean (95% CI).

\* Statistically significant difference between groups ( $P < 0.001$ ). †  $n = 31$  (data unavailable for one patient who died within 24 h). ‡  $n = 30$  (hemoglobin data were unavailable at this time point for two patients, including one who died within 24 h).

CPB = cardiopulmonary bypass.

the fibrinogen concentrate group (four patients *vs.* one patient, respectively). Although the relative risk was 0.3, there was no significant between-group difference. None of the serious adverse events was considered related to study medication. Cardiorespiratory arrest (day 1) and later death due to brain herniation and myocardial infarction (day 28) were reported in a 77-yr-old fibrinogen concentrate patient with a previous history of myocardial infarction, coronary angioplasty, and obesity who underwent repair of TAA and arch. Cardiorespiratory arrest was reported while the patient was being transferred for a computed tomography scan with unstable respiratory status. Resuscitation was successful.

Except for this patient, no potentially thromboembolic events were reported in the fibrinogen concentrate group. In the placebo group, two thromboembolic events were

reported (one pulmonary embolism and one air embolism). There were no viral transmissions in either study group.

## Discussion

This study demonstrates that fibrinogen concentrate, administered intraoperatively as targeted first-line hemostatic intervention, reduces the need for transfusion of allogeneic blood products in patients undergoing complex aortic surgery. The median transfusion of allogeneic blood components was reduced by 85% among patients receiving fibrinogen concentrate (2U transfused) compared with placebo (13U transfused). Total avoidance of allogeneic blood components in 13 of 29 patients (45%) treated with fibrinogen concentrate

**Table 4.** Major Safety Endpoints (Fibrinogen Concentrate, N = 29; Placebo, N = 32)

Safety Endpoint	Fibrinogen Concentrate	Placebo	Relative Risk
TEAEs (10-day follow-up)	24 (83%)	27 (84%)	1.0 (0.8–1.2)
SAEs (45-day follow-up)	5 (17%)	5 (16%)	1.1 (0.4–3.4)
SAEs leading to death	1 (3%)	4 (13%)	0.3 (0.0–2.3)
Myocardial infarction	1 (3%)*	0	NC
Cardiorespiratory arrest	0	1 (3%)	NC
Cerebral hemorrhage	0	1 (3%)	NC
Cerebral infarction	0	1 (3%)	NC
Operative hemorrhage	0	1 (3%)	NC
Reoperation because of surgical bleeding	4 (14%)	4 (13%)	1.1 (0.3–4.0)
Viral transmissions	0	0	NC

Data are presented as n (%) of patients, except for relative risk, which is presented with the 95% CI in parentheses.

\* Reported as follow-up TEAE, 28 days after infusion of fibrinogen concentrate; cause of death was brain herniation and myocardial infarction.

N = total number of patients; NC = not calculated; SAE = serious adverse event; TEAE = treatment-emergent adverse event.

is of high clinical relevance, considering that all patients in the placebo group received allogeneic blood components.

These outcomes may be attributable to plasma fibrinogen concentration being raised more quickly and to higher levels among patients receiving fibrinogen concentrate compared with those who received placebo. The use of fibrinogen concentrate instead of allogeneic blood products has important clinical implications. Fibrinogen concentrate is immediately available for reconstitution, with no need for thawing or blood group matching, and has a low administration volume.<sup>20</sup> It can therefore be administered in less time than either FFP or cryoprecipitate, decreasing the time to control bleeding. In the context of severe bleeding, the administration of 6 g fibrinogen concentrate in 1–2 min has been reported feasible.<sup>11</sup> In contrast, FFP administration causes hemodilution, and the concentration of fibrinogen in FFP (approximately 200 mg/dl)<sup>21</sup> limits the extent to which fibrinogen levels can be increased. Cryoprecipitate provides a considerably higher concentration of fibrinogen (300–600 mg in 20–50 ml,<sup>24</sup> corresponding to a range of 600–3000 mg/dl), but this concentration is more variable than with fibrinogen concentrate, meaning that doses cannot be controlled as accurately.<sup>25</sup> In addition, as with FFP, blood group matching is required before administering cryoprecipitate.<sup>25</sup> The use of fibrinogen concentrate along with other coagulation factor concentrates such as prothrombin complex concentrate, in the context of a treatment algorithm based on viscoelastic point-of-care coagulation testing, was recently shown to decrease blood transfusion and thrombotic events among cardiovascular surgery patients.<sup>19</sup> This approach is of particular interest because it enables the comprehensive treatment of coagulopathy using coagulation factor concentrates, with the potential for larger reductions in allogeneic blood transfusions than can be achieved with fibrinogen alone.

Postoperative data show that the increase in fibrinogen levels, relative to the placebo group, was only transitory (less than 24 h), and this may be viewed positively from a safety perspective. The safety profile of fibrinogen concentrate was similar to that of placebo. This study was not designed to detect differences between fibrinogen concentrate and placebo in morbidity or mortality. Nevertheless, the lack of conclusive between-group differences in safety is consistent with the previously reported safety of fibrinogen concentrate, including a very low risk of thromboembolic complications<sup>26</sup> and an excellent margin of virus safety.<sup>27</sup> Four patients in the fibrinogen concentrate group underwent reoperation compared with one in the placebo group. Surgical bleeding was identified in all five of these cases. In general, the possible reasons for reoperation are multifactorial, and there is no indication in this study that the reasons were related to study medication. The reoperation data are inconsistent with the overall reduction in the use of allogeneic blood products in the fibrinogen concentrate group. Importantly, all allogeneic blood products administered within 24 h of study

medication were included in the primary endpoint, including those used during and after reoperation, and all patients left the operating room with a 5-min bleeding mass less than 60 g.

This is the largest randomized, double-blind, placebo-controlled study of fibrinogen concentrate therapy. It is the first such study of fibrinogen concentrate administered intraoperatively as first-line hemostatic therapy, and the first placebo-controlled study of fibrinogen concentrate among patients undergoing complex cardiovascular surgery. Only two previous prospective randomized clinical studies of fibrinogen concentrate have been performed.<sup>14,16</sup> In the first one, patients undergoing cystectomy (n = 20) with blood loss substituted by hydroxyethyl starch exhibited a significant increase in MCF after fibrinogen supplementation (45 mg/kg) versus placebo. Transfusion and blood loss were similar in the two groups during surgery, whereas erythrocytes were administered to eight patients in the placebo group versus two in the fibrinogen group postoperatively ( $P < 0.05$ ). The second study, performed in 20 coronary bypass graft patients, showed that the preoperative, prophylactic administration of fibrinogen concentrate (2 g) reduced blood loss by 32% ( $P = 0.010$ ).<sup>16</sup> Blood transfusions were undertaken in one patient in the fibrinogen group compared with three in the placebo group. The present study cohort was relatively homogeneous, and the different types of operation were equally distributed between the two treatment groups, ensuring that they were comparable. Assessments such as fibrinogen concentration and prothrombin time confirm the comparability of the two groups before the administration of study medication. By administering study medication intraoperatively, patients received study medication during a period when, if treatment consisted only of allogeneic blood products, the patient would only have been monitored while blood products are prepared. Consequently, this placebo-controlled study may also be considered as a comparison of fibrinogen concentrate with standard hemostatic treatment based on FFP and platelet concentrate.

Dosing in our study was based on viscoelastic point-of-care coagulation testing to obtain results more quickly than standard laboratory parameters.<sup>13,22</sup> Bleeding management guidelines are beginning to advocate the use of viscoelastic methods.<sup>28–30</sup> However, consideration is required as to which viscoelastic methods are used: recent data showed that simultaneous performance of several ROTEM assays (*e.g.*, INTEM, EXTEM, FIBTEM, and HEPTM) is better able to distinguish different coagulopathies than a single kaolin-activated thrombelastography assay.<sup>31</sup> FIBTEM is a viscoelastic test designed to measure firmness of a clot formed in the presence of a platelet inhibitor (*i.e.*, fibrin-based clot firmness).<sup>11,23</sup> Importantly, FIBTEM does not provide a measurement of fibrinogen concentration. Firmness of the fibrin-based clot is measured in SI units (Système International d'Unités) that are completely different from those of fibrinogen concentration (dyne/cm<sup>2</sup> or gigapascal,

as opposed to g/l or mol/l).<sup>32</sup> Nevertheless, studies have shown correlation between FIBTEM MCF and measurements of fibrinogen concentration.<sup>13,22</sup> The aim was to restore FIBTEM MCF to 22 mm, higher than the mean preoperative baseline level of 16–18 mm and at the high end of the normal range (9–25 mm). This target was chosen to allow fibrinogen concentrate to be effective as a pharmacological hemostatic agent. There is evidence that fibrinogen deficiency is the primary problem associated with hemodilution,<sup>6</sup> and that fibrinogen supplementation may compensate for reduced platelet levels.<sup>8,33</sup> Twenty-two millimeters typically corresponds to a fibrinogen level of approximately 360 mg/dl,<sup>18</sup> which is within the normal range of 163–458 mg/dl<sup>34</sup> and similar to the normal value of 330 mg/dl reported for patients older than 60 yr.<sup>35</sup> Historically, the threshold plasma concentration for fibrinogen supplementation was set at 100 mg/dl.<sup>36,37</sup> Newer recommendations give this threshold as more than 150 mg/dl.<sup>20,30,38</sup> A higher target may be appropriate because clot firmness continues to increase with fibrinogen concentration throughout the normal range.<sup>7</sup>

The present study has several limitations. Our analysis does not strictly meet the definition for intention to treat. To avoid delaying treatment, patients were randomized to treatment before the presence of clinically significant coagulopathic bleeding was determined. Selection bias was not introduced because patients' entry to the study was based on predefined, objective criteria as opposed to clinical judgment, and because the criteria were the same for both study groups. The use of 5-min bleeding mass as a study inclusion criterion and as a trigger for the transfusion of allogeneic blood products provides an objective assessment of bleeding rate, but the authors recognize that it is not the current standard trigger for hemostatic intervention and that it may not be applicable in all clinical settings. However, we are confident this method allowed accurate and reproducible measurement of blood loss in the present setting. Patients with preoperative coagulation disorders or anticoagulation therapy were excluded, meaning that the applicability of the results to such patients may be limited. However, homogeneity of the study cohort was increased, enhancing comparison of the two treatment groups. This study is also limited by its small sample size and use of a single center. Thus, the robust assessment of safety (*i.e.*, low-frequency adverse events) is not possible. However, the sample size was determined statistically from an assumption that the use of allogeneic blood products would be reduced by 50%, a reduction of clinical and practical relevance. Also, while the use of a single center may limit applicability to other centers, it will also have reduced variability in personnel and clinical procedures. There is now a need for further studies of fibrinogen concentrate in different surgical fields, in patients with different preoperative conditions, and in larger numbers of patients to analyze factors that may influence efficacy and to confirm the observed safety profile. Ideally, such studies

would be randomized and controlled with true intention-to-treat analysis, while also remaining ethical.

In conclusion, this study in patients undergoing aortic replacement surgery has shown that targeted first-line treatment with fibrinogen concentrate is able to reduce the transfusion of allogeneic blood products. If confirmed in larger, multicenter studies, fibrinogen concentrate administration would provide a concrete means of reducing or eliminating transfusions. Consequently, this therapeutic approach has the potential to change the treatment paradigm for perioperative bleeding in patients with potentially life-threatening coagulopathy.

Medical writing support was provided by Ken Sutor, B.Sc. (Meridian HealthComms Ltd, Cheshire, United Kingdom), and Julia Forjanic Klapproth, Ph.D. (Trilogy Writing & Consulting GmbH, Frankfurt, Germany). The authors thank Brigitte Buchalik (Medical Technician, Clinic for Anesthesiology and Intensive Care Medicine, Hannover Medical School, Hannover, Germany) for her important contribution to this study: blood sample collection, performance of coagulation tests, and data collection.

## References

1. Diaz-Martin A, Escoresca-Ortega AM, Hernandez-Caballero C, Pena M, Adsuar-Gómez A, Herruzo-Aviles A, Hinojosa-Perez R: Considerations regarding major bleeding after cardiac transplantation. *Transplant Proc* 2010; 42:3204–5
2. Mannucci PM, Levi M: Prevention and treatment of major blood loss. *N Engl J Med* 2007; 356:2301–11
3. Murad MH, Stubbs JR, Gandhi MJ, Wang AT, Paul A, Erwin PJ, Montori VM, Roback JD: The effect of plasma transfusion on morbidity and mortality: A systematic review and meta-analysis. *Transfusion* 2010; 50:1370–83
4. Murphy GJ, Reeves BC, Rogers CA, Rizvi SI, Culliford L, Angelini GD: Increased mortality, postoperative morbidity, and cost after red blood cell transfusion in patients having cardiac surgery. *Circulation* 2007; 116:2544–52
5. Spiess BD: Platelet transfusions: The science behind safety, risks and appropriate applications. *Best Pract Res Clin Anaesthesiol* 2010; 24:65–83
6. Bolliger D, Szlam F, Molinaro RJ, Rahe-Meyer N, Levy JH, Tanaka KA: Finding the optimal concentration range for fibrinogen replacement after severe haemodilution: An *in vitro* model. *Br J Anaesth* 2009; 102:793–9
7. Dempfle CE, Kälsch T, Elmas E, Suvajac N, Lücke T, Münch E, Borggrefe M: Impact of fibrinogen concentration in severely ill patients on mechanical properties of whole blood clots. *Blood Coagul Fibrinolysis* 2008; 19:765–70
8. Lang T, Johanning K, Metzler H, Piepenbrock S, Solomon C, Rahe-Meyer N, Tanaka KA: The effects of fibrinogen levels on thromboelastometric variables in the presence of thrombocytopenia. *Anesth Analg* 2009; 108:751–8
9. Solomon C, Rahe-Meyer N, Sørensen B: Fibrin formation is more impaired than thrombin generation and platelets immediately following cardiac surgery. *Thromb Res* 2011; 128:277–82
10. Blome M, Isgro F, Kiessling AH, Skuras J, Haubelt H, Hellstern P, Saggau W: Relationship between factor XIII activity, fibrinogen, haemostasis screening tests and postoperative bleeding in cardiopulmonary bypass surgery. *Thromb Haemost* 2005; 93:1101–7
11. Solomon C, Pichlmaier U, Schöchel H, Hagl C, Raymondos K, Scheinichen D, Koppert W, Rahe-Meyer N: Recovery of

- fibrinogen after administration of fibrinogen concentrate to patients with severe bleeding after cardiopulmonary bypass surgery. *Br J Anaesth* 2010; 104:555–62
12. Karlsson M, Ternström L, Hyllner M, Baghaei F, Nilsson S, Jeppsson A: Plasma fibrinogen level, bleeding, and transfusion after on-pump coronary artery bypass grafting surgery: A prospective observational study. *Transfusion* 2008; 48:2152–8
  13. Reinhöfer M, Brauer M, Franke U, Barz D, Marx G, Lösche W: The value of rotation thromboelastometry to monitor disturbed perioperative haemostasis and bleeding risk in patients with cardiopulmonary bypass. *Blood Coagul Fibrinolysis* 2008; 19:212–9
  14. Fenger-Eriksen C, Jensen TM, Kristensen BS, Jensen KM, Tønnesen E, Ingerslev J, Sørensen B: Fibrinogen substitution improves whole blood clot firmness after dilution with hydroxyethyl starch in bleeding patients undergoing radical cystectomy: A randomized, placebo-controlled clinical trial. *J Thromb Haemost* 2009; 7:795–802
  15. Fenger-Eriksen C, Lindberg-Larsen M, Christensen AQ, Ingerslev J, Sørensen B: Fibrinogen concentrate substitution therapy in patients with massive haemorrhage and low plasma fibrinogen concentrations. *Br J Anaesth* 2008; 101:769–73
  16. Karlsson M, Ternström L, Hyllner M, Baghaei F, Flinck A, Skrtic S, Jeppsson A: Prophylactic fibrinogen infusion reduces bleeding after coronary artery bypass surgery. A prospective randomised pilot study. *Thromb Haemost* 2009; 102:137–44
  17. Rahe-Meyer N, Pichlmaier M, Haverich A, Solomon C, Winterhalter M, Piepenbrock S, Tanaka KA: Bleeding management with fibrinogen concentrate targeting a high-normal plasma fibrinogen level: A pilot study. *Br J Anaesth* 2009; 102:785–92
  18. Rahe-Meyer N, Solomon C, Winterhalter M, Piepenbrock S, Tanaka K, Haverich A, Pichlmaier M: Thromboelastometry-guided administration of fibrinogen concentrate for the treatment of excessive intraoperative bleeding in thoracoabdominal aortic aneurysm surgery. *J Thorac Cardiovasc Surg* 2009; 138:694–702
  19. Görlinger K, Dirkmann D, Hanke AA, Kamler M, Kottenberg E, Thielmann M, Jakob H, Peters J: First-line therapy with coagulation factor concentrates combined with point-of-care coagulation testing is associated with decreased allogeneic blood transfusion in cardiovascular surgery: A retrospective, single-center cohort study. *ANESTHESIOLOGY* 2011; 115:1179–91
  20. Fenger-Eriksen C, Ingerslev J, Sørensen B: Fibrinogen concentrate—a potential universal hemostatic agent. *Expert Opin Biol Ther* 2009; 9:1325–33
  21. Ketchum L, Hess JR, Hiippala S: Indications for early fresh frozen plasma, cryoprecipitate, and platelet transfusion in trauma. *J Trauma* 2006; 60(6 Suppl):S51–8
  22. Rugeri L, Levrat A, David JS, Delecroix E, Floccard B, Gros A, Allaouchiche B, Negrier C: Diagnosis of early coagulation abnormalities in trauma patients by rotation thrombelastography. *J Thromb Haemost* 2007; 5:289–95
  23. Solomon C, Sørensen B, Hochleitner G, Kashuk J, Ranucci M, Schöchl H: Comparison of whole blood fibrin-based clot tests in thrombelastography and thromboelastometry. *Anesth Analg* 2012; 114:721–30
  24. Summary information about blood products and haemostatic agents, Handbook of Transfusion Medicine. 4th edition. Edited by McClelland DBL. Norwich: The Stationery Office, 2007, pp 7–15
  25. Sørensen B, Bevan D: A critical evaluation of cryoprecipitate for replacement of fibrinogen. *Br J Haematol* 2010; 149:834–43
  26. Dickneite G, Pragst I, Joch C, Bergman GE: Animal model and clinical evidence indicating low thrombogenic potential of fibrinogen concentrate (Haemocomplettan P). *Blood Coagul Fibrinolysis* 2009; 20:535–40
  27. Manco-Johnson MJ, Dimichele D, Castaman G, Fremann S, Knaub S, Kalina U, Peyvandi F, Piseddu G, Mannucci P; Fibrinogen Concentrate Study Group: Pharmacokinetics and safety of fibrinogen concentrate. *J Thromb Haemost* 2009; 7:2064–9
  28. Craig J, Aguiar-Ibanez R, Bhattacharya S, Downie S, Duff S, Kohli H, Nimmo A, Trueman P, Wilson S, Yunni Y: The clinical and cost effectiveness of thromboelastography/thromboelastometry. *Health Technol Assess* 2008; 11:1–70
  29. Fries D, Innerhofer P, Perger P, Gütl M, Heil S, Hofmann N, Kneifel W, Neuner L, Pernerstorfer T, Pfanner G, Schöchl H, Ziegler B, Kölblinger C, Kozek-Langenecker S: [Coagulation management in trauma-related massive bleeding. Recommendations of the Task Force for Coagulation (AGPG) of the Austrian Society of Anesthesiology, Resuscitation and Intensive Care Medicine (OGARD)]. *Anesthesiol Intensivmed Notfallmed Schmerzther* 2010; 45:552–61
  30. Rossaint R, Bouillon B, Cerny V, Coats TJ, Duranteau J, Fernández-Mondéjar E, Hunt BJ, Komadina R, Nardi G, Neugebauer E, Ozier Y, Riddez L, Schultz A, Stahel PF, Vincent JL, Spahn DR; Task Force for Advanced Bleeding Care in Trauma: Management of bleeding following major trauma: An updated European guideline. *Crit Care* 2010; 14:R52
  31. Larsen OH, Fenger-Eriksen C, Christiansen C, Ingerslev J, Sørensen B: Diagnostic performance and therapeutic consequence of thromboelastometry activated by kaolin *versus* a panel of specific reagents. *ANESTHESIOLOGY* 2011; 115:294–302
  32. Solomon C, Cadamuro J, Ziegler B, Schöchl H, Varvenne M, Sørensen B, Hochleitner G, Rahe-Meyer N: A comparison of fibrinogen measurement methods with fibrin clot elasticity assessed by thromboelastometry, before and after administration of fibrinogen concentrate in cardiac surgery patients. *Transfusion* 2011; 51:1695–706
  33. Velik-Salchner C, Haas T, Innerhofer P, Streif W, Nussbaumer W, Klingler A, Klima G, Martinowitz U, Fries D: The effect of fibrinogen concentrate on thrombocytopenia. *J Thromb Haemost* 2007; 5:1019–25
  34. Lowe GD, Rumley A, Woodward M, Morrison CE, Philippou H, Lane DA, Tunstall-Pedoe H: Epidemiology of coagulation factors, inhibitors and activation markers: The Third Glasgow MONICA Survey. I. Illustrative reference ranges by age, sex and hormone use. *Br J Haematol* 1997; 97:775–84
  35. Coppola L, Caserta F, De Lucia D, Guastafierro S, Grassia A, Coppola A, Marfella R, Varricchio M: Blood viscosity and aging. *Arch Gerontol Geriatr* 2000; 31:35–42
  36. Practice Guidelines for Perioperative Blood Transfusion and Adjuvant Therapies: An updated report by the American Society of Anesthesiologists Task Force on Perioperative Blood Transfusion and Adjuvant Therapies. *ANESTHESIOLOGY* 2006; 105:198–208
  37. Ciavarella D, Reed RL, Counts RB, Baron L, Pavlin E, Heimbach DM, Carrico CJ: Clotting factor levels and the risk of diffuse microvascular bleeding in the massively transfused patient. *Br J Haematol* 1987; 67:365–8
  38. Thomas D, Wee M, Clyburn P, Walker I, Brohi K, Collins P, Doughty H, Isaac J, Mahoney PM, Shewry L; Association of Anaesthetists of Great Britain and Ireland: Blood transfusion and the anaesthetist: Management of massive haemorrhage. *Anaesthesia* 2010; 65:1153–61