Efficacy and safety of recombinant factor XIII on reducing blood transfusions in cardiac surgery: A randomized, placebo-controlled, multicenter clinical trial

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Objectives: Cardiac surgery with cardiopulmonary bypass frequently leads to excessive bleeding, obligating blood product transfusions. Because low factor XIII (FXIII) levels have been associated with bleeding after cardiac surgery, we investigated whether administering recombinant FXIII after cardiopulmonary bypass would reduce transfusions.

Methods: In this double-blinded, placebo-controlled, multicenter trial, 409 cardiac surgical patients at moderate risk for transfusion were randomized to receive an intravenous dose of recombinant FXIII, 17.5 IU/kg (n = 143), 35 IU/kg (n = 138), or placebo (n = 128) after cardiopulmonary bypass. Transfusion guidelines were standardized. The primary efficacy outcome was avoidance of allogeneic blood products for 7 days postsurgery. Secondary outcomes included amount of blood products transfused and reoperation rate. Serious adverse events were measured for 7 weeks.

Results: Study groups had comparable baseline characteristics and an approximately 40% decrease in FXIII levels after cardiopulmonary bypass. Thirty minutes postdose, FXIII levels were restored to higher than the lower 2.5th percentile of preoperative activity in 49% of the placebo group, and 85% and 95% of the 17.5- and 35-IU/kg recombinant FXIII groups, respectively ($P < .05$ for both treatments vs placebo). Transfusion avoidance rates were 64.8%, 64.3%, and 65.9% with placebo, 17.5 IU/kg, and 35 IU/kg recombinant FXIII (respective odds ratios against placebo, 1.05 [95% confidence interval, 0.61-1.80] and 0.99 [95% confidence interval, 0.57-1.72]). Groups had comparable adverse event rates.

Conclusions: Replenishment of FXIII levels after cardiopulmonary bypass had no effect on transfusion avoidance, transfusion requirements, or reoperation in moderate-risk cardiac surgery patients (ClinicalTrials.gov identifier: NCT00914589). (J Thorac Cardiovasc Surg 2013;146:927-39)

Cardiac surgery requiring the use of cardiopulmonary bypass (CPB) is frequently complicated by coagulopathy.[1] Timely control of the coagulopathy is essential if patients are to avoid excessive blood loss, red blood cell transfusions, or surgical reexploration, all of which have been linked to serious adverse outcomes and prolonged hospital stay.[1-4] The causes of coagulopathy after cardiac surgery are multifactorial and include excessive fibrinolysis, platelet dysfunction, and coagulation factor deficiency due to excessive consumption, dilution, or both.[1,2] The mainstay

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Abbreviations and Acronyms
AE = adverse event
CPB = cardiopulmonary bypass
FXIII = factor XIII

of therapy for coagulopathy in the setting of cardiac surgery includes antifibrinolytic drug therapy to prevent excessive fibrinolysis, platelet transfusions to increase the number of functioning platelets, and plasma transfusions to replenish coagulation factor levels.[1] These therapies, however, are frequently ineffective and are themselves associated with adverse outcomes.[5-7] Thus, there remains a need for novel therapies that can effectively and safely prevent or treat coagulopathy after cardiac surgery.

Several plasma-derived and recombinant coagulation factors are available for systemic administration, and their hemostatic effectiveness is under clinical investigation. One potential candidate is recombinant factor XIII (FXIII). FXIII is the terminal enzyme in the coagulation cascade, and is necessary for cross-linking of fibrin monomers to form a stable and strong fibrin clot.[8-10] Cardiac surgery with CPB leads to a marked reduction in plasma FXIII levels,[11-16] and an association between low FXIII levels and increased bleeding has been noted in cardiac surgery and other clinical settings,[12-17] but this finding is not consistent.[18] Recombinant FXIII has already undergone preliminary clinical trials,[19,20] and in cardiac surgery, it has been well tolerated in doses from 11.9 to 50 IU/kg, with 35 IU/kg suggested as possibly the most appropriate dose for replenishment of FXIII levels after cardiac surgery with CPB.[20] In the current clinical trial, we assessed the hemostatic efficacy of 2 doses of recombinant FXIII (17.5 and 35 IU/kg) administered at the conclusion of CPB in patients who were at moderate risk for requiring perioperative transfusions due to blood loss to determine whether such treatment would reduce transfusion needs.

METHODS

This was a randomized, double-blinded, placebo-controlled, multinational, multicenter, phase 2, dose-finding trial conducted from July 27, 2009, to February 23, 2011. The trial was performed in accordance with the Declaration of Helsinki, International Conference on Harmonization Good Clinical Practice, and was approved by the health authorities of each of the countries involved and by the independent ethics committees or institutional review boards of each center involved. All patients provided written informed consent before involvement in the study. Details of the centers are presented in Appendix 1. The trial was registered at http://www.clinicaltrials.gov (identifier: NCT00914589).

Patients

Adult patients (aged 18-80 years) who were scheduled to undergo nonemergent coronary artery bypass grafting requiring CPB, single heart valve replacement or repair requiring CPB, or both of these surgical interventions were eligible for inclusion. Intraoperative antifibrinolytic intervention was mandatory, and patients were required to be at moderate risk for transfusion. A moderate risk for transfusion was defined as meeting 2 or 3 of the following criteria: (1) nonelective surgery, (2) redo surgery, (3) combined coronary artery bypass grafting and valve surgery, (4) aged 69 years or older, (5) body surface area lower than 1.9 m^2, (6) creatinine level higher than 100 μmol/L if female and 110 μmol/L if male, (7) platelet count lower than 150,000/μL, and (8) hematocrit lower than 36% if female and 39% if male.[21,22] The validity of the criteria for identifying patients at moderate risk for transfusion was confirmed at 2 of the participating centers before trial initiation by retrospectively assessing the transfusion rates in patients who would have met the inclusion criteria. FXIII levels were not considered for inclusion of patients into the study.

Patients were excluded at screening if they had any of the following conditions: history of coagulation disorders, autoimmune diseases, thromboembolic events (excluding cardiac), stroke, or ST-elevated myocardial infarction within 7 days of surgery; received adenosine diphosphate receptor antagonists within 3 days of surgery, glycoprotein IIb/IIIa receptor blockers within 24 hours of surgery, low-molecular-weight heparin within 24 hours of surgery, or vitamin K antagonist therapy accompanied by an international normalized ratio higher than 1.5 on the day of surgery; weight of 140 kg or higher; left ventricular ejection fraction lower than 30%; liver dysfunction (aspartate aminotransferase or alanine aminotransferase level >2-fold higher than the upper limit of normal); platelet count lower than 100×10^9/L; creatinine level of 175 μmol/L or higher or receiving dialysis; and hematocrit predicted to be lower than 27% during CPB (based on starting hematocrit and CPB prime volume).

Eligibility for trial drug administration was reevaluated 15 minutes after initiation of protamine dosing for reversal of heparin, and patients were excluded before trial drug administration (but after randomization) if they had received any blood products or fibrin sealants. In addition, patients were excluded if they had a hematocrit lower than 22% before cross-clamp removal (because they would have a high chance of receiving packed red blood cells irrespective of the amount of post-CPB blood loss) or had experienced any of the following factors that would have changed their transfusion risk from moderate to high: CPB duration longer than 3 hours, surgery on the aortic arch or descending aorta, hypothermic (<28°C) circulatory arrest, or severe hemodynamic instability after CPB that may have necessitated reinstitution of CPB. Patients were also excluded if the trial drug could not be administered within 30 minutes of protamine administration.

Trial Drug Administration

Eligible patients were randomized (1:1:1) using an interactive voice/Web response system to a single intravenous dose of 17.5 or 35 IU/kg (according to lean body mass) of recombinant FXIII or placebo before the induction of anesthesia. Randomization was stratified according to trial site, transfusion risk score (2 or 3), age group (<65 or ≥65 years), and whether the patient had undergone previous cardiac surgery. The appearance and physical properties of recombinant FXIII and placebo were indistinguishable. Study drug/placebo was administered via a slow intravenous push (≤2 mL/min) at 15 to 30 minutes after initiation of protamine dosing after CPB but before any other hemostatic therapies. Induction of anesthesia, dosing with heparin and protamine (to induce and subsequently reverse anticoagulation), and administration of antifibrinolytic treatment (tranexamic acid) were performed according to standard clinical practice at each site.

TRANSFUSION MANAGEMENT

Autologous blood collected before surgery and acute normovolemic hemodilution were not permitted. Any shed blood collected in the operating room could be retransfused, washed or unwashed, depending on institutional practice. Retransfusion of blood collected in mediastinal drains was not permitted.

Perioperative transfusion practice was standardized. Red blood cell transfusion was mandatory for hemoglobin lower than 6.0 g/dL, optional for hemoglobin between 6.0 and 8.0 g/dL, acceptable for hemoglobin between 8.0 and 10.0 g/dL (provided there was evidence for end-organ ischemia), and not permitted for higher hemoglobin values. Transfusions were to be administered 1 U at a time, and the hemoglobin had to be rechecked after each unit. Coagulation products were to be administered if, after reversal of protamine, patients bled 2 mL/kg or more over 30 minutes or 1.5 mL/kg or more per hour over 2 consecutive hours. Fresh-frozen plasma (10-15 mL/kg) was to be administered if the international normalized ratio was 1.5 or higher. Platelets (1 apheresis unit or 4-6 pooled units) were to be transfused if the platelet count was 100×10^9/L or lower. Fibrinogen concentrate (2 g) or cryoprecipitate (8-10 U) was to be transfused if the fibrinogen concentration was 1.5 g/L or lower. An external adjudication committee was established to ensure compliance with the transfusion protocol at each center.

LABORATORY TESTS

Blood sampling for clinical laboratory tests was performed before surgery, after protamine, but before trial drug administration, and at the following times after trial drug administration: 30 ± 5 minutes, 8 ± 0.5 hours, 24 ± 2 hours, 48 ± 6 hours, 72 ± 6 hours, 7 days or discharge, and 5 to 7 weeks. Standard laboratory tests (hematology, coagulation parameters, biochemistry, and urinalysis) were performed by a local laboratory. Specialty laboratory assessments were performed by a central laboratory; these included FXIII activity (FXIII Berichrom activity assay), FXIII B-subunit (enzyme-linked immunosorbent assay), prothrombin fragment $1 + 2$, troponin T, fibrinogen, fibrin monomers, and recombinant FXIII antibodies (direct FXIII-antibody enzyme-linked immunosorbent assay).

Pharmacodynamic and Pharmacokinetic Outcomes

Pharmacodynamic and pharmacokinetic outcomes included plasma FXIII activity levels and the restoration of plasma FXIII activity to higher than the lower 2.5th percentile of the distribution of preoperative activity.

Efficacy Outcomes

The primary efficacy end point was the percentage of patients avoiding any allogeneic transfusions (red blood cell, fresh-frozen plasma, platelets, cryoprecipitate, or fibrinogen concentrate) up to postoperative day 7 or hospital discharge, whichever came first. Secondary efficacy end points, which were also assessed up to postoperative day 7 or hospital discharge, were the amount (units) of transfused blood products, the percentage of patients avoiding any red blood cell transfusion, and the percentage of patients avoiding massive red blood cell transfusion ($\geq$5 U). The incidence of reoperation was assessed until the end of the trial.

Safety

An external, independent safety data monitoring committee (Appendix 2) was established to ensure the safety of patients. All adverse events (AEs), including severity, relation to trial product, and patient outcome, were reported from the time of trial drug administration until postoperative day 7 or hospital discharge. For serious AEs, reporting continued until the follow-up visit at 5 to 7 weeks after trial drug administration. Prespecified critical AEs were thromboembolic events (including acute myocardial infarction [based on enzyme and electrocardiographic criteria, which were evaluated by an independent central laboratory], stroke, or transient ischemic attack, peripheral artery occlusion, deep venous thrombosis, and pulmonary embolism), renal dysfunction (doubling of creatinine or renal replacement therapy), reoperation, and death.

Statistical Analysis

The sample size of 409 patients (136 per group) was based on the following assumptions: transfusion avoidance rate of 40% in the placebo arm (based on the validation of the transfusion risk criteria) and 55% in each of the treatment arms, 70% power, and α level of .05 (2 sided). The null hypothesis was that placebo and recombinant FXIII administration resulted in the same avoidance rate (ie, odds ratio, 1).

Except where noted, all statistical analyses, including covariate selection, were planned a priori. Transfusion avoidance rates were compared between treatment groups using logistic regression analysis, adjusting for the following covariates: trial site, transfusion risk score (2 or 3), age group ($<$65 or $\geq$65 years), previous cardiac surgery, pretrial drug administration fibrinogen level, pretrial drug administration platelet count, urgency of surgery, and treatment arm. In sensitivity analyses, the primary analysis was repeated, adjusting only for statistically significant covariates and without adjusting for any covariates.

A blinded data review showed that the distribution of the amount of transfusions was not consistent with a Poisson distribution (ie, most patients did not receive any transfusions, and a few patients received many units, with 1 receiving $>$80 U). Therefore, the amount of transfusions was compared using a cumulative logit model on 0, 1, 2, 3, 4, and $>$4 U. This analysis was adjusted for covariates, as for the primary analysis.

The proportion of patients whose plasma FXIII activity level was restored to higher than the lower 2.5th percentile of the distribution of preoperative activity level was also compared between treatment groups using logistic regression analysis, adjusting for transfusion risk score (2 or 3), age, pretrial drug administration FXIII plasma activity,

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and treatment. The plasma concentration of FXIII at each time point was compared among treatment groups using analysis of variance, adjusting for these covariates.

The impact of the achieved FXIII activity level on the avoidance of any transfusions was analyzed in a manner analogous to the primary analysis, except that ''treatment'' was replaced by FXIII plasma activity at 30 min, 24 h, and 48 h in the respective analyses. The interaction effect between predose FXIII activity and treatment on avoidance of allogeneic transfusions was analyzed in a model similar to the primary analysis, but with predose FXIII activity as a covariate both on its own and in interaction with treatment.

The rate of critical AEs was compared between treatment groups using logistic regression analysis, adjusting for transfusion risk score (2 or 3), age, type of surgery (elective vs nonelective), and treatment.

The trial was sponsored by Novo Nordisk A/S (Bagsværd, Denmark). The authors designed the trial protocol, directed the statistical plan, and wrote the manuscript. The sponsor oversaw the trial operations, audited all data, and conducted the statistical analyses. Dr Karkouti signed the clinical trial report, and the authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree with the manuscript as written.

RESULTS

Patient Disposition, Demographics, and Operative Details

Patient disposition is shown in Figure 1. Of the 479 randomized patients, 409 were eligible to receive study drug and were included in the analyses. Within this group, 128 received placebo, 143 received 17.5 IU/kg of recombinant FXIII, and 138 received 35 IU/kg of recombinant FXIII. A total of 356 patients completed the entire trial, including the 5- to 7-week postoperative visit.

The 13 European hospitals randomized 61% of patients, followed by the 5 Canadian hospitals (28%), 7 US hospitals (5%), 4 Japanese hospitals (4%), and 2 Israeli hospitals (2%) (Appendix 1). This variability is, in part, the result of the study being initiated first in Europe, followed by Canada, and then in the other countries. Patients had similar characteristics, baseline laboratory values, and operative

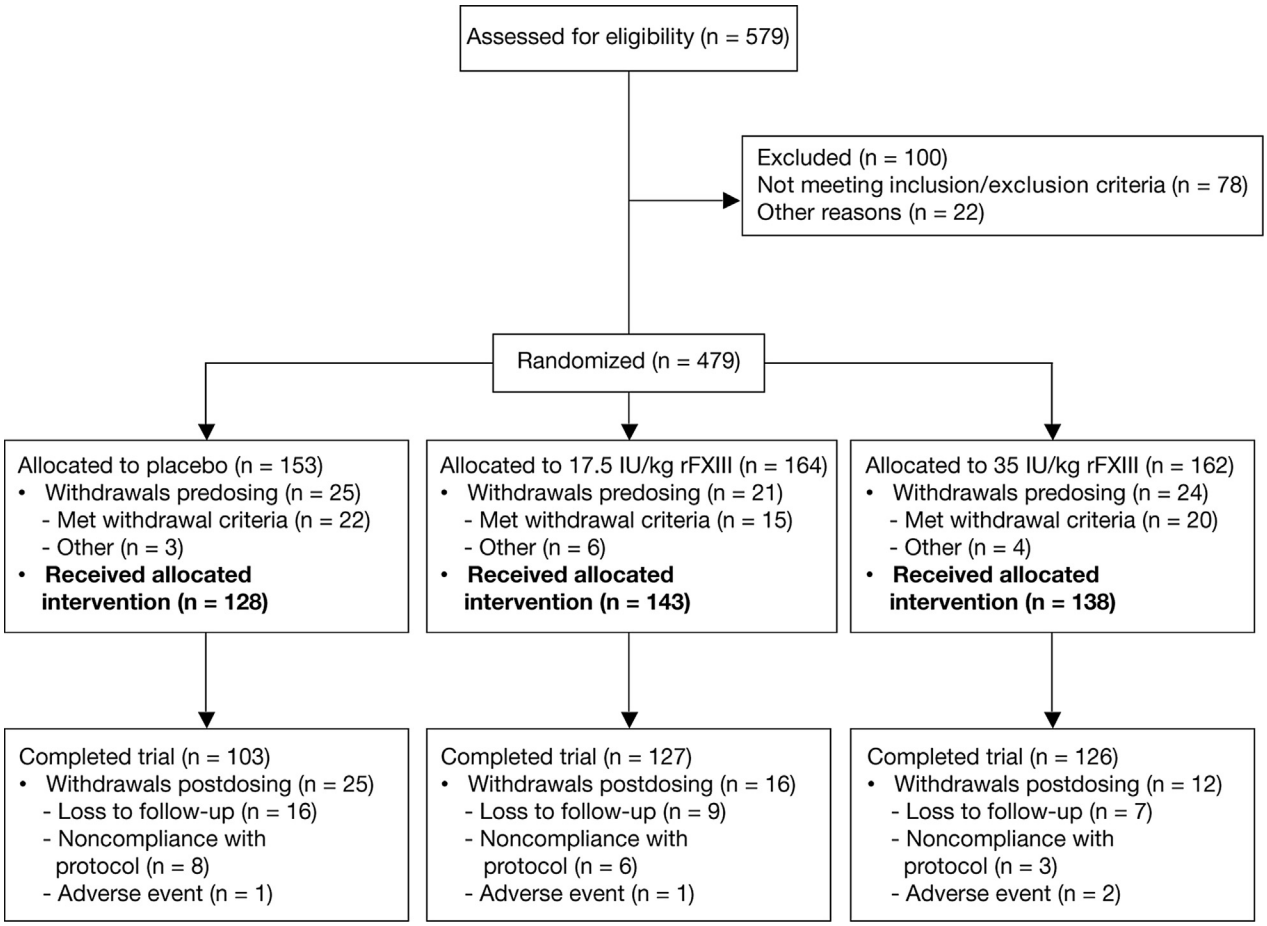

FIGURE 1. Patient disposition. *rFXIII*, Recombinant factor XIII.

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details across treatment groups (Table 1). All but 8 patients had a transfusion risk score of 2 or 3.

FXIII Activity Levels

Baseline FXIII activity levels were similar across treatment groups (Table 1). In addition, all 3 groups had an approximately 40% decrease in FXIII levels after CPB (Figure 2). Administration of recombinant FXIII increased FXIII levels in a dose-dependent manner, and the levels remained higher than in the placebo group across all 7-day postoperative time points (Figure 2). The proportion of patients who, at 30 minutes postdose, had achieved restoration of FXIII activity to higher than the lower 2.5th percentile of the preoperative activity was 49% in the placebo group, 85% in the 17.5-IU/kg recombinant FXIII group, and 95% in the 35-IU/kg recombinant FXIII group ($P < .05$ in both treatment groups vs placebo).

Efficacy Outcomes

The administration of recombinant FXIII had no effect on allogeneic transfusion avoidance (Table 2). The proportion of patients who avoided transfusion was 64.8% with placebo versus 64.3% with recombinant FXIII, 17.5 IU/kg, and 65.9% with recombinant FXIII, 35 IU/kg. This translated to an odds ratio close to unity for the comparisons of active treatments against placebo in the applied logistic regression analysis (1.05 [95% confidence interval, 0.61-1.80] for the 17.5-IU/kg recombinant FXIII group and 0.99 [95% confidence interval, 0.57-1.72] for the 35-IU/kg recombinant FXIII groups). Although transfusion practice was protocolized and there were few protocol violations (n = 30), transfusion rates varied substantially across sites; the median rate was 29%, and the 25th and 75th percentiles were 24% and 50% (sites with ≤3 randomized patients were considered a single site for this calculation), with a transfusion rate of 30%. Trial site ($P = .0001$) was the most important determinant of transfusion avoidance. The results of sensitivity analyses were similar to those of the main analysis.

Subgroup and interaction analyses showed no effect of recombinant FXIII on transfusion avoidance in any of the analyzed subgroups defined by predose FXIII activity, predose fibrinogen concentration, transfusion risk marker, and type of surgery. No interaction between treatment and predose FXIII activity level was identified from logistic regression analysis of transfusion avoidance ($P = .60$).

TABLE 1. Patient characteristics and operative details

	Placebo (n = 128)	rFXIII 17.5 IU/kg (n = 143)	rFXIII 35 IU/kg (n = 138)
Patient characteristics at screening			
Age, mean (SD), y	69 (9)	69 (9)	69 (8)
Male sex, no. (%)	100 (78.1)	119 (83.2)	117 (84.8)
White race, no. (%)	117 (91.4)	129 (90.2)	125 (90.6)
Body surface area, mean (SD), m^2	1.9 (0.2)	1.9 (0.2)	1.9 (0.2)
Left ventricular ejection fraction, mean (SD), %	56 (10)	57 (11)	56 (9)
Baseline assessments before anesthesia*			
Hemoglobin, mean (SD), g/dL	13.7 (1.3)	13.6 (1.3)	13.7 (1.2)
Creatinine, mean (SD), μmol/L	88 (22)	90 (23)	93 (22)
Platelet count, mean (SD), $\times 10^9$/L	210 (63)	210 (61)	212 (57)
INR, mean (SD)	1.1 (0.1)	1.1 (0.1)	1.1 (0.1)
Fibrinogen, mean (SD), mg/dL	406 (129)	401 (138)	426 (148)
FXIII activity, mean (SD), IU/mL	1.23 (0.26)	1.14 (0.28)	1.20 (0.29)
FXIII B-subunit, mean (SD), μg/mL	4.71 (1.65)	4.91 (1.88)	4.80 (1.92)
Operative details			
CABG, no. (%)	56 (43.8)	79 (55.2)	79 (57.2)
Valve repair or replacement, no. (%)	39 (30.5)	36 (25.2)	36 (26.1)
Combined CABG and valve, no. (%)	33 (25.8)	28 (19.6)	23 (16.7)
Redo surgery, no. (%)	6 (4.7)	3 (2.1)	11 (8.0)
Nonelective surgery, no. (%)	33 (25.8)	44 (30.8)	44 (31.9)
EuroSCORE, mean (SD)	4.3 (2.1)	4.1 (2.0)	4.3 (2.1)
No. of transfusion risk criteria, no. (%)†			
1	1 (0.8)	2 (1.4)	1 (0.7)
2	77 (60)	82 (57)	88 (64)
3	49 (38)	57 (40)	48 (35)
4	1 (0.8)	2 (1.4)	1 (0.7)

rFXIII, Recombinant factor XIII; *INR*, international normalized ratio; *CABG*, coronary artery bypass grafting. *Baseline values were collected before induction of anesthesia (preinduction). If data were not available, data from the screening visit were used. †See Methods for list of risk factors.

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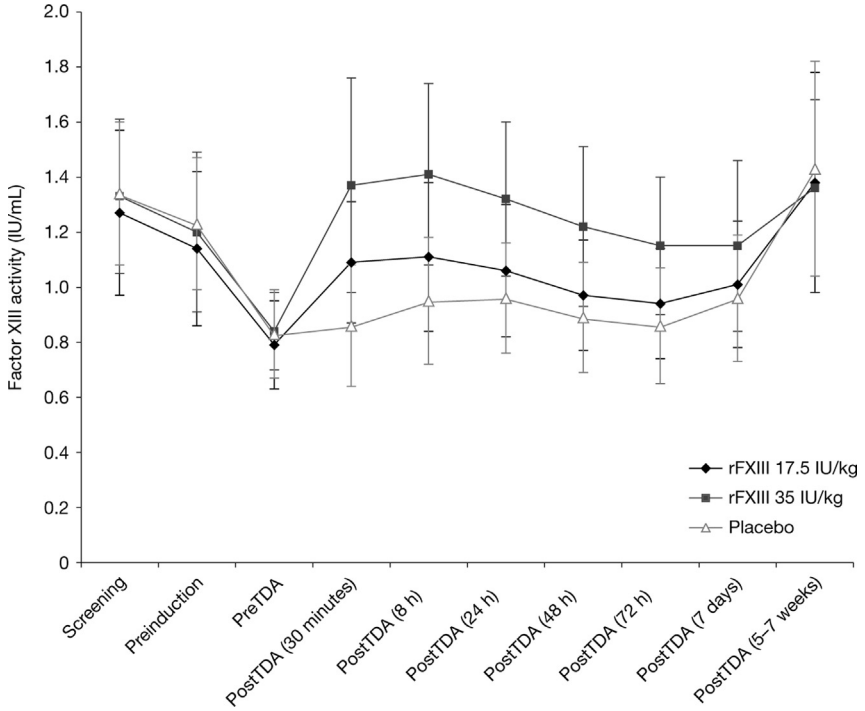

FIGURE 2. Factor XIII levels. Data shown are means and SDs. *rFXIII*, Recombinant factor XIII; *TDA*, trial drug administration.

Furthermore, there was no impact of FXIII activity level at 30 minutes postdose on avoidance of allogeneic transfusion ($P = .64$).

For the secondary transfusion end points, administration of recombinant FXIII did not reduce transfusion requirements, massive red blood cell transfusions, or the incidence of reoperation.

Safety

Administration of recombinant FXIII was not associated with an increased rate of AEs versus placebo, including all AEs, serious AEs, AEs possibly or probably related to study medication, and withdrawals due to AEs (Table 3). The most commonly reported AEs were pleural effusion (37%), atrial fibrillation (35%), procedural pain (30%), nausea (29%), anemia (17%), peripheral edema (16%), and hypotension (16%). Most AEs were mild or moderate in severity. More important, thromboembolic and predefined critical AEs occurred with similar frequencies in the FXIII and placebo groups. There were 2 fatal AEs (myocardial infarction and sepsis), which both occurred during FXIII treatment but were considered unlikely to be related to trial drug. None of the patients developed antibodies to recombinant FXIII.

DISCUSSION

In this randomized, double-blinded, placebo-controlled, multicenter clinical trial, we measured the hemostatic efficacy of 17.5 and 35 IU/kg of recombinant FXIII administered at the conclusion of CPB to patients who were at moderate risk for requiring perioperative transfusions because of blood loss. We found that FXIII levels were reduced by approximately 40% after CPB. We also found that, although both doses of recombinant FXIII

TABLE 2. Efficacy outcomes*

	Placebo (n = 128)	rFXIII 17.5 IU/kg (n = 143)	rFXIII 35 IU/kg (n = 138)
Patients who avoided allogeneic transfusions, no. (%)			
All transfusions	83 (64.8)	92 (64.3)	91 (65.9)
Red blood cell transfusions	85 (66.4)	94 (65.7)	92 (66.7)
Massive (≥5 U) red blood cell transfusion	125 (97.7)	140 (97.9)	136 (98.6)
Units of transfused blood products, median (25th, 75th percentiles)	0 (0, 1)	0 (0, 1)	0 (0, 1)
Incidence of reoperation, no. (%)	2 (1.6)	8 (5.6)	4 (2.9)

rFXIII, Recombinant factor XIII. *None of the prespecified comparisons was statistically significant.

TABLE 3. Adverse events*

	Placebo (n = 128)	rFXIII 17.5 IU/kg (n = 143)	rFXIII 35 IU/kg (n = 138)
All AEs	126 (98.4)	139 (97.2)	135 (97.8)
Serious AEs	35 (27.3)	43 (30.1)	32 (23.2)
AEs by severity			
Severe	24 (18.8)	31 (21.7)	23 (16.7)
Moderate	91 (71.1)	93 (65.0)	77 (55.8)
Mild	111 (86.7)	120 (83.9)	119 (86.2)
AEs probably/possibly related to study drug			
All	14 (10.9)	17 (11.9)	15 (10.9)
Serious AEs	5 (3.9)	6 (4.2)	4 (2.9)
Withdrawals due to AEs	1 (0.8)	1 (0.7)	1 (0.7)
Thromboembolic events	12 (9.4)	12 (8.4)	9 (6.5)
Perioperative acute myocardial infarction	8 (6.3)	10 (7.0)	7 (5.1)
Cerebrovascular thromboembolic event	3 (2.3)	2 (1.4)	0
Deep vein thrombosis	1 (0.8)	0	1 (0.7)
Peripheral artery occlusion	0	0	1 (0.7)
Pulmonary embolism	0	0	0
Prespecified critical AEs	19 (14.8)	24 (16.8)	16 (11.6)
Thromboembolic events	12 (9.4)	12 (8.4)	9 (6.5)
Renal dysfunction	9 (7.0)	10 (7.0)	5 (3.6)
Reoperation	2 (1.6)	8 (5.6)	4 (2.9)
Death	0	1 (0.7)	1 (0.7)

Results are reported as number (%), where number indicates patients with an AE. *rFXIII*, Recombinant factor XIII; *AE*, adverse event. *None of the prespecified comparisons was statistically significant.

significantly increased post-CPB FXIII levels, they had no effect on any of the efficacy end points, in either the entire sample or the subset of patients who had low predose FXIII activity. No safety issues were identified, because AEs were evenly distributed across treatment groups and individual types of AEs, including thromboembolic AEs and events predefined as critical for the trial, did not occur with higher frequency in the active dose groups relative to placebo.

The finding that FXIII levels are substantially reduced after CPB is consistent with those of previous studies, which have generally found there is approximately a 40% decrease in FXIII levels from baseline to after CPB.[11-16] Although other coagulation factor levels decrease by similar amounts, our hypothesis was based on several lines of evidence that suggest that FXIII substitution after CPB may reduce blood loss. First, several observational studies have found an inverse correlation between FXIII levels after CPB and clot strength or blood loss,[12-16] but this finding is not consistent.[18] Second, soluble fibrin monomer levels are increased after CPB, and this increase is independently associated with increased blood loss.[11] In the presence of adequate FXIII levels, soluble fibrin monomers are cross-linked into stable, insoluble fibrin clots.[23] Thus, the observed accumulation of soluble fibrin monomers after CPB suggests that there is a relative deficiency of FXIII levels that may contribute to inadequate clot formation and increased bleeding. Third, preliminary studies have shown that FXIII substitution is associated with improved clot strength and reduced blood loss.[12,14,24]

Nevertheless, this study's finding suggests that, at least in patients undergoing moderate-risk cardiac surgery and receiving concomitant antifibrinolytic drug therapy, replenishment of FXIII levels at the conclusion of CPB with recombinant FXIII does not reduce transfusion rates. To our knowledge, 2 other studies have explored the efficacy of FXIII replacement in cardiac surgery.[12,14] One study included 22 consecutive patients who underwent low-risk cardiac surgery and were alternately assigned to control or FXIII arms (who received 2500 U of FXIII postoperatively).[12] In that study, patients who received FXIII had lower blood loss and transfusion rates, but only the former achieved statistical significance, and patients were not properly randomized.[12] More recently, the same group conducted another trial that included 75 patients who underwent low-risk cardiac surgery and were randomized to placebo, low-dose (1250 U), or high-dose (2500 U) FXIII postoperatively (25 patients in each group).[14] In this blinded study, the amounts of blood loss and transfusion were highest in the placebo group and lowest in the high-dose group, but none of the between-group differences achieved statistical significance.[14] In both of these studies, patients received concomitant antifibrinolytic therapy (aprotinin), but the transfusion protocol was not standardized.

Several characteristics of this study must be considered when interpreting its negative results. First, based on the

requirements of some drug-regulating agencies, it was deemed necessary to determine if FXIII replenishment resulted in increased transfusion avoidance. As a result, the sample was limited to patients in whom there was a reasonable chance of transfusion avoidance, which (based on our inclusion criteria) placed them at moderate risk for transfusion. Moreover, despite validating the inclusion criteria at 2 of the participating centers, the transfusion avoidance rate was higher than anticipated, indicating that the transfusion risk in the sample was lower than anticipated. Second, the decision was made to determine the efficacy of FXIII when it is administered early after CPB, irrespective of FXIII levels. Third, the drug was tested in the context of concomitant antifibrinolytic therapy. Thus, it is possible that the drug may have better efficacy in patients whose transfusion risk is higher, in those with severe (<20%-30% activity levels) FXIII deficiency after CPB, or in place of antifibrinolytic drugs. Because FXIII is the terminal enzyme in the coagulation cascade,[8-10] targeting coagulation factors involved in the earlier stages of coagulation, such as fibrinogen, may be more effective.[25] Adequately powered randomized controlled trials are needed to examine these issues.

Although this study had many strengths (large, multicenter, placebo-controlled, blinded, randomized trial), it had 2 important weaknesses. First, because this was a phase 2 study, it was not adequately powered to prove drug safety and was only 70% powered for efficacy. Nevertheless, the efficacy results were clear and there were no major safety concerns detected. Second, despite attempts to standardize transfusion therapy, there were significant variations in transfusion rates across the sites. The reasons for these variations are unclear, but because randomization was stratified according to the site, it is unlikely that this limitation led to unmeasured biases.

In summary, in this multicenter, double-blinded, placebo-controlled, randomized trial, we found that administration of recombinant FXIII (17.5 or 35 IU/kg) after cardiac surgery and CPB in patients at moderate risk for transfusion restored FXIII activity to preoperative levels but had no hemostatic benefits, as measured by transfusion avoidance, transfusion requirements, or need for reoperation.

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APPENDIX TABLE 1. Study sites

Site and principal investigator	Subinvestigators	No. of patients randomized
Dr Jean-Yves Dupuis University of Ottawa Heart Institute 40 Ruskin St Room H545A Ottawa, ON K1Y 4W7 Canada	Dr James Robblee Dr Michael Bourke Dr Bernard McDonald	7
Dr Keyvan Karkouti Toronto General Hospital 200 Elizabeth St 3EN-402 Toronto, ON M5G 2C4 Canada	Dr Terrence Yau Dr Stuart McCluskey Dr Kathleen Dattilo Dr George Djaiani Dr Jacek Karski	18
Dr Louis Perrault Institut de Cardiologie de Montreal 5000 E Belanger Department of Anesthesia Montreal, QC H1T 1C8 Canada	Dr Michel Pellerin Dr Michel Carrier Dr Philippe Demers Dr Yves Hébert Dr Denis Bouchard Dr Antoine Rochon Dr Raymond Cartier	17
Dr Pierre Voisine Hospital Laval 2725 Chemin Sainte-Foy Institut Universitaire de Cardiologie et de Pneumologie de Québec Québec, QC G1V 4G5 Canada	Dr Richard Baillot Dr Eric Charbonneau Dr François Dagenais Dr Eric Dumont Dr Daniel Doyle Dr Patrick Mathieu Dr Jacques Métras Dr Jean Perron Dr Jean Bussières Dr Marcel Gilbert Dr Siamak Mohammadi	29
Dr C. David Mazer Saint Michael's Medical Center 30 Bond St Department of Anesthesia Toronto, ON M5B-1W8 Canada	Dr Mark Peterson Dr David Latter Dr Daniel Bonneau Dr Gregory Hare	62
Prof Dr Christian von Heymann Charité–Universitätsmedizin Berlin Campus Charité Mitte/Campus Virchow-Klinikum Klinik für Anästhesiologie mit Schwerpunkt Operative Intensivmedizin Charitéplatz 1 10117 Berlin Germany	Prof Dr Michael Sander Dr Marit Habicher Dr Torsten Geyer Dr Juliane Rau Dr Katharina Berger Dr Sibylle Semmler Dr Stephanie Scholz Dr Med. Birgit Puhlmann Dr Susanne Niederberger	31
PD Dr Med Frank Isgro Klinkum der Stadt Ludwigshafen am Rhein gGmbH Klinik für Herzchirurgie Bremserstrasse 79 67063 Ludwigshafen Germany	Dr Med Monica-Cristina Weber Dr Med Michael Neher Dr Helena Leitao Graca Jourdan Dr Med Angela Kornberger	31

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APPENDIX TABLE 1. Continued

Site and principal investigator	Subinvestigators	No. of patients randomized
Dr Med Hendrik Ruge Deutsches Herzzentrum München Klinik für Hertz-und Gefäßchirurgie Lazarettstrasse 36 80636München Germany	Dr Jacqueline Gümmer Dr Med Andrea Münsterer Dr Med Patrick Mayr	9
Dr Med Arndt-Holger Kiessling Universitätsklinikum Frankfurt am Main Theodor Stern Kai 7 60590 Frankfurt am Main Germany	Dr Nadejda Monsefi	28
Dr Peter Skov Olsen Department of Cardiothoracic Surgery Copenhagen University Hospital Blegdamsvej 9, 2100 Copenhagen Denmark	Prof Daniel Steinbrüchel	58
Dr Alain Vuylsteke Papworth Hospital NHS Trust Cambridge, CB3 8RE UK	Dr Rhiannon Beaumont Dr Peter Faber Dr Andrew Klein Dr Choo Yen Ng Dr Kamen Valchanov Dr Stephen Webb Dr Francis Wells Dr Tharuka Wijesuriya	8
Mr Peter Braidley Northern General Hospital Sheffield, S5 7AU UK	Dr Patrick Knowles	2
Dr Ravi Singh Gill Southampton University Hospitals NHS Trust Southampton, SO16 6YD UK	Dr Charles Deakin Dr Michael Herbertson Dr Paul Diprose	7
Prof Jacob Lavee Department of Cardiac Surgery Sheba Medical Center Tel-Hashomer 52621 Israel	Dr Ehud Raanani Dr Erez Kachel Dr Alexander Kogan Dr Dan Loberman Dr Vera Coman Dr Sergey Preisman Dr Danny Spiegelstein Dr Leonid Sternik Dr Basheer Sheick-Yousif Dr Ami Shinfeld Dr Yigal Kassif	4
Dr Eyal Porat Department of Cardiothoracic Surgery Rabin Medical Center Jabotinski St, Petah Tikva, 49100 Israel	Dr Prof Aida Inbal Dr Alexander Lipey Dr Ehud Jacobzon Dr Benjamin Medalion Dr Ariel Farkash Dr Avi Fuks Dr Michael Fainblut Dr Philippe Biderman Dr Sammer Diab Dr Eitan Snir Dr Ram Sharoni Dr Erez Sharoni Dr Viacheslav Bobovnikov	4

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APPENDIX TABLE 1. Continued

Site and principal investigator	Subinvestigators	No. of patients randomized
Dr Marco Ranucci Department of Cardiothoracic Anaesthesia and Intensive Care Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Policlinico San Donato Via R. Morandi 30 20097 San Donato Milanese Italy	Dr Dario Melani Dr Walter Castracane Dr Donatella De Benedetti Dr Angela Satriano Dr Annalisa Parisi	57
Dr Giovanni Marino Unità Operativa di Anestesia e Rianimazione Cardiochirurgica IRCCS Fondazione Centro S. Raffaele del Monte Tabor Via Olgettina, 60 20132 Milano Italy	Dr Elena Bignami Dr Giuseppe Iaci Dr Giovanni Landoni Dr Anna Mizzi Dr Fabrizio Monaco Dr Paolo Prati Dr Leda Nobile Dr Massimiliano Nuzzi Dr Alessandro Oriani	28
Dr Atsushi Amano Department of Cardiovascular Surgery Tokyo Japan	Dr Toshimasa Akazawa Dr Shizuyuki Dohi Dr Naoki Fukuhara Dr Hirotaka Inaba Dr Eiichi Inada Dr Hitomi Iwata Dr Shiori Kawasaki Dr Keita Kikuchi Dr Osamu Kudo Dr Kenji Kuwaki Dr Takeshi Matsumura Dr Taira Yamamoto Dr Hironobu Yamaoka	1
Dr Junjiro Kobayashi Department of Cardiovascular Surgery National Cardiovascular Center Osaka Japan	Dr Tomoyuki Fujita Dr Masataka Kamei Dr Shigeki Miyata Dr Hiroyuki Nakajima Dr Tatsuya Ogawa Dr Yoshihiko Ohnishi Dr Yusuke Shimahara Dr Koichi Toda Dr Kenji Yoshitani	14
Dr Hitoshi Okabayashi Department of Cardiovascular Surgery Memorial Heart Center 19-1 Uchimaru, Morioka, Iwate 020-8505 Japan	Dr Mamoru Kadosaki Dr Takeshi Kamada Dr Kazuyoshi Kanno Dr Tsuyoshi Kataoka Dr Hajime Kin Dr Takashi Kobyashi Dr Junichi Koizumi Dr Yoshino Mitsunaga Dr Masayuki Mukaida Dr Hiroshi Sato Dr Junichi Tsuboi	3
Dr Tatsuhiko Komiya Department of Cardiovascular Surgery 1-1-1 Miwa, Kurashiki-shi, Okayama, 710-8602 Japan	Dr Jouji Ito Dr Hideyuki Katayama Dr Norio Mouri Dr Genichi Sakaguchi Dr Jiro Sakai	1

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APPENDIX TABLE 1. Continued

Site and principal investigator	Subinvestigators	No. of patients randomized
	Dr Takeshi Shimamoto	
	Dr Chikara Ueki	
	Dr Keisuke Watadani	
	Dr Shun Watanabe	
Francisco Javier Hortal Department of Cardiac Surgery Anaesthesiology and Resuscitation c/ Dr Esquerdo, 46 28007 Madrid Spain	Dr Mónica Barranco Dr Jose Maria Barrio Dr Alejandro Garrido Dr Mario Iglesias Dr Mario Begoña Quintana Dr Guillermo Rodriguez Dr Eduardo Sanchez	15
Dr José Anastasio Montero Department of Cardiovascular Surgery La Fe University Hospital 46009 Valencia Spain	Dr Ana Bel Minguez Dr Lucia Doñate Dr Tomás Heredia Dr Juan Antonio Margarit Dr Daniel Mata Dr Azucena Pajares Dr Manuel Pérez Dr Francisco José Valera Dr Rosario Vicente Dr Salvador Torregrosa	12
Dr Angel Candela-Toha Department of Cardiac Surgery Anaesthesiology and Resuscitation Hospital Ramón y Cajal Carretera Colmenar Viejo km, 9, 100 28034 Madrid Spain	Dr Ignacio Garcia Andrade Dr José Garrido Dr Marcos Martínez Dr Adolfo Martínez Pérez Dr Enrique Oliva Dr Diego Parise	8
Dr Jerrold Levy Emory University Hospital 1364 Clifton Road Northeast Atlanta, GA 30322	Dr Marc Azran Dr Roman Sniecinski Dr Gautam Sreeram Dr William Whitley	1
Dr Nanette Schwann Lehigh Valley Health Network 1245 S Cedar Crest Blvd, Suite 301, Allentown, PA 18103	Dr James Wu	4
Dr Frank Sellke Rhode Island Hospital 593 Eddy St/APC 425 Providence, RI 02903	Dr Nikola Dobrilovic Dr William Feng Dr James Fingleton Dr Victoria Miller Dr Arun Singh	1
Dr Howard Song Division of Cardiothoracic Surgery Oregon Health and Science University 3181 SW Sam Jackson Park Road, L 353 Portland, OR 97239	Dr Lynn Boshkov Dr Matthew Slater Dr Steven Guyton Dr Lauren Brunner Dr Deanna Cully Dr Frederick Tibayan Dr Paul Wacek	5
Dr Luis Velez-Pestana The Methodist Hospital 6565 Fannin B452 Houston, TX 77030	Dr Brian Bruckner Dr Nidal Abdel-Rahman Dr Nicolas Athanassiou Dr Ghazala Butt Dr James Carter Dr Diane Gibson Dr Elizabeth Herrera	6

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APPENDIX TABLE 1. Continued

Site and principal investigator	Subinvestigators	No. of patients randomized
	Dr Jessica Brown	
	Dr Joseph Naples	
	Dr Michael Reardon	
	Dr Hany Samir	
	Dr Karanbir Singh	
	Dr Zbigniew Wojciechowski	
Dr Linda Sundt Department of Anaesthesiology Thomas Jefferson University 111 South 11th St, Suite G8490 Philadelphia, PA 19107	Dr James Diehl Dr Elia Elia Dr Rehana Jan Dr Scott Silvestry	1
Dr Ian Welsby Department of Anaesthesiology, Duke University Medical Center, 2301 Erwin Rd, 5691-H HAFS Bldg Durham, NC 27710	Dr Joseph Mathew	7

APPENDIX 2. Data monitoring committee

Mark L. Barr, MD (Chair); Mark S. Slaughter, MD; Michael C. J. Wanscher, MD; Marc Buyse, Biostatistician.

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