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Original Article

Heparin Dose and Point-of-Care Measurements of Hemostasis in Cardiac Surgery—Results of a Randomized Controlled Trial

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Objective: High heparin doses during cardiopulmonary bypass (CPB) have been suggested to reduce thrombin activation and consumption coagulopathy and consequently bleeding complications. The authors investigated the effect of a high heparin dose during CPB on point-of-care measurements of coagulation. The authors hypothesized that during CPB a high heparin dose compared with a lower heparin dose would reduce thrombin generation and platelet activation and tested whether this would be reflected in the results of rotational thromboelastometry (TEM) and platelet aggregation, measured with multiple electrode aggregometry (MEA).

Design: Prospective, randomized, controlled, open single-center study.

Setting: University teaching hospital.

Participants: Sixty-three consecutive patients undergoing elective coronary artery bypass grafting with CPB were enrolled.

Interventions: Patients were randomly assigned to receive either a high (600 IU/kg, n = 32) or a low (300 IU/kg, n = 31) initial dose of heparin. Target levels of activated clotting time during CPB were >600 seconds in the high heparin dose group and >400 seconds in the low heparin dose group.

Measurements and Main Results: Blood samples were collected (1) preoperatively after induction of anesthesia, (2) 10 minutes after aortic declamping, (3) 30 minutes after protamine administration, and (4) 3 hours after protamine administration. TEM and MEA were then measured. There was no difference in blood loss up to 18 hours postoperatively (median 735 mL for high dose *v* 610 mL for low dose; $p < 0.056$) or transfusions between the groups. Total median heparin dose (54,300 IU *v* 27,000 IU; $p = 0.001$) and median antifactor Xa levels during CPB (9.38 U/mL *v* 5.04 U/mL; $p = 0.001$) were greater in the high than in the low heparin dose group. However, neither TEM nor MEA results differed significantly between the groups.

Conclusions: Compared with a lower dose of heparin during CPB, a high dose of heparin had little effect on the point-of-care measurements of hemostasis, TEM, and MEA. Based on the similarity of platelet and coagulation activity assessments, the higher heparin dose does not appear to offer benefit during CPB.

Key Words: heparin; dose; cardiopulmonary bypass; point-of-care; thromboelastometry; multiple electrode aggregometry

HEPARIN is the drug of choice for the mandatory anticoagulation during cardiopulmonary bypass (CPB), but the optimal heparin dose during CPB is not known. High heparin doses have been suggested to reduce thrombin activation and

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consumption coagulopathy and consequently bleeding tendency.[1] Previously, heparin dosing based on an automated heparin dose-response assay resulted in higher heparin doses during CPB but fewer transfusions.[2] Higher heparin doses during CPB also have been shown to reduce activation of coagulation during CPB, as evidenced by lower levels of biomarkers of thrombin generation and fibrinolysis.[3,4]

The effects of heparin on platelets are not straightforward. Heparin may activate platelets via glycoprotein IIb/IIIa and immunologic mechanisms.[5] Platelet factor 4 (PF4) released during platelet activation, in turn, can neutralize heparin effects and inhibits megakaryopoiesis.[6] Overall, high heparin doses might have detrimental effects on sustaining hemostasis. Heparin has profibrinolytic activity,[7,8] it impairs von Willebrand factor–dependent platelet functions,[9] and heparin-induced thrombin inhibition may indirectly impair platelet functions. An initial heparin dose of 600 IU/kg reduces platelet aggregation compared with a dose of 300 IU/kg.[10] Impairment of platelet function could be detrimental because a number of patients undergoing cardiac surgery also are undergoing strong adjunct antiplatelet and novel anticoagulant therapies.

Conventional coagulation tests have been shown to be insufficient to detect hyperfibrinolysis and platelet dysfunction, the most important causes of nonsurgical bleeding episodes after CPB.[11-13] At present, several point-of-care (POC) tests of hemostasis and platelet function are available to improve and accelerate coagulation monitoring. Two commonly used POC tests are rotational thromboelastometry (TEM) and multiple electrode aggregometry (MEA). There is evidence that the use of POC tests to guide transfusion management in cardiac surgery may reduce blood loss, transfusion requirements, and morbidity,[14-17] although contradicting results also have been published.[18]

Maintaining high heparin concentrations during CPB results in reduced coagulation activation and may correlate with reduced transfusion requirements.[19] Therefore, the authors performed a randomized trial (EudraCT number 2012-000449-11) comparing a high (600 IU/kg, target activated clotting time [ACT] during CPB >600 s) and low (300 IU/kg, target ACT during CPB >400 s) dose of heparin in patients undergoing CPB for coronary artery bypass grafting (CABG). The present investigation is a substudy of a larger ongoing randomized trial, which aims to recruit 200 patients. The present study's aim was to compare the effects of heparin dosing on TEM and MEA with their multiple assessments using distinct activators.

Patients and Methods

Patients

Sixty-three consecutive patients undergoing elective CABG with CPB were enrolled into this prospective, randomized, controlled, single-center study between February 3, 2012, and March 11, 2015. The Ethics Committee of the Hospital District of Helsinki and Uusimaa, Helsinki, Finland, approved the study. All patients gave written informed consent to participate in the study. Exclusion criteria were as follows: urgent surgery; redo surgery; concomitant cardiac surgery in addition to CABG; antiplatelet or anticoagulant medication other than aspirin; biological disease–modifying anti-inflammatory drugs, peroral glucocorticoids; cytostatic drugs, hemoglobin <12 g/dL; platelet count $<130 \times 10^9$/L; international normalized ratio >1.2; left ventricular ejection fraction <35%; estimated glomerular filtration rate <50 mL/min; and C-reactive protein >20 mg/L.

Anesthesia and CPB

Benzodiazepines (diazepam, 5-10 mg, or lorazepam, 1-3 mg) were administered orally as premedication. Beta-blockers, calcium channel blockers, and long-acting nitroglycerin were given on the morning of the surgery, but other cardiovascular medications were discontinued. A peripheral arterial line was positioned before induction of anesthesia. General anesthesia was induced with propofol or etomidate, fentanyl or sufentanil, and rocuronium and was maintained with sevoflurane, fentanyl or sufentanil, and rocuronium. After induction of anesthesia and tracheal intubation, a central venous catheter was placed in the internal jugular vein.

CPB was performed using a nonpulsatile pump (Stöckert S5, Munich, Germany), a membrane oxygenator (Dideco Avant; Sorin Group, Mirandola, Italy), and a phosphorylcholine-coated circuit (Phisio; Sorin Group). The CPB circuit was primed with 1,500 mL of Ringer's solution and 5,000 IU of heparin. Oxygen/air mixture was set to achieve a 70% oxygen concentration in the oxygenator. During perfusion, target values were as follows: perfusion flow >2.4 L/min/m^2, mean arterial pressure ≥70 mmHg, mixed venous oxygen saturation >70%, and arterial blood partial pressure of carbon dioxide 4.5 to 5.5 kPa. Mild hypothermia was induced during CPB, with a target nasopharyngeal temperature of 34°C. Postoperatively, the mean arterial pressure was kept ≥70 mmHg. Volume status was optimized with Ringer's solution, 6% hydroxyethyl starch solution, or 4% albumin solution. Norepinephrine and epinephrine infusions were used, if needed.

Study Intervention

Because of the distinctly different ACT values between the study groups, the study was inevitably unblinded by nature in the operating room. The personnel in the intensive care unit and the laboratory were blinded to the patient allocation into the treatment groups. Patients were randomly assigned immediately before surgery using a lottery box. Before cannulation for CPB, 300 IU/kg (low-dose group) or 600 IU/kg (high-dose group) of unfractionated heparin (Leo Pharmaceutical Products, Denmark) was administered intravenously as an initial bolus dose. In both study groups, 5,000 IU of heparin was added to the priming volume of the CPB circuit.

Kaolin-ACT (Medtronic, Minneapolis, MN) was measured before the onset of CPB, every 20 minutes during CPB, and 3 minutes after each additional heparin bolus. The target ACT was >400 seconds in the low-dose group and >600 seconds in the high-dose group. The additional bolus dose of heparin was 60 IU/kg in both treatment groups. In both treatment groups,

after CPB, 1 mg of protamine was administered for every 100 IU of the initial doses of heparin. Of the total dose of protamine, two-thirds was given within 10 minutes after CPB. The rest of the protamine dose was infused within 30 minutes at the same time as the blood recovered from the CPB circuit was transfused to the patient. Heparinase-ACT was measured after protamine was administered if the ACT was greater than the ACT before heparin administration to detect any possible residual heparin effect. If the heparinase-ACT was >10% than the conventional ACT, 50 mg of protamine was administered to both treatment groups.

In both study groups, 25 mg/kg of tranexamic acid was administered intravenously before the surgical incision, and a second dose of 10 mg/kg was administered after the main dose of protamine. During CPB, red blood cells were transfused if hemoglobin was <60 g/L. The patients were followed-up for 18 postoperative hours. During the first 4 postoperative hours, platelet count, prothrombin time, and heparinase-ACT were measured if the chest tube drainage exceeded 100 mL/15 minutes or 200 mL/h. Thereafter, the limit was 100 mL/h. Transfusion triggers were as follows: hemoglobin <80 g/L for red blood cells, platelet count $<100 \times 10^9$/L for platelets, and international normalized ratio >1.5 for solvent-detergent–treated standardized plasma (Octaplas; Octapharma AG, Lachen, Switzerland). When the transfusion trigger was met, 1 to 2 U of red blood cells or 8 U of platelets or 15 mL/kg of plasma were transfused.

Blood Samples and Analyses

Blood samples were drawn from the peripheral arterial catheter (off-CPB) or the arterial line of the CPB circuit (on-CPB) at the following time points: (1) preoperatively after induction of anesthesia, (2) 10 minutes after aortic declamping, (3) 30 minutes after protamine administration, and (4) 3 hours after protamine administration.

For whole blood TEM analysis (ROTEM; Pentapharm Co, Munich, Germany), blood samples were collected into tubes containing 3.2% sodium citrate (BD Vacutainer; Verum Diagnostica GmbH, Munich, Germany). ExTEM and FibTEM were performed within 2 hours of blood sampling. ExTEM clotting time (CT), ExTEM maximum clot firmness (MCF), and FibTEM MCF were a priori chosen as the outcome measures. TEM analysis was performed from samples of 29 of 31 patients in the low heparin dose group and of 30 of 32 patients in the high heparin dose group. In both groups, samples from 2 patients were deleted owing to technical reasons.

Samples for MEA analysis (Multiplate; Verum Diagnostica GmbH, Munich, Germany) were collected into tubes containing 15 μg/mL of hirudin (Vacutainer; Verum Diagnostica GmbH). MEA analyses were performed on whole blood after a resting period of 30 minutes using thrombin-receptor-activating-peptide (TRAPtest), arachidonic acid (ASPItest), and adenosine diphosphate (ADPtest) as agonists. MEA analysis was performed from samples of 29 of 31 patients in the low heparin dose group and of 31 of 32 patients in the high heparin dose group. Samples from the 3 patients were deleted owing to technical reasons.

Samples for antifactor Xa (anti-FXa) activity in plasma as the measure of heparin effects were collected into tubes (3.2% sodium citrate) (BD Vacutainer) on ice and centrifuged (2,000g/10 min) at +4°C. Plasma was separated and stored at −80°C. Plasma anti-FXa activity was determined with a chromogenic assay with standard supplementation concentration of antithrombin (Berichrom Heparin; Siemens Healthcare Diagnostics, Marburg, Germany).

Statistical Analysis

The sample size was calculated with MedCalc for Windows software, Version 15.0 (MedCalc Software, Ostend, Belgium). In a previous study, a 24% decrease in intraoperatively measured mean TEG maximum amplitude was associated with increased postoperative blood loss.[20] In order to be conservative in the sample size estimation, the sample size calculation in the present study was based on an expected 15% difference in FibTEM MCF and ExTEM MCF of the TEM tracing. For the power calculation, FibTEM MCF (mean 15.4 mm, standard deviation 3.0 mm) and ExTEM MCF (mean 58.5 mm, standard deviation 5.0 mm) values of the preoperative Ringer's group of the authors' previous publication were used.[21] The calculation based on FibTEM MCF yielded 28 patients per group to detect a difference of 8.8 mm (15%) between the study groups with an α- and -error of 0.05 and 0.2, respectively.[21] The study was not powered to detect differences in postoperative blood loss between the groups.

SPSS software, version 22 (IBM Corp, Armonk, NJ), was used for statistical analysis. Because logarithmic transformation failed to normalize skewed distributions and the sample size was small, nonparametric tests were used. Differences between the study groups were tested with the Mann-Whitney *U* test. Owing to repeated measurements at 4 time points, the Bonferroni correction was applied, and p values < 0.0125 were considered to be significant. The data are presented as numbers or as medians and interquartile ranges (IQR).

Results

Study Population

The patient characteristics were comparable between the study groups (Table 1). There were no differences in comorbidities, medications, or preoperative laboratory values between the study groups (data not shown). No major adverse events (eg, stroke, myocardial infarction, venous thromboembolism, acute kidney injury or resternotomy) occurred during the study. The surgical data are presented in Table 2.

Heparin and Protamine Dose

Total heparin dose (median 54,300 [IQR 46,950-62,250] IU *v* median 27,000 [IQR 24,420-34,920] IU; p < 0,001) and anti-FX activity (median 9.3 [IQR 8.3-9.9] U/mL *v* median 5.1

Table 1
Preoperative Patient Characteristics in the Study Groups

	Low Heparin Dose (31 patients)	High Heparin Dose (32 patients)	p Value
Male/female	29/2	29/3	0.67
Age (y)	64 (57-72)	70 (59-74)	0.36
Weight (kg)	83 (74-93)	88 (77-104)	0.26
Height (cm)	174 (170-180)	176 (170-180)	0.80

NOTE. Data are presented as numbers or median values and interquartile range.

[IQR 4.8-5.6] U/mL; $p < 0.001$) were significantly greater in the high-dose than in the low-dose group during CPB (Fig 1). A single additional heparin dose was administered to 17 of 31 (55%) patients in the low heparin dose group and to 4 of 32 (12.5%) patients in the high heparin dose group. As expected, the total protamine dose needed to neutralize heparin also was greater in the high heparin dose group (median 544 [IQR 468-628] mg) than in the low heparin dose group (median 259 [IQR 230-280] mg; $p = 0.01$).

TEM

All median TEM values (Fig 2) at all 4 time points in both study groups were within the reference ranges reported by the manufacturer.[22] ExTEM CT, ExTEM MCF, and FibTEM MCF values were comparable in the groups at all time points measured, except for the ExTEM CT values measured 3 hours after protamine administration (see Fig 2).

MEA

Preoperatively, median values of the MEA ASPItest were within the reference range in both the high and low heparin dose groups (Fig 3). During CBP, ASPItest aggregation responses were impaired compared with preoperative values and were below the lower limit of the reference range in both groups. After CPB, at 3 hours after protamine administration, ASPItest values recovered in both groups but remained below the lower limit of the reference range in the high heparin dose group but not in the low heparin dose group. However, the differences in the MEA ASPItest values between the groups did not reach statistical significance at any time points measured (see Fig 3). Median ADP-triggered aggregation rates of MEA were within the reference ranges at all 4 time points in both study groups and did not differ at any time point (see Fig 3). The median TRAP-induced aggregation did not differ at any time point between the study groups (see Fig 3).

Table 2
CPB-Related Variables in the Low- and High-Dose Heparin groups

	Low Heparin Dose (31 patients)	High Heparin Dose (32 patients)	p Value
Pre-CPB ACT (s)	131 (123-141)	129 (123-143)	0.78
ACT (s) after heparin	488 (439-564)	999 (892-999)	0.001
First ACT (s) on CPB	474 (422-538)	860 (757-999)	0.001
Post-CPB ACT (s)	126 (115-133)	128 (117-140)	0.32
CPB time (min)	97 (75-119)	95 (79-119)	0.90
Cross-clamp time (min)	70 (51-83)	66 (58-80)	0.84

NOTE. Data are presented as numbers or median values and interquartile range.
Abbreviations: ACT, activated clotting time; CPB, cardiopulmonary bypass.

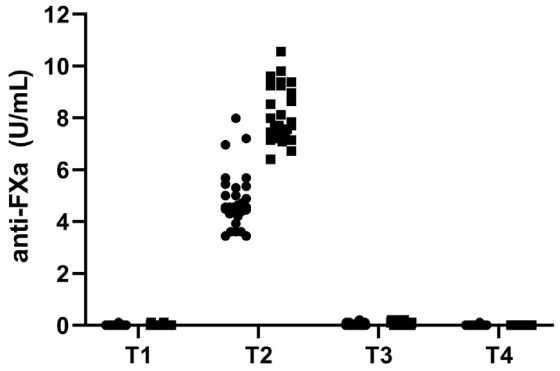

Fig 1. Antifactor Xa in the low *(circles)* and high *(squares)* heparin dose groups. Anti-FXa, antifactor Xa; T1, preoperatively after induction of anesthesia; T2, 10 minutes after aortic declamping; T3, 30 minutes after protamine administration; T4, 3 hours after protamine administration.

Blood Loss and Transfusions

There was no significant difference in chest tube output during the first 18 postoperative hours between the high-dose (median 735 [IQR 543-830] mL) and the low-dose (median 610 [IQR 510-720] mL) heparin groups ($p = 0.056$). There was no significant difference between the study groups in blood product transfusions up to the follow-up of 18 postoperative hours. One patient in both groups received 2 U of red blood cells in the intensive care unit, and 1 patient in the low heparin dose group received 2 U of platelets after CPB in the surgery room.

Discussion

In patients who received either a high or a conventional dose of heparin during CPB, there were no significant dose-dependent differences in TEM ExTEM CT, ExTEM MCF, or FibTEM MCF values, except for a small difference in ExTEM CT at 3 hours after protamine administration. Strikingly, all of the median TEM values studied in citrated blood were within the normal ranges reported by the manufacturer at all time points.[22] Likewise, platelet aggregation in hirudin-anticoagulated whole blood did not differ between the groups with any of the aggregation triggers.

There are at least 2 possible explanations for the present findings. First, the potential differences in hemostasis between the 2 heparin dose groups may be beyond the detection limit of TEM and MEA. This question could not be further addressed specifically in the present study, which focused on POC measurements in the absence of laboratory-based

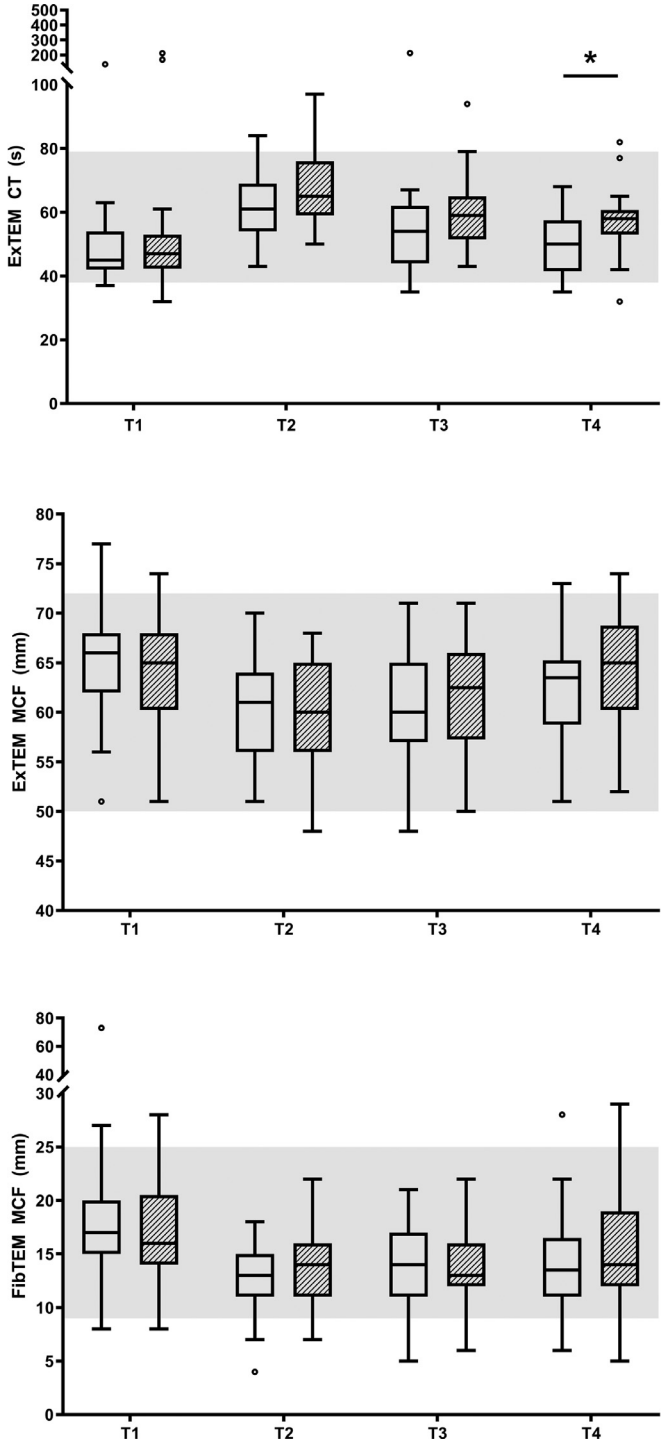

Fig 2. ExTEM clotting time, ExTEM maximum clot firmness, and FibTEM maximum clot firmness in the low *(white)* and high *(hatched)* heparin dose groups. *Gray background* denotes reference values reported by the manufacturer.[22] CT, clotting time; MCF, maximum clot firmness; T1, preoperatively after induction of anesthesia; T2, 10 minutes after aortic declamping; T, 30 minutes after protamine administration; T4, 3 hours after protamine administration. *$p < 0.05$.

measurements of coagulation and hemostasis. However, there have been previous concerns that TEM and MEA assays are not sensitive enough during cardiac surgery, during which strong dual extrinsic and intrinsic pathway activation occurs because of tissue factor exposure and neutrophil activation with netosis.[23] Furthermore, previously TEM failed to be sensitive enough to detect low platelet counts or platelet dysfunction in experimental hemodilution in vitro.[24] High heparin concentrations overall (plasma levels of anti-FXa >2-4 IU/mL) have been reported to alter TEM measurements.[25]

The second and alternative explanation is that the 2 heparin doses used in the present study indeed did not result in significant differences in hemostasis. In previous publications, the effect of heparin dosing on hemostasis during CPB was evaluated primarily by measuring biomarkers of thrombin activation and fibrinolysis. These biomarkers, however, do not directly reflect whole blood hemostasis. The interpretation of TEM is not without problems, either. It is an in vitro method with an unphysiologically high concentration of tissue factor as an initiator of coagulation. Still, different functional aspects of coagulation can be addressed with TEM. Importantly, application of validated algorithms of viscoelastometry is related to the reduced need of blood products in cardiac surgery.[16] In the present study, no differences between the study groups were observed in either initiation of coagulation or clot firmness. More specifically, no difference in FibTEM MCF, which was within the normal reference range in most patients in both study groups, was detected. This suggests that either a high or low heparin dose did not lead to significantly lowered fibrinogen levels. These results are compatible with the finding that there was no significant difference in blood loss or transfusions between the study groups, although the study was underpowered in this respect.

Again, no differences were observed between the high and low heparin dose groups in MEA platelet aggregation, irrespective of the aggregation trigger. Despite this, reduced platelet aggregation was detected with the MEA ASPItest during CPB in both groups. There are several possible explanations for the latter finding. First, heparin impairs von Willebrand factor–dependent platelet function,[9] and high doses of heparin have been shown to inhibit platelet aggregation and reduce clot strength.[26] Heparin also inhibits the formation of thrombin, the most potent in vivo platelet activator.[27] Very high heparin activity was measured in both treatment groups in the present study with median anti-FXa activity level during CPB >9 U/L in the high heparin dose group and >5 U/mL in the lower dose group. The authors of the present study are not aware of previous studies comparing platelet aggregation during CPB at such high heparin concentrations. The fact that this difference in heparin concentrations did not translate into a difference in MEA platelet aggregation between the groups is in agreement with the present TEM results. As previously stated, comparable FibTEM MCF between the study groups indicates that plasma fibrinogen concentration was comparable. Together with FibTEM data, ExTEM MCF also was comparable, suggesting that platelet engagement in whole blood fibrin formation did not deviate between the study groups.

In the high heparin dose group, median ASPItest results remained below the lower limit of the reference range, even after heparin reversal. It has been previously shown that at high heparin concentrations (4 U/mL), protamine concentrations,

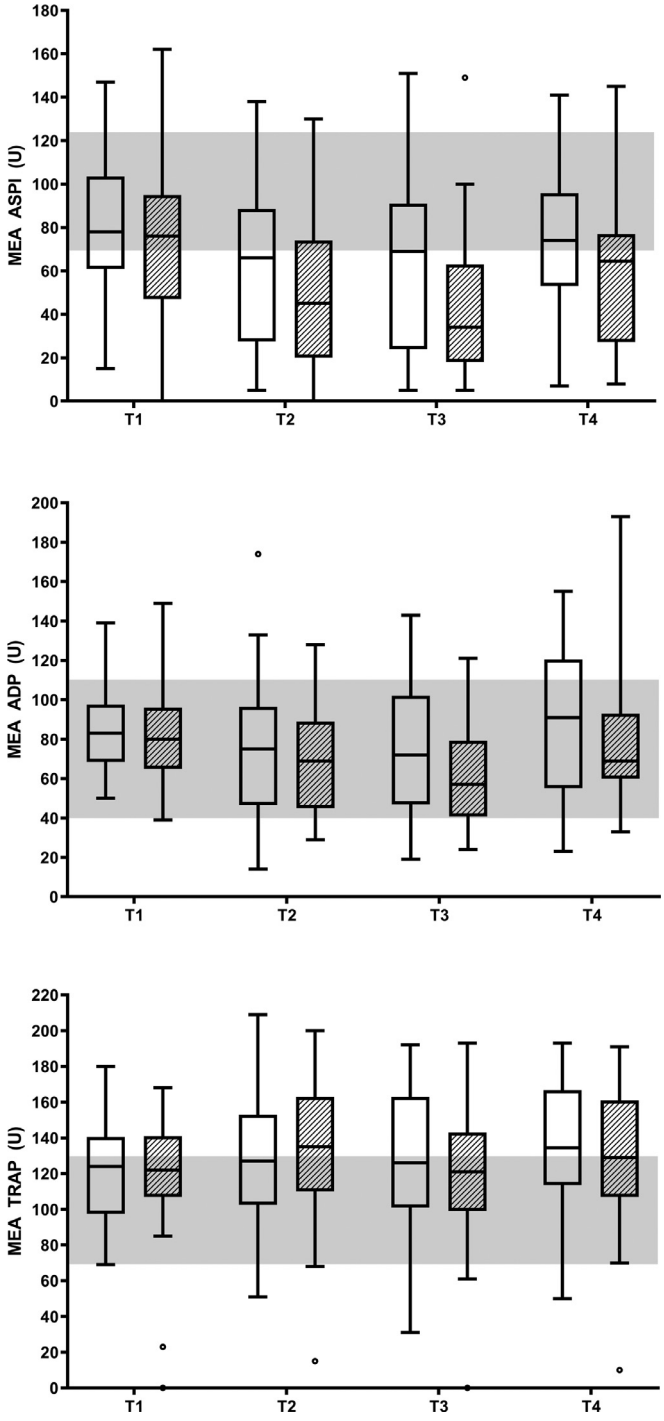

Fig 3. Multiple electrode aggregometry arachidonic acid test, adenosine diphosphate test, and thrombin-receptor-activating-peptide test in the low *(white)* and high *(hatched)* heparin dose groups. *Gray background* denotes reference values of the local clinical laboratory of the hospital. ADP, adenosine diphosphate; ASPI, arachidonic acid; MEA, multiple electrode aggregometry; T1, preoperatively after induction of anesthesia; T2, 10 minutes after aortic declamping; T3, 30 minutes after protamine administration; T4, 3 hours after protamine administration; TRAP, thrombin-receptor-activating-peptide test.

which correct the anticoagulant effects of heparin, were unable to reverse its antiplatelet effects[26] and that the CPB-induced impairment of platelet aggregation was not reversed at the time of protamine administration,[28] but only after 24 hours after CPB.[29] Furthermore, excess protamine itself can inhibit platelet functions.[30] Therefore, the higher protamine doses in the high heparin dose group may have affected the results after reversal of heparin. Indeed, the observed tendency to lower aggregation responses to arachidonic acid at 3 hours after heparin reversal in the high heparin dose group may have been influenced by both residual heparin effect and protamine dosing.

Despite its randomized setting, there are limitations in the present study. First, the trial was unblinded in the operating room. This could not be avoided because different heparin doses resulted in notably different ACT values. In the intensive care unit, on the other hand, all personnel were blinded with regard to the study group. Second, platelet counts were not monitored during surgery because the aim of the study was to assess the effects of heparin dosing on the POC measurements. It has been shown that the platelet count decreases during CPB and reaches a plateau at 2 hours after CPB.[31] In the present study, low platelet counts during the surgery may have affected the results.[32] However, based on the inclusion criterion, preoperative platelet counts were within normal range and the preoperative and postoperative platelet counts did not differ between the study groups. Third, AT3 levels were not measured. As a strength, the study groups differed substantially from each other in terms of the study intervention. During CPB with systemic heparinization, the anti-FXa activity in the high heparin dose group was >1.5-fold greater than the activity in the low heparin dose group.

Conclusions

In the present randomized clinical trial, the whole blood POC assays TEM and MEA did not demonstrate significant differences in either coagulation or platelet function in patients anticoagulated with a high or standard dose of heparin during cardiac surgery. Therefore, although the higher dose of heparin was as safe as the lower dose, regarding POC measurements, it does not seem to offer any benefit.

Conflict of Interest

None.

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