A Pharmacokinetic Model for Protamine Dosing After Cardiopulmonary Bypass

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

Design: **Retrospective case-control study.**

Setting: **Tertiary university hospital.**

Participants: **Patients undergoing elective cardiac surgery with cardiopulmonary bypass.**

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

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

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

KEY WORDS: protamine, heparin, cardiac surgery, cardiopulmonary bypass, hemostasis, coagulation

PROTAMINE IS USED to reverse heparin after termination of cardiopulmonary bypass (CPB). The anticoagulant properties of heparin are antagonized by the formation of a neutral 1:1 protamine-heparin complex. Protamine is commonly administered in a fixed protamine-to-heparin ratio based on the intraoperative dose of heparin, irrespective of the patient's heparin consumption. Protamine dosing usually ranges from 0.5 to 1.3 mg of protamine per 100 IU of heparin administrated.[1,2]

Yet, during CPB, heparin is broken down, with a half-life of approximately 4 hours in high dosage, and a small part of heparin is lost by blood loss. When these factors were taken into account, protamine dosing based on a fixed ratio was inadequate for the great majority of patients, and might lead to unantagonized heparin or protamine overdose, both causing coagulopathy.[3,4]

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

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

METHODS

Study Population

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

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

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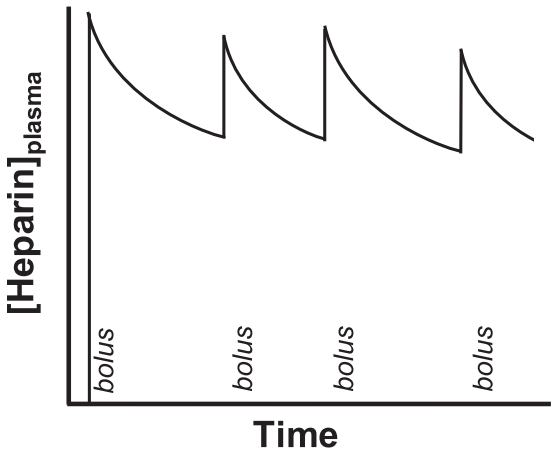

Fig 1. Schematic representation of the pharmacokinetic heparin model. Prior to CPB a bolus of 300 IE/kg of heparin is administered to achieve an ACT >480 s. During CPB, when the heparin concentration drops due to its half-life, the ACT will shorten. When the ACT falls below 480 seconds an additional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

therapy, and the preoperative use of heparin or heparin-like medication.

Study Procedure

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

Protamine Dosing Model

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

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

C_t is the residual heparin in the patient at the moment of protamine dosing (t). A and B represent the distribution over the subsequent compartments of the initial dose (C_0), 0.1 and 0.9, respectively,[7] and e is the base of the natural logarithm (2.718) with α and β being the time constants of both compartments (α = 10 minutes and β = 250 minutes). The half-life of heparin increases by incremental dosage. The highest dose investigated was 400 IU and showed a half-life of 150 minutes in humans.[8] Patients receive an average of 500 IU of heparin during a cardiac procedure in this center, leading to a half-life greater than 150 minutes. The half-life is expected to increase further with cooling (to approximately 34°C during CPB). As the detrimental effect of heparin overdosing is greater than protamine overdosing, the half-life of heparin was rounded up to avoid a residual heparin effect. In the absence of evidence about the half-life of high-dose heparin during CPB, an empirical half-life (β) of 250 minutes was chosen taking all the above-mentioned factors into consideration.

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

Anesthesia and Cardiopulmonary Bypass

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

CPB was performed at a blood flow of (2.2-2.4 L/min/m^2) during normo- or mild hypothermia (>34°C) using either a C5 or S5 heart–lung machine (Stöckert Instrumente GMBH, Munich, Germany) with a centrifugal pump and biocompatible coated circuit. Cell saving was applied throughout the surgical procedure. Shed blood from the thoracic cavity and residual blood that remained in the extracorporeal system after bypass were sucked up into the cell-saver reservoir using a dual-lumen tube connected to a vacuum pump, and subsequently transferred to the centrifuge bowl of the cell saver (Autolog, Medtronic, Minneapolis, MN). After plasma removal, red blood cells were washed, and concentrated red blood cells were retransfused.

Heparin and Protamine Management

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

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

Local Blood Transfusion Algorithm

The transfusion of packed red blood cells (PRBC) was based on the Dutch transfusion guidelines, recommending transfusion if hemoglobin levels are below 9.7 g/dL (6 mmol/L), in American Society of Anesthesiologists IV patients, and patients with symptomatic cerebrovascular disease, sepsis, heart failure, or severe pulmonary disease. Packed red blood cells transfusion is advised in cases of hemoglobin levels below 8.1 g/dL (5 mmol/L), in cases of acute blood loss from one bleeding focus in a normovolemic American Society of Anesthesiologists I patient aged >60 years, and in cases of fever or following cardiac surgery. Fresh frozen plasma or platelet transfusion was performed in patients with clinical signs of persistent nonsurgical bleeding (oozing). When laboratory coagulation test results were available, fresh frozen

Table 1. Patient Demographics and Surgical Characteristics

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

NOTE. Data represent frequencies or mean ± standard deviation.

Abbreviations: BMI, body mass index; CABG, coronary artery bypass graft; CD, conventional dosing; ECC, extracorporeal circulation; PK, pharmacokinetic dosing.

plasma was transfused when the international normalized ratio of the prothrombin time exceeded 1.5. Additional platelet concentrate administration was indicated for extended bypass times, clinical signs of bleeding, and/or a platelet count below 75×10^9/L.

Blood Sampling

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

Thromboelastometry

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

Other Study Parameters

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

Statistical Analysis

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

RESULTS

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

Figure 2 shows heparin and protamine dosing in the CD and PK dosing groups. Implementation of the PK model did not alter heparin dosing, but significantly reduced protamine dosing from 416 ± 82 mg to 186 ± 42 mg ($p = 0.001$) compared with the CD group. This resulted in a major reduction in the protamine-to-heparin dosing ratio from 1.1 ± 0.3 for the CD to 0.5 ± 0.1 for the PK dosing ($p < 0.001$).

Table 2 shows whether reduced protamine dosing in the PK group was associated with alterations in patient hemostasis, differences for hemoglobin, aPTT, prothrombin time, and platelet count between groups before surgery and at 3 minutes following protamine administration. Figure 3 shows the thromboelastometric clotting times for the subsequent tests 3 minutes after protamine administration. Furthermore, INTEM MCF was

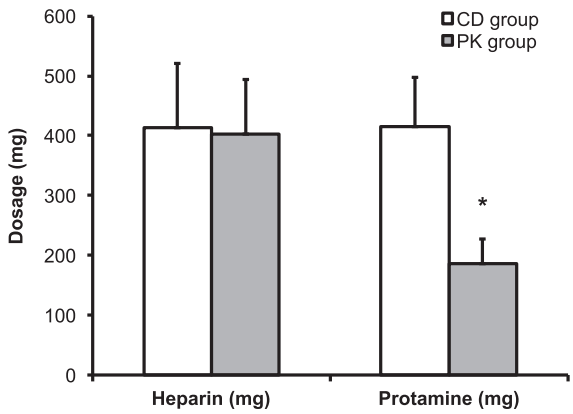

Fig 2. Protamine and heparin dosage. The protamine-to-heparin dosing ratio resulted in 1.1 ± 0.3 for the CD, and 0.5 ± 0.1 for the PK dosing ($p < 0.001$). PK, pharmacokinetic dosing.

Table 2. Laboratory Findings

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

NOTE. Data represent frequencies or mean ± standard deviation.

Abbreviations: ACT, activated coagulation time; aPTT, activated partial thrombin time; CD, conventional dosing; INR, international normalized ratio; PK, phramacokinetic dosing; PT, prothrombin time.

52 ± 8 in the CD group compared with 59 ± 9 in the PK dosing group, $p < 0.01$. EXTEM MCF was higher in the PK group (60 ± 9) when compared with the CD group (54 ± 8, $p = 0.02$), respectively. The fibrin part of the clot (fibrin polymerization test MCF) was 11 ± 4 in the CD group and 14 ± 6 in the PK group, $p = 0.01$.

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

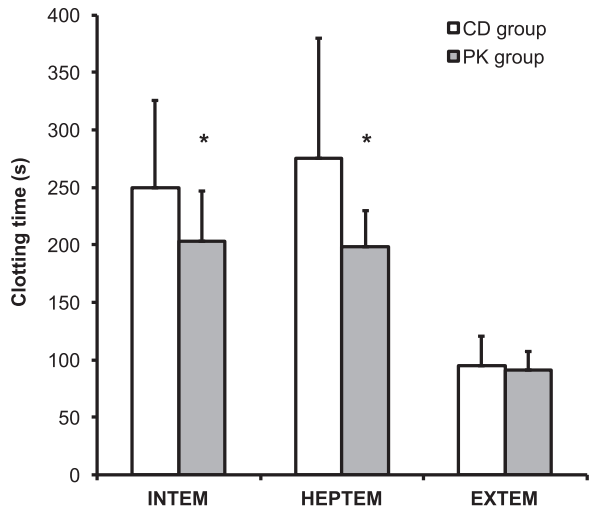

Fig 3. Thromboelastometric test results at three minutes following protamine administration. PK, pharmacokinetic dosing; INTEM, intrinsic coagulation cascade; HEPTEM, intrinsic coagulation cascade in the presence of heparinase; EXTEM, extrinsic coagulation cascade. *p < 0.05.

Table 3. Postoperative Outcome

	CD	PK	p Value
Postoperative transfusion			
PRBC	0 (0-2)	0 (0-0)	<0.001
0 units (n [%])	27 (52%)	58 (93%)	
1-2 units (n [%])	13 (25%)	3 (5%)	
≥ 3 units (n [%])	12 (23%)	1 (2%)	
Fresh frozen plasma	1 (0-2)	0 (0-0)	<0.001
0 units (n [%])	26 (50%)	61 (98%)	
1-2 units (n [%])	17 (32%)	1 (2%)	
≥ 3 units (n [%])	9 (18%)	0 (0%)	
Platelet concentrate	0 (0-1)	0 (0-0)	<0.001
0 units (n [%])	27 (52%)	59 (95%)	
1-2 units (n [%])	23 (44%)	3 (5%)	
3 units (n (%))	2 (4%)	0 (0%)	
Fibrinogen concentrate	0 (0-0) n=3	0 (0-0) n=4	0.98
Prothrombin complex concentrate	0 (0-0) n=1	0 (0-0) n=8	0.48

NOTE. Data are represented as median with interquartile range.

Abbreviations: CD, conventional dosing; PK, pharmacokinetic dosing; PRBC, packed red blood cells.

Multiple Linear Regression

Multiple linear regression was performed to evaluate the possible confounding effect of the type of surgery on thromboelastometric results and postoperative blood loss for both groups (Tables S1 and S2). The effect of PK dosing remained significant after correcting for the type of surgery for the INTEM, and HEPTEM was improved by PK dosing after correcting for the type of surgery when compared with the CD group. There were no differences in EXTEM or postoperative blood loss 6 hours postoperative after correcting for the type of surgery.

DISCUSSION

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

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

result in residual heparin, and protamine overdosing, which may result in impaired hemostasis, warrants better tools for protamine dosing.

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

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

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

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

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

APPENDIX A. SUPPLEMENTARY MATERIAL

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1053/j.jvca.2016.04.021.

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