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ORIGINAL ARTICLE

Impact of protamine dose on activated clotting time and thromboelastography in infants and small children undergoing cardiopulmonary bypass

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Summary

Objectives: To study the effect of two protamine-dosing strategies on activated clotting time (ACT) and thromboelastography (TEG).
Background: Protamine dosage based on neutralizing heparin present in the combined estimated blood volumes (EBVs) of the patient and cardiopulmonary bypass (CPB) pump may result in excess protamine and contributes toward a coagulopathy that can be detected by ACT and TEG in pediatric patients.
Methods: A total of 100 pediatric patients 1 month to $\leq$5 years of age undergoing CPB were included in this retrospective before/after design study. Combined-EBV group consisted of 50 consecutive patients whose protamine dose was calculated to neutralize heparin in the combined EBVs of the patient and the pump. Pt-EBV group consisted of the next 50 consecutive patients whose protamine dose was calculated to neutralize heparin in the patient's EBV.
Results: Baseline and postprotamine ACTs were similar between groups. Postprotamine heparin assay (Hepcon) showed the absence of residual heparin in both groups. Postprotamine kaolin-heparinase TEG showed that *R* was prolonged by 7.5 min in the Combined-EBV group compared with the Pt-EBV group (mean *R* of 20.17 vs 12.4 min, respectively, $P < 0.001$). Increasing doses of protamine were associated with a corresponding, but nonlinear increase in *R*. There was no significant difference in the changes for *K*, alpha, and MA between the groups.
Conclusion: Automated protamine titration with a protamine dosage based on Pt-EBV can adequately neutralize heparin as assessed by ACT while minimizing prolonging clot initiation time as measured by TEG.

Introduction

Protamine sulfate exerts its action by binding ionically to unfractionated heparin (UFH) and dissociates UFH from the heparin–Antithrombin complex. Protamine in excess can paradoxically impair coagulation and is a preventable cause of bleeding after cardiac surgery (1–7). The anticoagulant effect of protamine can be attributed to its polycationic structure that inhibits serine proteases resulting in weakened clot strength, clot kinetics and decreases platelet sensitivity to ADP receptors and collagen (2–5).

Heparin–protamine mismatch can occur following pediatric cardiopulmonary bypass (CPB) considering large variations in hemodilution related to patient/pump blood volumes and in the presence of an immature coagulation system makes maintenance of hemostasis challenging (8–12). To alleviate some of these effects, a patient-specific heparin concentration (PSHC)-based management of anticoagulation has been suggested to be superior, resulting in less activation of the coagulation cascade, less fibrinolysis, reduced blood loss and reduced need for transfusions in both pediatric and adult cardiac surgery (13–15). However, protocols for heparin reversal with protamine are not standardized and vary per institutional protocol.

In studies using automated protamine titration, the calculated protamine dose depends on the estimated heparinized blood volume. Some strategies approximate the protamine dose to neutralize the residual heparin circulating in the estimated blood volume of the patient (Pt-EBV) post-CPB and others recommend to neutralize the heparin in the combined EBVs of the patient and the CPB machine (Combined-EBV) (13–16). The latter strategy provides heparin reversal for nearly all of the heparin in the CBP machine that could be delivered to the patient by hemoconcentration with modified ultrafiltration (MUF) and/or transfusion of the remaining CPB machine volume. Williams, *et al*. (17) suggested the use of additional protamine after MUF to compensate for the increase in heparin activity after MUF. Gruenwald, *et al*. (16) studied effects of a modified heparin-dosing protocol and proposed protamine neutralization by a factor of 1.5 times the protamine dose for Combined-EBVs. Previous studies have analyzed the effects of varying amounts of protamine added to blood samples to study its impact on activated clotting time (ACT) or thromboelastography (TEG) (18–20). The objectives of this study were to retrospectively examine the impacts of two protamine dosage strategies for infants and children on efficacy of heparin neutralization based on ACT and changes in TEG variables.

Methods

The Institutional Review Board approved this retrospective, observational study, which analyzed data before and after a change in clinical practice concerning protamine dosage. All data were gathered from anesthesia and perfusion records. The inclusion criteria were as follows: (i) children undergoing CPB between ages of 1 month to ≤ 5 years; (ii) CPB <120 min; (iii) baseline and postprotamine ACT and UFH levels (Hepcon® HMS Plus™, Medtronic, Minneapolis, MN, USA); (iv) baseline and postprotamine TEG (TEG® 5000; Thromboelastography® Hemostasis Analyzer, Haemoscope Corporation, Niles, IL, USA); and (v) postprotamine ACT and TEG blood samples drawn within 15 min of administration of protamine. The exclusion criteria were as follows: (i) patients with known coagulation abnormalities such as hemophilia or protein C or S deficiency; (ii) patients who received more than one CPB run; (iii) patients who received fresh frozen plasma (FFP) (other than in the CPB priming volume), platelets, cryoprecipitate or recombinant factor 7 during MUF, or immediately after termination of CPB, but prior to protamine reversal. Baseline and intraoperative variables collected are listed in Table 1.

All TEG measurements were performed with addition of kaolin as the activator and heparinase per institutional protocol. CPB tubing biosurfaces were nonheparin coated and were the same through the time period of the study.

Heparin–protamine management

The initial UFH dose was based on the Hepcon® heparin dose response (HDR), a 6 channel test that calculates a three-point curve to estimate the amount of heparin required to initiate CPB. The patients activated whole blood clotting time is measured at baseline (channels 5,6- devoid of heparin) and in the presence of the different concentrations of heparin (channels 1–2 have 2.5 units·ml^{-1} and channel 3–4 have 1.5 units·ml^{-1} of heparin activity). The difference in the clotting times generates the patient's response to heparin and from the slope of this curve the amount of UFH to achieve an ACT of 480 s is determined. In addition to initial UFH dose, patients <10 kg receive 1000 units of UFH in CPB prime and those >10 kg receive 2000 units of UFH in CPB prime as per institutional protocol.

While on CPB, heparin activity is quantitatively determined by neutralization methods utilizing automated heparin–protamine titration (HPT) on the Hepcon® HMS Plus™ machine and is performed every 30–60 min. The HPT is a four-channel test, with each channel of the cartridge containing known but incremental amounts of protamine and a constant amount of thromboplastin for activating the patients heparinized blood. The first channel to clot as detected by a photo-optical system provides information on the lowest concentration of protamine required to neutralize the heparin in the blood sample. The amount of heparin present in the patient's blood sample is quantified based on this titration and the test may recommend additional UFH to maintain the patient at a stable heparin concentration during CPB. Our protocols during the time period of

Table 1 Demographics and procedural characteristics

	Combined-EBV (Mean ± SD)	Pt-EBV (Mean ± SD)	*P*-value*
Demographics			NS
Age (months)	15.7 ± 15.8	13.3 ± 14.2	0.43
Weight (kg)	9.0 ± 5.6	7.9 ± 4.0	0.24
Estimated blood volume (ml)	789.7 ± 401.1	709.1 ± 302.8	0.13
Body surface area (m^2)	0.400 ± 0.166	0.366 ± 0.138	0.27
Procedures			0.13
Atrial or ventricular septal defect repair	18	22	
Valves/conduits/aortic arch or Pulmonary artery repairs	12	10	
BCPC/Fontan/Shunts	16	14	
Others – CAVC/PAPVR Repair	4	4	
Preoperative Oxygen saturation <88%	14	14	1.0
Preoperative aspirin	14	14	1.0
Preoperative UFH iv infusion	1	1	1.0
Operative			NS
CPB time (min)	70.1 ± 20.6	75.3 ± 22.2	0.23
PRBC added to CPB prime [*n* (%)]	48 (96)	44 (88)	0.14
FFP added to CPB prime [*n* (%)]	14 (28)	11 (22)	0.49
Patient's EBV+ Crystalloid in prime+ PRBCpre+ FFPpre (ml)	1330 ± 552	1180 ± 413	0.97
Initial heparin dose (units·kg^{-1})	488.8 ± 155.2	496.4 ± 150.1	0.80
Total heparin dose (units·kg^{-1})	877.8 ± 238.9	880.6 ± 291.1	0.96
Protamine dosage			
(mcg·ml^{-1} of estimated blood volume)	77 ± 21 mcg·ml^{-1}	44 ± 19 mcg·ml^{-1}	<0.001
(mg·kg^{-1})	7.0 ± 1.9 mg·kg^{-1}	3.9 mg ± 0.9 mg·kg^{-1}	

Combined-EBV, sum of estimated blood volumes of the patient and cardiopulmonary bypass machine; Pt-EBV, estimated blood volume of the patient; mcg, micrograms; CPB, cardiopulmonary bypass; BCPC, bidirectional cavo-pulmonary connection; CAVC, complete atrioventricular canal; PAPVR, partial anomalous pulmonary venous return; FFP, fresh frozen plasma; PRBC, packed red blood cells.
*Based on an unpaired *t*-test for continuous variables and Fischer's exact test for categorical variables.

this study utilized a lower limit heparin concentration on CPB of 2.7 units·ml^{-1} and an upper limit of 5.4 units·ml^{-1}.

Prior to termination of CPB, a blood sample is drawn from the CPB circuit and the HPT test determines the protamine concentration to neutralize the heparin present in the combined EBVs of the patient and the pump. From this protamine concentration, protamine dosage in milligrams is calculated as a patient dose and a pump dose and this extrapolation is based on the respective EBVs of the patient and the pump (21). Postprotamine, a HR-ACT and a heparin assay is obtained within 5 min of administration of protamine.

Blood product management

As per our institution protocol, all infants less than 4 kg received FFP in the CPB prime to compensate for the reduced antithrombin III (AT) to maintain heparin sensitivity in this age group (8,22,23). Packed red blood cells (PRBC) were added in the CPB prime to maintain a target hematocrit above 28%. Use of aprotinin in our institution had been discontinued prior to this study period, and all patients per protocol had received epsilon aminocaproic acid (75 mg·kg^{-1} load prior to incision, 75 mg·kg^{-1} in CPB prime and 25 mg·kg·h^{-1} infusion) as an antifibrinolytic.

Protamine dose groups

Prior to November 2008, we based our protamine dose to neutralize heparin present in the Combined-EBV of the patient and the pump to theoretically provide reversal for the increase in heparin activity that could occur after MUF (17,24). During a quality improvement study in November 2008, kaolin-heparinase TEGs analyzed at 3 additional points (pre-MUF, post-MUF & postprotamine) revealed a sudden prolongation of *R* with the postprotamine TEG. We speculated that MUF had not contributed toward an increase in *R* and that excess protamine could have resulted in this finding. After November 2008, we altered the protocol such that the protamine dosage was based only on the Pt-EBV. Our intent was to administer additional protamine if the postprotamine ACT did not return to baseline or if residual heparin activity was detected with the HMS-based heparin assay. The Combined-EBV group included 50 consecutive patients before November 2008, and the Pt-EBV group included 50 consecutive patients after November 2008.

Data analysis

We used the method proposed by Cohen, *et al.* (25) to determine sample size needs based on a multiple regression analysis with an alpha of 0.05, power of 0.8. We used proportions and frequency counts for categorical variables and means and standard deviations for continuous variables. Groups were compared using Fischer's exact tests for categorical variables (due to small cell counts) and unpaired *t*-tests for continuous measures. We computed changes in ACT and TEG for each patient from baseline (b) to postprotamine (pp), (ΔACT = ACTpp − ACTb; ΔR = *R*pp − *R*b etc.). The normality of baseline and postprotamine ACT values was tested using the Shapiro–Wilk test. Wilcoxon sign rank test was used for non-normally distributed data. We compared between protamine dose groups the differences in TEG value changes using ANOVA, controlling for several important baseline and intraoperative covariates (listed in Table 1). Both groups were analyzed for age-related difference by stratifying the groups by age categories of 1–3 months, 3 months to <1 year and >1 year. Important presurgical covariates for the multivariate models were identified through rigorous crosstabulations. Covariates that were associated with the outcome of interest in the multivariate analysis at $P < 0.05$ were included in the final multivariate models.

Results

Subject population and heparin–protamine dosage

A total of 450 patient records were retrospectively screened until the target sample size meeting the inclusion criteria was reached. Of the 158 patients that met the inclusion criteria initially, 58 were further excluded for missing data (e.g., incomplete TEG data or other missing data). Both groups were comparable in terms of patient characteristics and type of surgeries (Table 1). Both groups were similar for patients with aspirin therapy and the presence of preoperative oxygen saturations on room air $\leq 88\%$. There were no changes in the heparin-dosing strategies during the period of review, and no differences were noted in either the initial UFH dose before CPB or the total UFH dose for CPB between groups. Patients in the Combined-EBV group received significantly more protamine than those in the Pt-EBV group, 77 ± 21 mcg·ml^{-1} vs 44 ± 19 mcg·ml^{-1} ($P < 0.001$). For perspective, ignoring age-dependent EBV differences, the Combined-EBV group received protamine 7 ± 1.9 mg·kg^{-1} vs 3.9 mg ± 0.9 mg·kg^{-1} for the Pt-EBV group.

The groups were stratified by age (1–3 months, 3 months to <1 year, and >1 year of age) (Table 2). Within the age categories, the groups were similar in patient weight, the effect of hemodilution (estimated by the sum of blood volumes of the patient, crystalloid in pump prime and PRBC and FFP in pump prime), the initial UFH and total UFH doses. (Table 2) Between the groups, there was a significant difference in protamine dose in all age categories (Table 2).

Efficacy of heparin neutralization via ACT

Although both groups varied significantly in the amount of protamine they received, the effect on postprotamine kaolin-activated HR-ACTs were similar in both groups. Baseline ACT (Combined-EBV 131 ± 15.7 vs Pt-EBV 126.8 ± 12.3, $P = 0.14$) and postprotamine ACT (Combined-EBV 119.8 ± 30.1 vs Pt-EBV 131.6 ± 83.9, $P = 0.35$) were similar when compared between groups (Table 3). When compared within the groups, Wilcoxon sign rank test reveals significant changes regarding return of ACT to baseline. Excluding a single outlier in Pt-EBV group (ACTpp of 700 s after 4 mg·kg^{-1} of protamine), postprotamine ACT was significantly lower than baseline in both groups ($P = 0.02$, both groups). Postprotamine Hepcon heparin assay determined zero heparin concentration and did not recommend additional protamine for patients in either group.

Impact on TEG

There were several statistically significant univariate associations between baseline variables and each kaolin-heparinase TEG outcome, as listed in Tables 4 and 5. The postprotamine clot initiation time (Rpp) averaged 20.17 min in the Combined-EBV group, a significantly longer time than 12.41 min for the Pt-EBV group ($P < 0.001$) (Table 4). The average ΔR (=*R*pp − *R*b) was 10.8 min for the Combined-EBV group and 3.4 min for the Pt-EBV group ($P < 0.001$). Stratified for age, ΔR was statistically significant in the Combined-EBV group in the 3 month to <1 year of age category ($P = 0.007$) (Table 5). On regression analysis, addition of FFP to the CPB prime was associated with a 7.39 min decrease in ΔR ($P = 0.002$). Further, to analyze the protective effect of FFP, regression analysis on children who received PRBCprime and no FFPprime revealed the mean ΔR was 13.2 min for the Combined-EBV group vs 3.3 min for the Pt-EBV group ($P = 0.0003$).

Between the groups, there were statistically significant differences in the average baseline values of α (Combined-EBV, 59 ± 11 vs Pt-EBV, 64 ± 10, $P = 0.05$) and MA (Combined-EBV, 65 ± 7 vs Pt-EBV, 68 ± 6, $P = 0.02$). Corresponding significant differences were seen in postprotamine averages of α and MA. Stratified

Table 2 Patient characteristics, heparin, protamine dosage per age group

	Combined-EBV group	Pt-EBV group	*P*-value*
1 month to <3 months of age			
n	9	15	
Weight (kg)	4.5 ± 1.0	4.4 ± 0.5	0.65
Patient's EBV + Crystalloid in prime + PRBCpre + FFPpre (ml)	910.7 ± 194.5	795.1 ± 125	0.09
Initial heparin (units·kg^{-1})	547.4 ± 275.8	532.2 ± 165.6	0.87
Total heparin (units·kg^{-1})	1037.7 ± 275.8	965.5 ± 285.1	0.55
Protamine dose (mg·kg^{-1})	7.5 ± 2.0	3.9 ± 0.7	**0.01**
3 months to <1 year of age			
n	23	17	
Weight (kg)	6.6 ± 1.3	6.5 ± 1.7	0.63
Patient's EBV + Crystalloid in prime + PRBCpre + FFPpre (ml)	1096.3 ± 195.4	1084.6 ± 167.1	0.84
Initial heparin (units·kg^{-1})	493.8 ± 158.1	524 ± 138.8	0.53
Total heparin (units·kg^{-1})	891.6 ± 231.7	985.9 ± 314.7	0.28
Protamine dose (mg·kg^{-1})	7.3 ± 2.0	3.9 ± 1.1	**<0.001**
>1 year of age			
n	18	18	
Weight (kg)	14.3 ± 5.5	12.1 ± 3.1	0.19
Patient's EBV + Crystalloid in prime + PRBCpre + FFPpre (ml)	1838.7 ± 609	1593.3 ± 364.6	0.15
Initial heparin (units·kg^{-1})	453.2 ± 146.9	440.1 ± 137.5	0.78
Total heparin (units·kg^{-1})	780.1 ± 188.2	710.3 ± 189.2	0.27
Protamine dose (mg·kg^{-1})	6.2 ± 1.4	3.7 ± 0.6	**<0.001**

Combined-EBV, sum of estimated blood volumes of the patient and cardiopulmonary bypass machine; Pt-EBV, estimated blood volume of the patient; PRBC, packed red blood cells.
*Based on unpaired *t*-test for continuous variables.
Bold values denote statistical significance.

Table 3 Efficacy of Heparin Neutralization on Kaolin HR-ACTs between and within groups

	Combined-EBV group (mean ± SD), *n* = 50	Pt-EBV group (mean ± SD), *n* = 50	*P*-value*
ACT baseline (s)	131.0 ± 15.7	126.8 ± 12.3	0.14
ACT postprotamine (s)	119.8 ± 30.1	131.6 ± 83.9	0.35
P-value†	0.02	0 < 0.001	
Excluding the single outlier in Pt-EBV group	Combined-EBV group (mean ± SD), *n* = 50	Pt-EBV group (mean ± SD), *n* = 49	*P*-value*
ACT baseline (seconds)	131.0 ± 15.7	126.7 ± 12.4	0.15
ACT postprotamine (seconds)	119.8 ± 30.1	120.0 ± 17.8	0.98
P-value†	0.02	0.02	

Combined-EBV = sum of estimated blood volumes of the patient and cardiopulmonary bypass machine; Pt-EBV = estimated blood volume of the patient; ACT = activated clotting time; ΔACT = ACT postprotamine ACT baseline.
*Calculated using an unpaired *t*-test between groups.
†Calculated using Wilcoxon sign rank test within each group.

for age, baseline MA was the only significant parameter in the 1–3 month age group (Table 5). There were no statistically significant differences between the groups for baseline and postprotamine values of *K*. No statistical differences were spotted in TEG changes as measured by Δ*K*, Δα, and ΔMA (Tables 4 and 5).

Figure 1 demonstrates TEG results in a patient in the Combined-EBV group, with a baseline TEG, a rewarming TEG drawn just prior to termination of CPB (patient core temperature of approximately 35°C), and a post-MUF, postprotamine TEG drawn within 5 min of protamine administration, showing a lengthening of *R* between the period just before termination of CPB and a few minutes after protamine administration after CPB.

Discussion

We observed that utilizing a strategy to neutralize the heparin present in the combined EBVs of the patient and the pump resulted in excess protamine and caused a significant prolongation of clotting time (*R*) on TEG but showed minimal effect on ACT. We employed the Combined-EBV strategy of protamine reversal prior to November 2008 secondary to data suggesting that there is a substantial (approximately 25%) increase in heparin concentration after MUF following CPB (6,17,24,26).

We were unable to demonstrate an increase in the postprotamine ACT or a dose-related effect on postprotamine ACT with either protamine strategy. Comparing within the groups, after removal of the single outlier, we

Table 4 Impact of Protamine on kaolin, heparinase Thromboelastogram

Thromboelastogram values	Combined-EBV group (mean ± SD)	Pt-EBV group (mean ± SD)	*P*-value*
R b (min)	9.35 ± 4.20	8.98 ± 3.56	0.64
R pp	20.17 ± 11.95	12.41 ± 7.16	**<0.001**
Δ*R*	10.8 ± 13.1	3.4 ± 7.1	**<0.001**
K b (min)	2.41 ± 1.78	1.96 ± 1.21	0.14
K pp	6.78 ± 4.28	4.81 ± 6.55	0.08
Δ*K*	4.4 ± 4.8	2.9 ± 5.7	0.15
α b (degrees)	59.71 ± 11.75	64.16 ± 10.98	**0.05**
α pp	35.85 ± 15.80	44.37 ± 14.05	**0.005**
Δα	23.9 ± 19.2	19.8 ± 14.2	0.23
MA b (mm)	65.02 ± 7.19	68.35 ± 6.27	**0.02**
MA pp	47.47 ± 10.31	53.03 ± 9.69	**0.007**
ΔMA	17.5 ± 11.8	15.3 ± 9.7	0.30

Univariate mean changes in thromboelastogram values.
b = baseline thromboelastogram value; pp = postprotamine thromboelastogram value; Δ = postprotamine (value) − baseline (value); Combined-EBV = sum of estimated blood volumes of the patient and cardiopulmonary bypass machine; Pt-EBV = estimated blood volume of the patient; MA = maximum amplitude.
*Unpaired *t*-test between groups.
Bold values denote statistical significance.

Table 5 Age-stratified thromboelastograph (TEG) parameters

Thromboelastogram values	Combined-EBV group (mean ± SD)	Pt-EBV group (mean ± SD)	*P*-value*
1–3 months			
Δ*R*	7.4 ± 11.8	2.3 ± 4.0	0.13
Δ*K*	3.1 ± 3.8	1.9 ± 1.6	0.29
Δα	19.9 ± 17.0	20.4 ± 16.8	0.94
ΔMA	14.1 ± 12.3	13.6 ± 10.2	0.90
3 months to <1 year			
Δ*R*	12.2 ± 14.6	1.2 ± 5.4	**0.007**
Δ*K*	5.4 ± 6.3	2.3 ± 1.5	0.06
Δα	26.1 ± 24.2	20.0 ± 9.0	0.35
ΔMA	19.0 ± 14.8	18.5 ± 7.0	0.89
>1 year			
Δ*R*	10.9 ± 12.1	6.2 ± 9.4	0.20
Δ*K*	3.8 ± 2.8	4.1 ± 9.0	0.90
Δα	23.1 ± 13.1	19.1 ± 16.3	0.40
ΔMA	17.4 ± 6.7	14.1 ± 10.9	0.26

Univariate mean changes in thromboelastogram values.
Δ = postprotamine (value) − baseline (value); Combined-EBV = sum of estimated blood volumes of the patient and cardiopulmonary bypass machine; Pt-EBV = estimated blood volume of the patient; MA = maximum amplitude.
*Unpaired *t*-test between groups.
Bold value denotes statistical significance.

observed that the average ACTpp was less than baseline ACT in both groups (Table 3). The heparin assay by HPT indicated the absence of residual heparin after protamine reversal and did not recommend an additional protamine dose in any patients regardless of group. We recognize that relying only on ACT in predicting return of hemostasis has its pitfalls (13–15), but there are overall advantages in using a PSHC-based anticoagulation management that utilizes ACT within its test as an end point. First, the HDR determines the patient's responsiveness to UFH and demonstrates linear correlation to the anticoagulant effects of heparin and helps determine UFH dose to initiate CPB (27,28). Second, the HPT test maintains stable heparin concentrations on CPB, as opposed to stable ACTs, minimizing the total UFH dose and calculates UFH levels independent of all factors except UFH level. Whole blood heparin concentrations determined by HPT also appear to correlate with measurements of the functional assay using antifactor Xa than with ACTs in adults and children undergoing CPB (15,29).

Our study differs from previous studies in that we were unable to illustrate a protamine excess related increase in ACT (18,30). Ni Ainle, *et al.* (30) demonstrated that excess protamine reduces the rate of factor V activation by thrombin and factor Xa, suggesting at least a partial mechanism for impairment of coagulation tests that utilize the intrinsic coagulation pathway such as the ACT and the activated partial thromboplastin time. Mochizuki, *et al.* (18) studied the effect of excess protamine on samples of blood from the extracorporeal reservoir of adults having CPB-assisted surgery. They were able to show that higher doses of protamine resulted in statistically significant lengthening of ACT values for protamine/heparin ratios greater than 2.6 : 1. Heparin concentrations in our patients at the termination of CPB were not consistently documented, but based on our protocol of maintaining the lower limits of heparin concentration at 2.7 units·ml^{-1}, we can speculate that the *maximum* possible protamine/heparin ratio received by Pt-EBV group was 1.6 : 1 vs 2.8 : 1 for the Combined-EBV group. Although the Combined-EBV group received large protamine doses of 7.0 ± 1.9 mg·kg^{-1}, we might not have traversed the 2.6 : 1 ratio to appreciate a consistent effect on ACT as suggested by Mochizuki *et al.* It is also possible that the kaolin-activated HR-ACT might not be as sensitive as the TEG for detecting subtle anticoagulant effects of excess protamine.

Comparing between and within groups, our findings are consistent with protamine excess induced prolongation of TEG's clot initiation time (*R*) as demonstrated by previous studies (19,20,31). When compared between the groups, with exception of Δ*R* (Δ*R* = *R*pp − *R*b), other TEG changes (Δα, Δ*K*, and ΔMA) did not reach statistical significance, differing from these previous

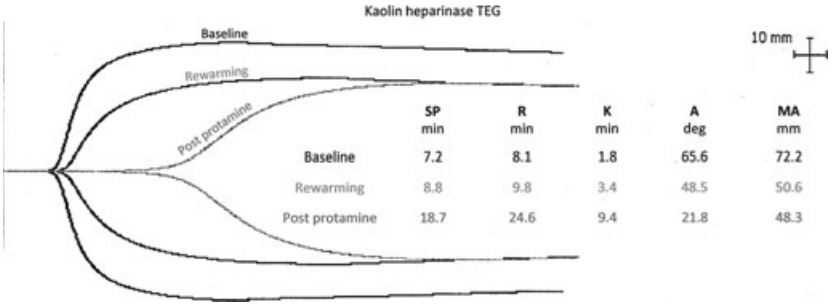

Figure 1 Example of a set of thromboelastographs (TEGs) from a patient who received protamine based on a dose to reverse the combined blood volumes of the cardiopulmonary bypass machine and the patient.

studies (20,31). While these previous studies have used blood samples from adults, we investigated the coagulation parameters of a physiologically different population of infants and young children, and the results may not be directly comparable. Our findings reflect results of protamine/heparin interactions that occurred from direct administration of these drugs to pediatric patients. Also, our CPB times were 29% shorter than Gibbs, *et al*. (average 94 min, range 54–154 min), and perhaps more functional platelets were present at the end of CPB for our patients accounting for the nonsignificant changes in $\Delta\alpha$, ΔK, and ΔMA. On fractional polynomial analysis, we did not notice a dose-dependent effect of excess protamine on the *R*-value of TEG, instead we observed disproportionate increase in ΔRs with smaller increases in protamine as protamine dose increased.

We analyzed the TEG results stratified by patient's age. In the age category of 3 months to <1 year, we observed a significant difference in ΔR between the groups (Combined-EBV $\Delta R = 12.2 \pm 14.6$, Pt- EBV $\Delta R = 1.2 \pm 5.4$, $P = 0.007$) (Table 5). To explain for this occurrence, we assessed the impact of FFP that was routinely administered to infants <4 kg as a part of the CPB prime on TEG. By regression analysis, addition of FFP to the CPB prime was associated with a decrease in ΔR by 7.39 min ($P = 0.002$) suggesting that FFP added in the CPB prime had a protective effect on the clot initiation time on TEG. In contrast, children (>4 kg) who did not receive FFP in CPB prime, but received PRBC in pump and those exposed to higher doses of protamine demonstrated significantly longer postprotamine clot initiation times (ΔR was 13.2 min for the Combined-EBV group vs 3.3 min for the Pt-EBV group, $P = 0.0003$). Recent findings from Bolliger, *et al*. (32) indicated that a protamine dose-dependent anticoagulant effect exists on the rate of thrombin generation, thromboelastometry parameters, specifically clotting time. Bolliger *et al*. (32) also state that the presence of normal functioning platelets or an increased factor VIII/von Willebrand factor concentration would mitigate effect of excess protamine anticoagulation *in vitro*. Our findings are in agreement with the study of Bolliger, *et al*., in that the addition of FFP in CPB prime to children <4 kg may have contributed to exogenous factor VIII/von Willebrand factor concentrations in the 1 month to <3 months age category and despite receiving excess protamine did not demonstrate a statistical significance in clot initiation time (Table 4).

We also assessed the groups for transfusion administration after protamine administration but prior to surgery completion. In the Pt-EBV group, 14% (7/50) patients received FFP or cryoprecipitate for clinical bleeding prior to the completion of surgery, while 20% (10/50) patients in the Combined-EBV group received similar blood products during surgery after protamine administration. This difference was not significant ($P = 0.42$).

We did not find any changes in clinical management or protocols within the interval selected for study that may have affected our outcomes. Likewise, we controlled for effects of hemodilution with CPB prime on clotting factors and platelets by comparing similar groups with similar hemodilution. The limitations of the study were the before/after design. We chose this design because these time periods represented a surrogate for protamine dose as well as a representation of two different dose strategies. Between the groups, there were statistical differences for baseline and α and MA despite their average absolute values being in the clinically normal ranges. We do not have a good explanation for the baseline differences in α and MA between the groups, and we can speculate the before/after design to contribute to this variation. We used baseline values as a control and differences existed in α and MA, but the impact of excess protamine on *R* was remarkable. We did not assess for fibrinolysis, as we had no control on how long TEGs were run after achieving a MA. We did not analyze postoperative clinical parameters such as transfusion amount, hematocrit, chest tube output, coagulation profiles, platelet counts, functional heparin concentrations, or inotropic scores. We did not measure platelet counts or factor VIII activity at the time of protamine administration, but attempted to minimize all

these variables by comparing similar groups. We did exclude patients who underwent CPB for more than 120 min to limit the effects of prolonged CPB and complex repairs on hematologic outcomes. Neonates were not included because of the longer CPB run times, the complexity of cases (RACHS 4–6) and also per institutional policy platelet transfusions are started during MUF for all neonates, prior to start of protamine thus excluding these patients from the study.

Excessive protamine, a condition suggested by our findings when protamine dose was calculated based on neutralizing UFH present in the Combined-EBVs of patient and the pump, was associated with prolongation of clotting time (*R*) measured by TEG and not reflected as a prolongation in ACT. Addition of FFP in CPB prime was protective by mitigating the effects of excess protamine on clot initiation. In summary, automated protamine titration with a protamine dosage based on Pt-EBV can adequately neutralize heparin as assessed by ACT while minimizing prolonging clot initiation time as measured by TEG.

Disclosures

Institutional IRB approval was procured prior to recording all the retrospective data of the study. No funding was used for the study.

Conflict of Interest

None of the authors have any conflicts of interests pertaining to this study.

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