



The association of thrombin generation with bleeding outcomes in cardiac surgery: a prospective observational study

L'association de la génération de thrombine avec les issues hémorragiques en chirurgie cardiaque : une étude observationnelle prospective

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Abstract

Background Cardiac surgery with cardiopulmonary bypass (CPB) is associated with coagulopathic bleeding. Impaired thrombin generation may be an important cause of coagulopathic bleeding but is poorly measured by existing hemostatic assays. We examined thrombin generation during cardiac surgery, using calibrated automated thrombography, and its association with bleeding outcomes.

Methods We conducted a prospective observational study in 100 patients undergoing cardiac surgery with CPB. Calibrated automated thrombography parameters were expressed as a ratio of post-CPB values divided by pre-CPB values. The association of thrombin generation parameters for bleeding outcomes was compared with conventional tests of hemostasis, and the outcomes of patients with the most severe post-CPB impairment in thrombin generation ($\geq 80\%$ drop from baseline) were compared with the rest of the cohort.

Results All 100 patients were included in the final analysis, with a mean age of 63 (12) yr, 31 (31%) female, and 94 (94%) undergoing bypass and/or valve

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surgery. Post-CPB, peak thrombin decreased by a median of 73% (interquartile range [IQR], 49–91%) ($P < 0.001$) and total thrombin generation, expressed as the endogenous thrombin potential (ETP), decreased 56% [IQR, 30–83%] ($P < 0.001$). In patients with $\geq 80\%$ decrease in ETP, 21% required re-exploration for bleeding compared with 7% in the rest of the cohort ($P = 0.04$), and 48% required medical or surgical treatment for hemostasis compared with 27% in the rest of the cohort ($P = 0.04$).

Conclusions Thrombin generation is significantly impaired by CPB and associated with higher bleeding severity. Clinical studies aimed at the identification and treatment of patients with impaired thrombin generation are warranted.

Résumé

Contexte La chirurgie cardiaque avec circulation extracorporelle (CEC) est associée à des saignements sur coagulopathie. L'altération de la génération de thrombine peut constituer une cause importante de saignement sur coagulopathie, mais elle est mal mesurée par les tests d'hémostase existants. Nous avons examiné la génération de thrombine pendant la chirurgie cardiaque à l'aide d'une thrombographie automatisée calibrée ainsi que son association avec les issues hémorragiques.

Méthode Nous avons réalisé une étude observationnelle prospective portant sur 100 patients bénéficiant d'une chirurgie cardiaque sous CEC. Les paramètres de thrombographie automatisée calibrée ont été exprimés sous forme du rapport entre les valeurs post-CEC divisées par les valeurs pré-CEC. L'association des paramètres de génération de thrombine pour les issues hémorragiques a été comparée aux tests conventionnels de l'hémostase, et les issues des patients présentant l'altération post-CEC la plus prononcée dans la génération de thrombine (baisse $\geq 80\%$ par rapport aux valeurs de base) ont été comparées au reste de la cohorte.

Résultats Les 100 patients ont tous été inclus dans l'analyse finale, avec un âge moyen de 63 (12) ans, 31 (31 %) femmes et 94 (94 %) subissant une chirurgie de pontage et / ou une chirurgie valvulaire. Après la CEC, le pic de thrombine a diminué d'une médiane de 73 % (écart

interquartile [ÉIQ], 49 à 91 %) ($P < 0,001$) et la génération de thrombine totale, exprimée en potentiel de thrombine endogène (PTE), a diminué de 56 % [ÉIQ, 30–83 %] ($P < 0,001$). Chez les patients présentant une diminution $\geq 80\%$ du PTE, 21 % ont nécessité une nouvelle exploration pour dépister les saignements, comparativement à 7 % dans le reste de la cohorte ($P = 0,04$), et 48 % ont nécessité un traitement médical ou chirurgical pour l'hémostase, comparativement à 27 % dans le reste de la cohorte ($P = 0,04$).

Conclusion La génération de thrombine est significativement altérée par la CEC et associée à des saignements plus graves. Des études cliniques visant à identifier et à traiter les patients présentant une altération de la génération de thrombine sont recommandées.

Keywords Blood coagulation disorders · Cardiac surgical procedures · Hemostasis · Perioperative care · Thrombin

Coagulopathy and bleeding are common during cardiac surgery with cardiopulmonary bypass (CPB), with up to 10% of patients experiencing major bleeding.^{1–3} Higher levels of coagulopathic bleeding and transfusion are associated with increases in major morbidity and mortality.³ Coagulopathy after CPB is due to a combination of factors, including consumptive coagulopathy, hemodilution, and prothrombin activation during CPB despite systemic anticoagulation.^{4,5} These processes interact to cause platelet consumption and dysfunction, hyperfibrinolysis, and depletion of coagulation factors, leading to profoundly impaired thrombin generation.^{6,7}

Thrombin generation, a downstream event facilitated by the intrinsic and extrinsic coagulation systems and a key component of an effective hemostatic response, has a central role in CPB-associated coagulopathy. Nevertheless, there are few clinical data assessing thrombin generation in this context, since conventional laboratory measures of hemostasis (such as the prothrombin time, PT, or the international normalized ratio, INR, and the activated prothrombin time, aPTT) generally reflect the initiation of clot formation, but not propagation or the dynamics of clot formation.^{7,8} Point-of-care coagulation assays, such as viscoelastic testing (ex., Thromboelastography [TEG®; Haemonetics Corporation, Boston, MA, USA] and rotational thromboelastometry [ROTEM®; TEM International GmbH, Mississauga, ON, Canada]) may be more predictive of coagulopathic bleeding than conventional testing, yet still inadequately reflect thrombin generation.^{7,9} There is limited evidence

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clarifying the best method to measure changes in thrombin generation and how to incorporate such measurements to improve clinical management and patient outcomes.¹⁰

Calibrated automated thrombography (CAT) measures thrombin generation in a plasma sample *in vitro*, providing information not otherwise captured by standard laboratory tests. A few prior studies using CAT have shown that thrombin generation decreases significantly during CPB and is associated with significant bleeding, but existing data are sparse, and it is unclear what threshold of thrombin generation impairment is associated with bleeding.^{7,11–13} We conducted a nested prospective cohort study within a randomized trial comparing heparin management in 100 patients undergoing cardiac surgery with cardiopulmonary bypass. The objectives of this study were to determine the effects of CPB on thrombin generation as measured by CAT and standard coagulation assays and to explore the association between post-CPB thrombin impairment and clinical bleeding outcomes. We hypothesized that increased bleeding will be observed in a subset of patients with the lowest thrombin function post-CPB and that a threshold of thrombin impairment could be identified that facilitates risk stratification and may help guide management of coagulopathy.

Methods

Study population

This was a prospective cohort study nested in a single institution randomized trial examining the impact of heparin management on thrombin generation in 100 consenting adult patients undergoing cardiac surgery with CPB (NCT03347201) between 2 October 2017 and 28 February 2019 (Electronic Supplementary Material [ESM] eFig. 1). The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines were adhered to for the reporting of this study.¹⁴ Ethics approval was obtained from the University Health Network Research Ethics Board (REB 15-9761) on 3 January 2017. Patients were eligible for inclusion if they were > 18 yr and scheduled to undergo coronary artery bypass grafting, valve repair or replacement, or a combination of procedures requiring CPB. Patients were excluded if they were anticipated to undergo circulatory arrest, ventricular assist device insertion or explant, heart transplant, or correction of complex congenital cardiac disease. Patients were also excluded if they had significant liver dysfunction (liver enzymes greater than two times the upper limit of normal), pre-existing coagulopathy (INR >

1.5, aPTT > 45 sec, fibrinogen < 1.0 g·L⁻¹, or platelet count < 100 × 10⁹·L⁻¹), non-discontinuation of preoperative anticoagulants, hereditary bleeding disorders, or a previous diagnosis of heparin-induced thrombocytopenia.

Clinical procedures and standardized heparin management protocol

Anesthesia was induced routinely at the discretion of the attending anesthesiologist. The CPB circuit was primed with PLASMA-LYTE A solution (Baxter Corporation, Mississauga, ON, Canada), 25 g of mannitol, and 5000 U of heparin, utilizing retrograde autologous priming if appropriate to a total volume of 930 mL. Adequate inhibition of the clotting cascade prior to CPB was monitored with the HemochronTM activated clotting time (ACT) assay (International Technidyne Corporation [ITC]; Edison, NJ, USA). Half of patients ($n = 50$) received an initial heparin bolus of 350–400 U·kg⁻¹ targeting an ACT > 480 sec prior to initiation of CPB, with ongoing ACT monitoring guiding additional 5,000–10,000 U boluses, and an empiric heparin to protamine reversal ratio ≤ 1:1. The other half ($n = 50$) were managed using the HMS Plus system (Medtronic; Minneapolis, MN, USA) with heparin protamine titration, which calculates individual patient heparin concentrations to dose heparin and protamine. In both groups, ACT was performed every 30 min while on CPB and additional heparin given if the ACT fell below 480 sec. Standard non-pulsatile CPB with a membrane oxygenator and roller pumps was utilized with moderate hypothermia (core temperature 26–34°C) and crystalloid prime. Patients received tranexamic acid accounting for baseline renal function (ESM eFig. 2),¹⁵ and cell salvage was standardized as per institutional practices. Blood product transfusions were administered according to standardized protocols, with balanced crystalloid typically employed as the non-blood resuscitation fluid of choice (ESM eFigs 2 and 3).^{16,17}

Sample size

The primary outcome of this nested cohort study was detection of a difference in peak thrombin formation from pre- to post-CPB after protamine administration similar to that observed by Bosch *et al.*,¹² where the pre-CPB group mean peak thrombin was 321 nM, and the post-protamine CPB peak thrombin group mean was 241 nM. Assuming a similar result, our power to detect a difference with a sample size of 100 patients and a pre-specified two-sided alpha = 0.05 is > 0.90, not accounting for multiple comparisons.

Measurements

Samples were collected via a syringe from an arterial line placed for the procedure. Pre-CPB measurements were collected at the time of anesthesia induction prior to heparin administration. Samples for post-CPB measurements were taken approximately five minutes after heparin reversal with protamine.

Thrombin generation

Specimens were collected in sodium citrate tubes (0.13 M, nine parts blood, one part sodium citrate) containing corn trypsin inhibitor ($20 \mu\text{g}\cdot\text{mL}^{-1}$) and centrifuged twice at 2,900 g for ten minutes at room temperature.¹⁸ Platelet-poor plasma was collected from the upper three quarters of the supernatant.¹¹ Calibrated automated thrombography was performed as described by Hemker *et al.*,^{19,20} with the standardization of reagents suggested by Dargaud *et al.*²¹ Calibrated automated thrombography assays were performed using a thrombinoscope system based on a Fluoroskan Ascent® fluorometer (Thermo Fisher Scientific Inc.; Waltham, MA, USA). Citrated plasma samples (80 μL) were combined in wells of 96-well plates with 20 μL trigger solution (thrombinoscope PPP or PPP-Low reagent; Stago Canada Ltd., Mississauga, ON, Canada) containing phospholipids ($\sim 4 \text{ pM}$) and tissue factor (TF) at two concentrations ($\sim 5 \text{ pM}$ for one run and $\sim 1 \text{ pM}$ for a

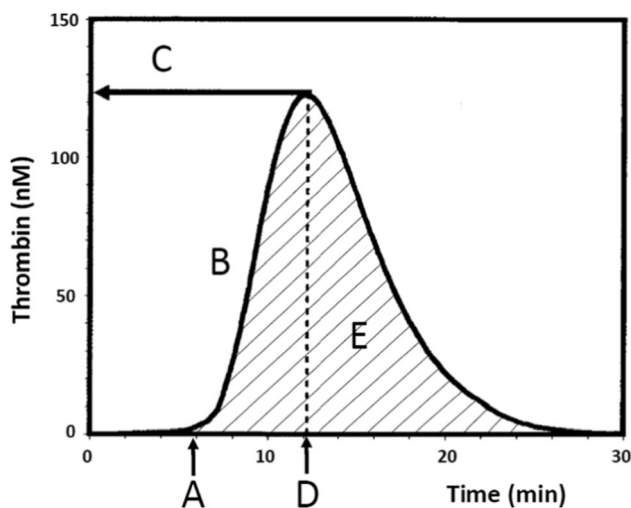


Fig. 1 Thrombogram parameters. A=Lag Time (min); B=Maximal Rate (nM/min); C=Peak Height (nM); D=Time to Peak (min); E=Endogenous Thrombin Potential (ETP) which is equivalent to the area under the curve. The parameters correspond to the following phases of thrombin generation: Phase 1 (initiation and amplification phases of coagulation), A; Phase 2 (maximum rate of increase in thrombin generation), B; Phase 3 (time to peak thrombin generation), C and D; and Phase 4 (shaded area: total amount of thrombin generation), E.(19, 26, 37)

second run).^{22–25} The plate was then moved to the fluorometer and 20 μL of FluCa solution added containing fluorogenic substrate Z-Gly-Gly-Arg-AMC and CaCl_2 . The thrombin generation reaction was resulted via a thrombin-specific fluorogenic substrate¹⁸. Thrombin generation was measured in samples taken at baseline before heparinization and CPB (pre) and five minutes after reversal of heparin with protamine (post). Summary statistics derived from thrombin generation data included: 1) lag time (min): the time until thrombin is first detected; 2) peak thrombin (nM): the maximum thrombin concentration achieved, a key indicator of the thrombin generating capacity of the sample;²⁵ 3) time to peak (min): the time when peak thrombin is measured, representing the latency of thrombin generation; and 4) endogenous thrombin potential, or ETP in $\text{nM}\cdot\text{min}^{-1}$: the area under the curve, representing the total amount of thrombin that a given plasma sample generated (Fig. 1).²⁶

Rotational thromboelastometry (ROTEM®) and platelet function analysis

Rotational thromboelastometry (ROTEM® delta; TEM International GmbH, Mississauga, ON, Canada) was used for measurements with 300 μL citrated whole blood.²⁷ The tests included INTEM (intrinsic coagulation system – contact phase activation) and EXTEM (extrinsic system – TF activation) performed at the same time points as CAT measurements. Measures analyzed included the 1) clotting time measured in seconds; 2) clot formation time in seconds. Platelet function was assessed using Plateletworks (Helena Laboratories Corporation, Beaumont, TX, USA) five minutes after protamine reversal using a total platelet count collected in an ethylenediaminetetraacetic acid (EDTA) tube, which was compared with a count obtained after exposure to 10 μg collagen agonist in 3.2% sodium citrate, with the difference corresponding to the functional platelet count.²⁸

Outcomes

The primary outcome of interest was the change in thrombin generation from baseline to after CPB. In a secondary analysis, measures of thrombin generation were examined as predictors for outcomes utilizing parameters from the low TF (1 pM) analysis, as lower levels of TF are more sensitive in detecting hemostatic differences between patients.¹⁸ All thrombin generation parameters were standardized to pre-CPB bypass values (post-CPB measurements divided by pre-CPB measurements, or post/pre). Secondary outcomes included: 1) any hourly chest drain output $> 200 \text{ mL}$ in the first six postoperative hours, a critical threshold often used as a decision point for

return to the operating room and associated with major morbidity and mortality;²⁹ 2) return to the operating room for bleeding, with typical triggers including several consecutive hours of postoperative chest tube drainage $\geq 200 \text{ mL}\cdot\text{hr}^{-1}$ and/or hemodynamic instability (i.e., high volume transfusion requirements, tamponade); 3) transfusion of platelets, frozen plasma (FP), prothrombin complex concentrate (PCC), fibrinogen concentrate, cryoprecipitate, or recombinant FVIIa; 4) the composite of return to the operating room or transfusion of any hemostatic product listed above; 5) the absolute numbers of all products transfused; 6) the total volume of chest drain output in the first six and 24 postoperative hours; 7) chest drain output $> 450 \text{ mL}$ in 24 hr, a criterion against safe chest tube removal.³⁰

Statistical analysis

Continuous and categorical variables are reported as means and standard deviations for parametric data and medians with interquartile ranges for nonparametric data. The Wilcoxon ranked sum, *t* test, or Chi square test were applied as appropriate. Since missing data were uncommon ($< 2\%$), a complete case analysis was employed. Spearman correlation coefficients for nonparametric data were used to characterize unadjusted correlations between continuous variables.

Logistic regression models were used to determine the association of predictors with clinical bleeding endpoints while adjusting for heparin management strategy (the intervention in the original study).^{31–33} Adjusted associations were expressed as odds ratios (ORs) with 95% confidence intervals (CIs). Assumptions of logistic regression modeling were verified; model discrimination was evaluated using the c-statistic and goodness of fit with the Hosmer-Lemeshow statistic. Unadjusted logistic regression was used to assess optimal cut-off points for the continuous variables post/pre ETP and post/pre peak thrombin generation. Equal weighting was given to sensitivity and specificity in selecting cut-offs. The Youden Index (J) was calculated for post/pre ETP and peak thrombin, where $J = 0$ indicates that a diagnostic test gives the same proportion of positive results for the group with and without the disease (i.e., is not clinically useful) and $J = 1$ indicates that the test has perfect performance. Sensitivities, specificities, and positive predictive values (PPVs) were calculated for a range of cut-off points representing plausible thresholds for clinical intervention. The combination of these parameters was used to select a cut-off for the post/pre ETP and peak thrombin ratio.¹⁶ To understand the association of thrombin generation with clinical outcomes, the unadjusted and adjusted association of post/pre ETP and peak thrombin with the composite

endpoint of any hemostatic product or return to the operating room was examined. Logistic regression models were adjusted for baseline differences in study group, INR, aPTT, and CPB time. SAS University Edition (release version June 2017, SAS Institute Inc., Cary, NC, USA) was used for all analyses. A two-sided $\alpha \leq 0.05$ was considered statistically significant.

Results

A total of 100 patients were eligible for inclusion in the final analysis (Table 1, ESM eFig. 1),¹⁴ with a mean age of 63 (12) yr, 31 (31%) female, and 94 (94%) undergoing bypass and/or valve surgery. A total of 41 (41%) cases involved aortocoronary bypass and 72 (72%) valve intervention. Of the patients undergoing bypass, 16 had one to two bypasses and 25 had three to four bypasses. The average length of time from surgical incision to leaving the operating room (OR) was 3.4 (1.0) hr, and the average length of CPB was 93 (37) min. Within 24 hr of surgery, 11 (11%) returned to the OR for re-exploration due to bleeding, and 48 (48%) experienced the composite endpoint of any transfusion or return to the operating room within 24 hr.

Post-CPB changes in thrombin generation parameters

A significant decrease in thrombin generation was seen after CPB across all thrombin parameters (Table 2, Fig. 2, Fig. 3). The lag time increased by a median of 76% (95% CI, 48 to 117) ($P < 0.001$), peak thrombin decreased by 73% (95% CI, -49 to -91) ($P < 0.001$), endogenous thrombin potential decreased by 56% (95% CI, -30 to -83) ($P < 0.001$), and the time to peak thrombin increased by 72% (95% CI, 43 to 104) ($P < 0.001$).

Receiver-operating characteristic curve (ROC) analysis examining threshold cut-offs for decreases in thrombin generation predictive of clinical bleeding outcomes

A post-CPB thrombin generation decrease of $\geq 80\%$ (or post/pre ETP and post/pre peak ratio ≤ 0.20) was selected for further analysis based on the sensitivity, specificity, and Youden Index to maximize the positive predictive value (PPV) and thereby the clinical usefulness of the test. For the outcome of blood loss $> 200 \text{ mL}\cdot\text{hr}^{-1}$ in the first 6 hr, PPV was moderate at 64% for post/pre ETP and 61% for post/pre peak ratio. For the composite endpoint of any transfusion or return to the OR, the PPV was 48% for post/pre ETP and 41% for post/pre peak ratio. Positive predictive values were lower for the prediction of return to the OR (ESM eTables 1–3).

Table 1 Descriptive characteristics of the study population

Characteristics	<i>N</i> = 100
Age (yr), mean (SD)	63.4 (12.1)
Sex, <i>n</i> (%)	
<i>Female</i>	31 (31%)
<i>Male</i>	69 (69%)
Height (cm), mean (SD)	171 (11)
Weight (kg), mean (SD)	84 (20)
BMI (kg·m ⁻²), mean (SD)	28.6 (6.0)
Ejection fraction (%), mean (SD)	58.0 (9.8)
eGFR (mL·min ⁻¹ ·1.73 m ⁻²), mean (SD)	77.1 (17.8)
Preoperative laboratory values	
Hemoglobin (g·L ⁻¹), mean (SD)	138 (15)
Platelets (× 10 ⁹ ·L ⁻¹), median [IQR]	212 [180–254]
PT, median [IQR]	11.7 [11.3–12.4]
aPTT, median [IQR]	24.0 [22.8–25.7]
INR, median [IQR]	1.00 [0.93–1.03]
ACT (sec), median [IQR]	105.5 [100.5–114.5]
Fibrinogen (g·L ⁻¹), median [IQR]	3.40 [2.80–3.88]
Preoperative thrombin generation parameters	
Lag time (min), median [IQR]	5.56 [5.01–6.33]
Peak thrombin (nM), median [IQR]	311.8 [283.3–343.6]
Endogenous thrombin potential (nM·min ⁻¹), median [IQR]	1566.5 [1467.5–1775.7]
Time to peak (min), median [IQR]	8.1 [7.4–9.1]
Baseline clinical characteristics	
Presence of at least 1 comorbidity, <i>n</i> (%)	90 (90%)
Hypertension, <i>n</i> (%)	69 (69%)
Dyslipidemia, <i>n</i> (%)	57 (57%)
Smoker, <i>n</i> (%)	11 (11%)
Obstructive lung disease, <i>n</i> (%)	8 (8%)
Diabetes, <i>n</i> (%)	22 (22%)
Prior myocardial infarction, <i>n</i> (%)	7 (7%)
Cerebrovascular disease, <i>n</i> (%)	4 (4%)
Peripheral vascular disease, <i>n</i> (%)	5 (5%)
Prior cardiac surgery, <i>n</i> (%)	4 (4%)
Preoperative medications	
Aspirin, <i>n</i> (%)	47 (47%)
Clopidogrel, <i>n</i> (%)	7 (7%)
Other antiplatelets, <i>n</i> (%)	1 (1%)
Warfarin, <i>n</i> (%)	3 (3%)
Other anticoagulation, <i>n</i> (%)	13 (13%)
CPB medications	
Total heparin administered (IU), median [IQR]	52,000 [45,000–62,000]
Total protamine administered (mg), median [IQR]	340 [285–400]
Surgical procedure performed	
Valve replacement or repair, <i>n</i> (%)	55 (55%)
Aortocoronary bypass, <i>n</i> (%)	26 (26%)
Aortocoronary bypass plus valve, <i>n</i> (%)	13 (15%)
Aortic root and valve, <i>n</i> (%)	3 (3%)
Aortocoronary bypass plus aortic root, <i>n</i> (%)	1 (1%)

Table 1 continued

Characteristics	<i>N</i> = 100
Aortic root, <i>n</i> (%)	1 (1%)
Intraoperative products	
RBC (units), median (range)	0 (0–5)
Platelets (units), median (range)	0 (0–2)
FFP (units), median (range)	0 (0–4)
Cryoprecipitate (IU), median (range)	0 (0–10)
Fibrinogen (g), median (range)	0 (0–4)
PCC (IU), median (range)	0 (0–2,000)
Intraoperative fluid balances	
CPB volume (mL), mean (SD)	761 (245)
CPB balance (mL), mean (SD)	1,377 (950)
Total fluid intake (mL), mean (SD)	2,283 (1,065)
Total fluid balance (mL), median [IQR]	50 [35–172]
Blood loss in cell saver (mL), median [IQR]	0 [0–554]
Cell saver returned (mL), mean (SD)	113 (191)
Blood loss in suction (mL), mean (SD)	1,234 (571)
Blood loss in OR sponges (mL), mean (SD)	330 (171)
Post-heparin reversal laboratory values	
ACT post heparin reversal in OR (sec), median [IQR]	113.0 [105.0–125.0]
INTEM-CT (sec), median [IQR]	215.0 [181.5–261.5]
EXTEM-CT (sec), median [IQR]	92.5 [84.0–103.0]
INTEM-CFT (sec), median [IQR]	95 [79–110]
EXTEM-CFT (sec), median [IQR]	87 [73–100]
Postoperative laboratory values on ICU arrival	
Hemoglobin (g·L ⁻¹), mean (SD)	104 (15)
Platelets (× 10 ⁹ ·L ⁻¹), median [IQR]	156.9 [44.39]
PT (sec), median [IQR]	15.0 [13.9–15.7]
aPTT (sec), median [IQR]	26.7 [24.4–29.0]
INR, median [IQR]	1.3 [1.2–1.4]
Fibrinogen (g·L ⁻¹), median [IQR]	2.0 [1.7–2.2]

aPTT = activated partial thromboplastin time; BMI = body mass index; CPB = cardiopulmonary bypass; CFT = clot formation time; CT = clotting time; eGFR = estimated glomerular filtration rate; FFP = fresh frozen plasma; ICU = intensive care unit; INR = international normalized ratio; IQR = interquartile range; IU = international units; OR = operating room; PCC = prothrombin complex concentrate; PT = prothrombin time; RBC = red blood cell; SD = standard deviation; nM = nmol·L⁻¹

Table 2 Changes in hemostatic parameters after CPB compared with baseline

Hemostatic parameter	Pre-CPB value	Post-protamine reversal value	Percentage change from baseline	<i>P</i> value (signed-rank)
Thrombin generation				
Lag time (min), median [IQR]	5.6 [5.0–6.3]	9.9 [8.5–12.1]	+76% [48–117]	< 0.001*
Peak thrombin (nM), median [IQR]	312 [283–344]	81 [25–179]	– 73% [-49–91]	< 0.001*
Endogenous thrombin potential (nM·min ⁻¹), median [IQR]	2567 [1,468–1,776]	703 [268–1,192]	– 56% [-30–83]	< 0.001*
Time to peak (min), median [IQR]	8.1 [7.4–9.1]	14.7 [12.2–16.7]	+72% [43–104]	< 0.001*

CPB = cardiopulmonary bypass; IQR = interquartile range; nM = nmol·L⁻¹

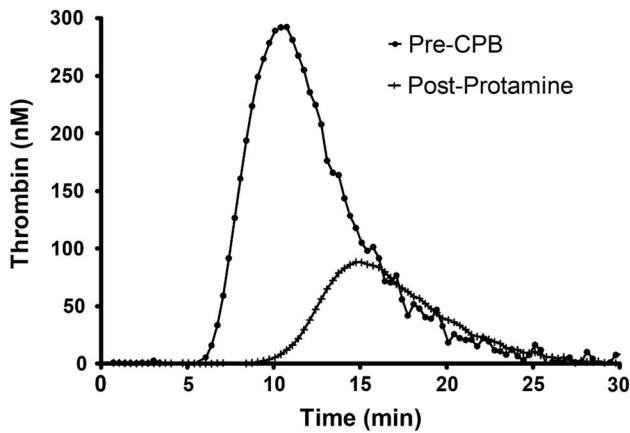


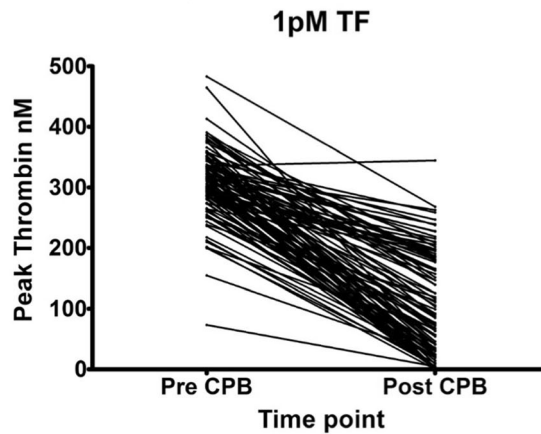
Fig. 2 Representative Thrombograms Illustrating Typical Changes Post-Protamine at the time of Conclusion of Cardiopulmonary Bypass (CPB) Compared with Baseline (Pre-CPB)

Association of selected post/pre ETP and post/pre peak thrombin ratios with patient characteristics, laboratory values, and clinical bleeding outcomes

There were no clear associations between impaired thrombin generation and baseline patient characteristics, but there were small between-group differences in INR, aPTT, and CPB duration (Table 3). In patients with $\geq 80\%$ decrease in ETP post-CPB, rates of return to the operating room within 24 hr for bleeding were higher ($P = 0.05$), as was total chest drain output at six hours ($P = 0.04$) and 24 hr ($P = 0.05$). The proportion of patients experiencing > 200 mL chest drain output in any one hour within the first six postoperative hours was also higher ($P = 0.04$). Additionally, patients with $\geq 80\%$ decrease in ETP post-CPB had higher rates of platelet transfusion and a higher incidence of the primary composite endpoint ($P = 0.04$). The association between patients with a $\geq 80\%$ decrease in peak thrombin generation post-CPB and clinical bleeding outcomes showed similar trends that did not achieve statistical significance.

Fig. 3 Change in thrombin generation Parameters in Relation to Cardiopulmonary Bypass. CPB, Cardiopulmonary Bypass; ETP, Endogenous Thrombin Potential; Peak, Peak Thrombin Generation. Results are shown for PPP-Low ~ 1 pM TF samples

(a) Change in Peak Thrombin After Cardiopulmonary Bypass (Post CPB) compared with Baseline (Pre)



(b) Changes in Endogenous Thrombin Potential (ETP) and Peak Thrombin (Peak) Expressed as Ratios of Post/Pre Cardiopulmonary Bypass (CPB) Measurements

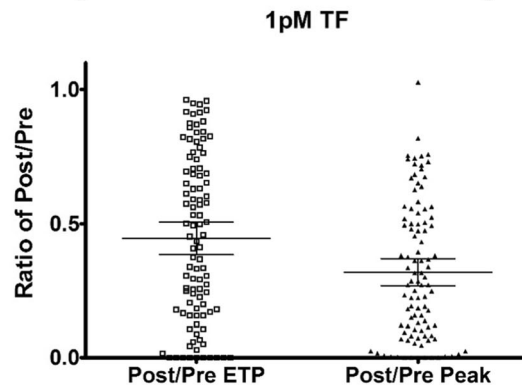


Table 3 Unadjusted association of the CAT parameters endogenous thrombin potential (ETP) and peak thrombin generation with patient characteristics, laboratory values, and postoperative outcomes

Variable	Endogenous thrombin potential (ETP) parameter		P value	Peak thrombin generation parameter		P value
	POST/PRE CPB ratio \geq 0.20 (<i>n</i> = 71)	POST/PRE CPB ratio < 0.20 (<i>n</i> = 29)		POST/PRE CPB ratio \geq 0.20 (<i>n</i> = 58)	POST/PRE CPB ratio < 0.20 (<i>n</i> = 42)	
Group characteristics						
Age (yr), median [IQR]	73 [54–73]	67 [55–73]	0.76	73 [54–73]	66 [55–73]	0.68
Sex, <i>n</i> (%)	48 (68%)	21 (72%)	0.64	42 (72%)	27 (64%)	0.39
Male	23 (32%)	8 (28%)		16 (28%)	15 (36%)	
Female						
eGFR (mL·min ⁻¹ ·1.73 m ⁻²), median [IQR]	78 [66–90]	77 [69–89]	0.86	80 [66–90]	77 [68–89]	0.85
BMI (kg·m ⁻²), median [IQR]	28 [25–31]	27 [25–28]	0.35	28 [25–31]	27 [24–29]	0.25
Baseline hemoglobin (g·L ⁻¹), median [IQR]	139 [125–152]	142 [134–148]	0.40	140 [127–152]	141 [131–148]	0.94
Baseline platelets ($\times 10^9 \cdot L^{-1}$), median [IQR]	217 [182–263]	198 [175–240]	0.12	207 [177–260]	218 [185–249]	0.78
Baseline INR, median [IQR]	1.00 [0.93–1.00]	0.97 [1.03–1.10]	0.01*	1.00 [0.95–1.00]	1.00 [0.93–1.10]	0.16
Baseline aPTT (sec), median [IQR]	23.7 [22.4–25.1]	25.2 [23.8–27.4]	0.002*	23.6 [22.3–25.3]	24.7 [23.4–26.4]	0.01*
Baseline fibrinogen (g·L ⁻¹), median [IQR]	3.48 [3.03–3.85]	3.19 [2.37–3.90]	0.39	3.48 [3.03–3.80]	3.26 [2.59–3.90]	0.69
CPB time (min), median [IQR]	80 [64–103]	92 [75–114]	0.06	80 [66–95]	92 [75–117]	0.04*
CPB pump balance (mL), mean (SD)	1,352 (871)	1,440 (1,134)	0.70	1,281 (835)	1,510 (1,086)	0.24
Total intraoperative fluid balance (mL), median [IQR]	48 [35–130]	61 [40–250]	0.74	46 [35–130]	60 [36–250]	0.65
Total intraoperative intake (mL), mean (SD)	2,302 (1,058)	2,239 (1,102)	0.80	2,242 (993)	2,341 (1,168)	0.65
Post-heparin reversal laboratory parameters						
ACT post heparin reversal in OR (sec), median [IQR]	112 [104–125]	117 [110–125]	0.05	112 [104–122]	115 [109–125]	0.07
Post-protamine ACT (sec), median change from baseline [IQR]	8 [1–16]	6 [0–15]	0.99	6 [0–15]	9 [1–17]	0.34
HEPTEM-CT (sec), median [IQR]	205 [177–249]	232 [193–282]	0.08	204 [174–226]	233 [187–305]	0.03*
INTEM-CT (sec), median [IQR]	200 [180–249]	234 [192–266]	0.17	198 [179–236]	237 [192–280]	0.02*
EXTEM-CT (sec), median [IQR]	92 [83–102]	95 [88–103]	0.49	91 [83–103]	96 [87–103]	0.33
INTEM-CFT (sec), median [IQR]	92 [77–108]	101 [88–112]	0.15	92 [78–108]	99 [83–112]	0.24
EXTEM-CFT (sec), median [IQR]	86 [71–97]	92 [78–107]	0.07	86 [72–96]	91 [76–102]	0.12
Post-CPB functional platelet count/pre-op platelet count, median [IQR]	60% [47–69]	58% [47–63]	0.47	61% [50–72]	56% [47–63]	0.10
Outcomes						
Suction blood loss (mL), median [IQR]	1,300 [700–1,600]	1,400 [1,000–1,600]	0.25	1,300 [700–1,600]	1,250 [800–1,500]	0.70
Cell saver blood loss (mL), median [IQR]	0 [0–500]	0 [0–600]	0.42	0 [0–466]	0 [0–649]	0.49
Return to the OR for re-exploration, <i>n</i> (%)	5 (7%)	6 (21%)	0.05*	5 (9%)	6 (14%)	0.37
Chest drain output > 200 mL any hour within 6 hr, <i>n</i> (%)	44 (62%)	24 (83%)	0.04*	35 (60%)	33 (79%)	0.05*
Chest drain output > 450 mL within 24 hr, <i>n</i> (%)	27 (38%)	15 (52%)	0.21	21 (36%)	21 (50%)	0.17

Table 3 continued

Variable	Endogenous thrombin potential (ETP) parameter		<i>P</i> value	Peak thrombin generation parameter		<i>P</i> value
	POST/PRE CPB ratio \geq 0.20 (<i>n</i> = 71)	POST/PRE CPB ratio < 0.20 (<i>n</i> = 29)		POST/PRE CPB ratio \geq 0.20 (<i>n</i> = 58)	POST/PRE CPB ratio < 0.20 (<i>n</i> = 42)	
Chest drain output at 6 hr (mL), median [IQR]	190 [145–260]	243 [178–450]	0.04*	195 [150–280]	230 [160–340]	0.21
Chest drain output at 24 hr (mL), median [IQR]	410 [320–600]	465 [393–805]	0.05*	410 [325–600]	460 [355–700]	0.26
Transfusion (intraoperative and within 24 hr of CPB)						
Recombinant factor VIIa, <i>n</i> (%)	0 (0.0%)	1 (3.5%)	0.29	0 (0.0%)	1 (2.4%)	0.42
Fibrinogen, <i>n</i> (%)	2 (2.8%)	3 (10.3%)	0.14	2 (3.5%)	3 (7.1%)	0.65
Packed red cells, <i>n</i> (%)	21 (29.6%)	10 (34.5%)	0.63	16 (27.6%)	15 (35.7%)	0.39
Frozen plasma, <i>n</i> (%)	4 (5.6%)	2 (6.9%)	1.00	3 (5.2%)	3 (7.1%)	0.69
Prothrombin complex concentrate, <i>n</i> (%)	6 (8.5%)	6 (20.7%)	0.09	6 (10.3%)	6 (14.3%)	0.55
Platelets, <i>n</i> (%)	8 (11.3%)	11 (37.9%)	0.002*	7 (12.1%)	12 (28.6%)	0.04*
Cryoprecipitate, <i>n</i> (%)	4 (5.6%)	4 (13.8%)	0.22	3 (5.2%)	5 (11.9%)	0.27
Composite outcome (hemostatic therapies and return to the OR)						
Number, <i>n</i> (%)	19 (26.8%)	14 (48.3%)	0.04*	17 (29.3%)	16 (38.1%)	0.36

* Due to non-normality in the distributions of the above data, the Kruskal-Wallis or Wilcoxon rank sum test was used to calculate the *P* values presented. For categorical variables, the Chi square test was used for cell counts >5, otherwise Fisher's exact test was used.

ACT = activated clotting time; aPTT = activated partial thromboplastin time; BMI = body mass index; CAT = calibrated automated thrombography; CPB = cardiopulmonary bypass; ETP = endogenous thrombin potential; eGFR = estimated glomerular filtration rate. eGFR was estimated using the CKD-EPI formula,³⁸ INR = international normalized ratio; OR = operating room

Unadjusted and adjusted association of post/pre ETP and post/pre peak thrombin ratios with the composite outcome of administration of any hemostatic therapies or return to the OR

For patients with \geq 80% decline in ETP post-CPB, there was an association with the composite outcome of return to the operating room or hemostatic therapies within 24 hr (OR, 2.55; 95% CI, 1.04 to 6.27; *P* = 0.04). After adjustment for baseline INR, aPTT, total CPB time, and study group, this association no longer persisted (adjusted odds ratio [aOR], 2.11; 95% CI, 0.77 to 5.79; *P* = 0.15). For patients with \geq 80% decline in peak thrombin post CPB, there was no significant unadjusted (OR, 1.48; 95% CI, 0.64 to 3.44; *P* = 0.36) or adjusted association (aOR, 1.07; 95% CI, 0.42 to 2.71; *P* = 0.89) observed with the composite outcome.

Discussion

We observed significant impairments in thrombin generation post-CPB, which were associated with clinical bleeding and greater need for hemostatic therapies. Patients

with a \geq 80% decline in ETP had higher chest drain output and a higher incidence of return to the operating room and need for hemostatic therapies.

Conventional hemostatic tests measure the time from coagulation activation to fibrin clot formation and largely reflect the initiation phase of coagulation corresponding to about 5% of the total thrombin potential.¹⁸ They do not capture ongoing thrombin and fibrin formation in the amplification and propagation phases, where the majority of thrombin formation occurs.²² Peak thrombin and the ETP, which better capture these aspects of thrombin generation, offer additional information to conventional or viscoelastic tests that can help characterize coagulopathy.

Among the parameters obtained from CAT, ETP is thought to be the most comprehensive, since it reflects the net amount of thrombin that a patient can generate and reflects how thrombin is regulated.¹⁸ In a previous study, Coakley *et al.* found that the postoperative ETP and peak thrombin were associated with a higher risk of bleeding in the first 24 postoperative hours.¹³ In our study, ETP was the variable from CAT that exhibited the strongest association with bleeding outcomes.

A general strength of our study is our larger sample size, prospective design, and analysis method, which

incorporated individual patient-level change in thrombin generation parameters.^{11–13} Limitations pertain to our post hoc analysis and multiple comparisons. Furthermore, our sample size calculation was based on an ability to detect differences in thrombin generation post-CPB. This may have resulted in our study being relatively under-powered to detect a difference in clinical bleeding outcomes, such as our composite endpoint. Larger studies powered to detect differences in bleeding outcomes may offer additional insights regarding the optimal thresholds for the prediction of morbid bleeding events and treatment.

Thrombin generation testing is currently limited largely to the research setting because of existing methodological limitations, which preclude point-of-care application and require a relatively long time for result output. Next-generation thrombin assays that are amendable to point-of-care application are currently under development.³⁴ These assays can potentially (once validity and reliability are determined) further our understanding of the role of impaired thrombin generation in perioperative coagulopathy. Importantly, these novel assays can be conducted in whole blood in a point-of-care manner, similar to viscoelastic tests. Whole blood testing provides results reflecting the important platelet contribution to thrombin generation. Accounting for platelets may improve the moderate PPV values we observed in our study using CAT and increase the clinical applicability of the test.

Early detection of thrombin generation impairment with prompt thrombin repletion therapy may help prevent highly morbid postoperative bleeding complications. In cardiac surgical patients experiencing a decrease in thrombin generation, recovery of thrombin generation was seen with the equivalent of 15 mL·kg⁻¹ fresh frozen plasma, 25 IU·kg⁻¹ of PCCs, or 45 µg·kg⁻¹ of recombinant FVIIa.³⁵ While further research is needed, the optimal treatment of decreased thrombin generation in cardiac surgical patients may eventually include low-dose coagulation factor isolates.³⁶ Our study suggests the ETP in particular may be a useful therapeutic target.

Conclusions

Large impairments in thrombin generation are observed after CPB and may be associated with increases in hemostatic product transfusion and clinical bleeding outcomes. The development of timely, easily accessible point-of-care thrombin generation testing has the potential to reduce bleeding complications and major morbidity and mortality in bleeding cardiac surgical patients.

Author contributions Justyna Bartoszko was involved in data analysis and interpretation and prepared the first draft of the

manuscript. Han Li was involved in data interpretation and assisting with manuscript revisions. John Fitzgerald was involved in study design and data collection and assisted with manuscript revisions. Loretta T.S. Ho assisted with manuscript revisions. Cyril Serrick was involved in study design and organization, data collection, and provided revisions to the manuscript. Walter H.A. Kahr was involved in data collection and sample processing, and manuscript editing for important intellectual content. Peter L. Gross was involved in data interpretation and manuscript revision. Keyvan Karkouti was involved in study inception and design, data analysis and interpretation, and manuscript revisions.

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