Augmentation of thrombin generation in neonates undergoing cardiopulmonary bypass

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Editor’s key points

- Bleeding after cardiac surgery with cardiopulmonary bypass is common in neonates, but the role of factor concentrate therapy is unclear.
- Computer simulation and ex vivo factor supplementation of plasma from 11 neonatal subjects undergoing cardiac surgery was used to model thrombin generation (TG).
- A three-factor prothrombin complex concentrate was more effective than recombinant activated factor VII in improving TG.
- Further studies to assess clinical efficacy and safety are warranted.

Introduction. Factor concentrates are currently available and becoming increasingly used off-label for treatment of bleeding. We compared recombinant activated factor VII (rFVIIa) with three-factor prothrombin complex concentrate (3F-PCC) for the ability to augment thrombin generation (TG) in neonatal plasma after cardiopulmonary bypass (CPB). First, we used a computer-simulated coagulation model to assess the impact of rFVIIa and 3F-PCC, and then performed similar measurements ex vivo using plasma from neonates undergoing CPB.

Methods. Simulated TG was computed according to the coagulation factor levels from umbilical cord plasma and the therapeutic levels of rFVIIa, 3F-PCC, or both. Subsequently, 11 neonates undergoing cardiac surgery were enrolled. Two blood samples were obtained from each neonate: pre-CPB and post-CPB after platelet and cryoprecipitate transfusion. The post-CPB products sample was divided into control (no treatment), control plus rFVIIa (60 nM), and control plus 3F-PCC (0.3 IU ml\(^{-1}\)) aliquots. Three parameters of TG were measured ex vivo.

Results. The computer-simulated post-CPB model demonstrated that rFVIIa failed to substantially improve lag time, TG rate and peak thrombin without supplementing prothrombin. Ex vivo data showed that addition of rFVIIa post-CPB significantly shortened lag time; however, rate and peak were not statistically significantly improved. Conversely, 3F-PCC improved all TG parameters in parallel with increased prothrombin levels in both simulated and ex vivo post-CPB samples.

Conclusions. Our data highlight the importance of prothrombin replacement in restoring TG. Despite a low content of FVII, 3F-PCC exerts potent procoagulant activity compared with rFVIIa ex vivo. Further clinical evaluation regarding the efficacy and safety of 3F-PCC is warranted.

Keywords: bleeding; cardiopulmonary bypass; coagulation/anticoagulation; neonate; thrombin

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Bleeding after cardiopulmonary bypass (CPB) is associated with substantial morbidity and mortality.1,2 Of patients requiring CPB, neonates are most likely to benefit from efforts to reduce post-CPB blood loss.3,4 Their immature coagulation system at birth is further compromised by surgery and CPB. Circuit priming volumes are often as large as circulating blood volume and produce severe haemodilution of platelets and coagulation factors. Additionally, neonates often undergo long, complex cardiac repairs with extensive suture lines again contributing to excessive post-CPB bleeding.

Blood coagulation is a complex physiologic process in which platelets and coagulation proteins interact to generate thrombin and fibrin. Thrombin generation (TG) is a critical event in this process. Fresh frozen plasma (FFP) is a blood product conventionally used to restore procoagulant factors; however, large volumes of plasma (15 ml kg\(^{-1}\)) are required to increase
coagulation factor levels by as little as 20%. Such a volume overload is not well tolerated in post-CPB neonates with limited cardiopulmonary reserve. Purified or recombinant factor concentrates have advantages compared with FFP in that they can be administered irrespective of blood type, have rapid availability, and involve only a small volume of administration.

In the USA, recombinant activated factor VII (rFVIIa; Novoseven®, Novo Nordisk, Bagsvaerd, Denmark) and three-factor prothrombin complex concentrate (3F-PCC) are currently available. 3F-PCC contains low levels of factor (F) VII and therapeutic levels of FII (prothrombin), FIX, and FX. This is in contrast to 4F-PCC, which contains therapeutic amounts of all vitamin K-dependent factors and is available in major European countries. Although their primary indications are for the prevention and treatment of bleeding episodes in patients with specific bleeding disorders, it is becoming increasingly common that they are administered off-label during complex cardiac surgery when conventional haemostatic treatment is inadequate. However, there is a paucity of data on the haemostatic effects of these agents in paediatric patients. In neonates especially, it is difficult to conduct comprehensive coagulation studies because of the limited amount of blood that can be collected from each patient.

In this investigation, we hypothesized that replacement of prothrombin is pivotal to the restoration of TG in neonatal plasma after CPB. Thus, we compared rFVIIa, which does not contain prothrombin, with 3F-PCC, which contains ample prothrombin but little FVII. In order to circumvent the small amount of blood obtainable from each subject, we initially simulated the impact of rFVIIa and 3F-PCC in neonatal plasma post-CPB using a computer-simulated coagulation model. Subsequently, we measured TG in neonatal plasma post-CPB after platelet and cryoprecipitate transfusion and after adding rFVIIa or 3F-PCC ex vivo.

**Methods**

**Simulation of thrombin generation**

The coagulation reactions included in the computer-simulated model are based on published literature, and have been used in our previous experiments. Briefly, coagulation reactions are modelled as a system of coupled ordinary differential equations. Each equation relates the rate of production (or decay) of a protein (or protein complex) with the current concentration of each protein (or protein complex). Readers are referred to the Appendix for a description of the model and equations.

The model of TG mimics the extrinsic pathway and is triggered by tissue factor (TF) at 5 pM. A rapid binding of activated FVII (FVIIa) to TF catalyses the initial activation of FX (FXa). When a sufficient amount of thrombin (0.1–1 nM) is generated, FV, FVIII, and FXI are engaged via thrombin-mediated feedback activation to sustain procoagulant reactions on activated platelets. The propagation of thrombin results in fibrin formation. The thrombomodulin-mediated protein C anticoagulant pathway was omitted because the main focus of this simulation was to evaluate procoagulant reactions at the site of vascular injury and thrombomodulin is mostly depleted by endothelial disruption at the site of vascular injury.

Coagulation factor levels, expressed in nanomolar concentration (nM), in 10 umbilical cord blood samples were used to compute baseline TG profiles: FII 616 ± 87.3, FV 19.5 ± 3.4, FVII 6.6 ± 1.4, FVIII 0.33 ± 0.18, FIX 40.0 ± 12.9, FX 67.1 ± 24.8, FXI 30.3 ± 10.8, antithrombin (AT) 1760 ± 307 (mean ± s). Post-CPB coagulant factor levels, including AT, were set as 50% of the baseline values except for FVIII, which was set at 80%. TG was simulated without and with the addition of a therapeutic dose of 3F-PCC (0.3 IU ml⁻¹ or ~25 IU kg⁻¹). The per cent activity of FII, FVII, and FX relative to FIX (100%) in 3F-PCC was set at 148, 11, and 64%, respectively. The effect of rFVIIa (60 nM (3 μg ml⁻¹)) was also simulated without changing other factor levels in the post-CPB set. The impact of restoring FII to pre-CPB levels was also tested with rFVIIa. In summary, TG patterns were simulated (n=10 each) at (i) baseline, (ii) post-CPB, (iii) post-CPB + 3F-PCC 0.3 IU ml⁻¹, (iv) post-CPB + rFVIIa 60 nM, and (v) post-CPB + rFVIIa 60 nM + FII (baseline). By convention, lag time for the simulation is defined as the time (in s) required to form 5 nM of thrombin.

**Ex vivo experiments**

After approval by the Institutional Review Board, 11 neonates undergoing elective cardiac surgery requiring CPB at Children’s Healthcare of Atlanta at Egleston were enrolled in this prospective study. Premature neonates (~36 weeks gestational age) and neonates with known coagulopathy, coagulation defect, or mother with known coagulation defect were excluded.

**Cardiopulmonary bypass management**

Non-pulsatile hypothermic CPB with a non-heparin-coated system consisting of a Terumo RX-05 hollow-fibre membrane oxygenator (Terumo Cardiovascular Systems, Ann Arbor, MI, USA) and COBE SMArt neonatal circuit tubing (Sorin Group USA, Inc., Arvada, CO, USA) was used for all neonates. Standard anticoagulation protocol consisted of a heparin bolus of 400 units kg⁻¹ with 1000 units added to the CPB prime. Circuits contained a 250 ml priming volume with packed red blood cells (RBCs) added to the circuit as needed to achieve and maintain a haematocrit of 30% for the duration of CPB. No FFP was included in the priming volume. All neonates received tranexamic acid (100 mg kg⁻¹ as a load to the patient, 100 mg kg⁻¹ as a load to the pump, and a continuous infusion of 10 mg kg⁻¹ h⁻¹ for the duration of the operation). After heparin neutralization with protamine, post-CPB bleeding was initially treated with 0.25 U of apheresis platelets and 3 U of cryoprecipitate (standard protocol). Further blood product administration for continued bleeding deemed clinically significant was at the discretion of the attending anaesthesiologist or intensivist.

**Blood sampling**

Two blood samples were obtained from each neonate. The first sample was collected pre-CPB and provided a baseline
measurement of TG in neonatal plasma. The second was collected post-CPB after heparin reversal with protamine and standard protocol blood product replacement. Pre-CPB and post-products blood samples were centrifuged to yield platelet poor plasma (PPP) and stored at −80°C until analysis. At the time of analysis, the following samples were prepared: (i) baseline; (ii) control post-products; (iii) post-products + 60 nM rFVIIa (3 µg ml⁻¹); and (iv) post-products + 0.3 IU ml⁻¹ of 3F-PCC (Profivine from Grifols Biologicals, Inc., Los Angeles, CA, USA). We chose Profivine because it contains only low levels of factor VII (<35 U per 100 U of FIX) and does not contain heparin. Prothrombin (FII) and AT levels were also measured in each sample.

**Thrombin generation ex vivo**

TG assays were performed using a calibrated automated thrombography technique as originally described. Briefly, 80 µl of thawed PPP was added to a microtitre plate well (Thermolabsystems, Franklin, MA, USA) followed by 20 µl of tissue factor (TF)-based activator (PPP reagent: final concentration 5 pM TF with 4 µM phospholipid). The plate was incubated 7–10 min at 37°C. Next, 20 µl of fluorogenic substrate-CaCl₂ mixture was added to each well to start the reaction. All TG reagents were from Diagnostica Stago, Parsippany, NJ, USA. A thrombin calibrator with a known constant thrombin-like activity was used in parallel wells to eliminate signal differences due to the light absorption characteristics of different plasmas, inner filter effects, and non-linearity of the emission signal. The TG reaction was monitored using a microplate fluorometer (Fluoroskan Ascent, Labsystems, Finland) set at 390 nm excitation and 460 nm emission wavelengths. Fluorescence was recorded every 20 s for 90 min. Acquired data were automatically processed by the Thrombinscope software (Thrombinscope, Stago, Maastricht, Netherlands) and TG parameters were calculated. The following parameters were evaluated: time to initiate TG or lag time, rate of TG, and peak amount of thrombin generated. By convention, lag time for ex vivo TG is defined as the time (in min) required for thrombin concentration to reach one-sixth of the peak amount.

**Factor II and AT measurements**

Residual plasma was used to measure FII and AT activity in each neonate for each condition. FII and AT levels were measured using a Stago Compact analyser with the Stago kits and reagents (Diagnostica Stago) according to the manufacturer’s directions. For AT measurements we used the Stachrom ATIII kit (chromogenic assay).

**Statistical analysis**

Median values with range were used for all continuous variables. Because normality assumptions were not upheld, we used the Wilcoxon signed-rank test to compare continuous variables between different conditions. All P-values are two-sided.

**Results**

**Simulated thrombin generation**

The numerical simulation of factor levels in neonates post-CPB revealed that no factors were restored to adult levels after 3F-PCC or rFVIIa except for supra-physiological FVII activity after rFVIIa addition. After 3F-PCC addition, levels of FII, FX, and FIX (not shown) were significantly higher than post-CPB with or without rFVIIa. AT levels remained lower than baseline (Fig. 1).

Simulation of TG is shown in Table 1 and Figure 2. Post-CPB lag time was prolonged by 93% relative to baseline; rate and peak also decreased by 46 and 42%, respectively. Simulated addition of 3F-PCC shortened post-CPB lag time by 61% and increased the rate and peak. In contrast, simulated addition of rFVIIa shortened the post-CPB lag time by only 6.3% and failed to increase rate and peak. Only when FII was restored to the baseline concentration in addition to rFVIIa were lag time, rate and peak restored to pre-CPB levels.

**Thrombin generation in plasma samples**

Eleven neonates were enrolled in this prospective observational study. Subject characteristics and CPB-related data are given in Table 2.

The median lag time was significantly prolonged after CPB despite heparin reversal and the transfusion of platelets and cryoprecipitate (Table 3). The addition of either rFVIIa or 3F-PCC to the post-transfusion products sample resulted in a statistically significant decrease in lag time, but was unable to return lag time to pre-CPB value. The median peak thrombin level was significantly elevated above pre-CPB levels after the termination of CPB, protamine administration, and the transfusion of platelets and cryoprecipitate. The addition of rFVIIa did not result in a further increase in peak thrombin generated. However, the addition of 3F-PCC produced a statistically significant increase in the peak thrombin level. Similarly, the rate was significantly increased from pre-CPB values by transfusion of platelets and cryoprecipitate. 3F-PCC but not rFVIIa was able to further augment the rate above that of the post-transfusion products level.

**Factor II and AT measurements**

After the transfusion of platelets and cryoprecipitate, FII activity did not significantly differ from baseline (Table 4). Conversely, AT activity post-transfusion was significantly decreased from baseline. The addition of both rFVIIa and 3F-PCC to the post-transfusion products sample significantly increased FII activity, but did not affect AT activity. The increase in FII was substantially greater with 3F-PCC than with rFVIIa (P<0.01).

**Bleeding outcomes**

Five of 11 neonates (46%) received additional blood product transfusion after the initial round to treat continued bleeding (Table 5), resulting in less 24 h chest tube output (non-significant at P=0.6). The median (range) amounts of additional products received were platelets 19.4 (0–80.3) ml
kg\(^{-1}\), cryoprecipitate 0 (0–4.8) ml kg\(^{-1}\), and FFP 25 (0–54.6) ml kg\(^{-1}\).

**Discussion**

We demonstrate the critical importance of prothrombin in restoring TG in a computer-simulated coagulation model and in post-CPB neonatal (ex vivo) blood samples. Baseline levels of vitamin K-dependent factors in neonates are \(\approx\)50% of adult levels, thus predisposing neonates to coagulopathy from haemodilution and blood loss during CPB.\(^{15,16}\) The addition of 3F-PCC, which is rich in prothrombin, was capable of restoring all three parameters of TG in both simulated and ex vivo post-CPB models, whereas the effect of rFVIIa was limited only to a faster onset of TG.

Initially, we evaluated the effects of 3F-PCC and rFVIIa using a computer-simulated model\(^{8–10}\) based on presumed neonatal plasma coagulation factor levels. Despite low prothrombin levels, baseline simulated TG peak was normal because of low AT activity. However, the simulated 50% haemodilution post-CPB led to significant prolongation in lag time and rate and decrease in peak. In the simulation of 3F-PCC addition to post-CPB plasma, all the parameters of TG were restored. In contrast, simulation of rFVIIa addition minimally improved

**Table 1** Computer-simulated thrombin generation with and without added haemostatic agents. Values expressed as median (range). 3F-PCC, three-factor prothrombin complex concentrate (0.3 IU ml\(^{-1}\) in FIX unit); rFVIIa, recombinant factor VIIa (60 nM); FII, factor II (prothrombin restored to baseline)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Post-CPB</th>
<th>Post-CPB + rFVIIa</th>
<th>Post-CPB + 3F-PCC</th>
<th>Post-CPB + rFVIIa + FII</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lag time (s)</td>
<td>78.5 (72–84)</td>
<td>151.5 (133–157)</td>
<td>142 (125–148)</td>
<td>59 (59–60)</td>
<td>63.5 (56–70)</td>
</tr>
<tr>
<td>Rate (nM s(^{-1}))</td>
<td>3.5 (3.3–3.6)</td>
<td>1.9 (1.7–2.0)</td>
<td>1.9 (1.7–2.0)</td>
<td>10.6 (10.3–10.6)</td>
<td>4.8 (4.5–6.6)</td>
</tr>
</tbody>
</table>

**Fig. 1** Computer-simulated factor levels with and without added haemostatic agents. CPB, cardiopulmonary bypass; PCC, prothrombin complex concentrate; rFVIIa, recombinant activated factor VII. (a) Prothrombin levels; (b) Factor VII levels; (c) Factor X levels; (d) Antithrombin levels; x axis denotes time, y axis denotes concentration.
lag time but not rate or peak. The haemostatic activity of rFVIIa improved in the simulation model only when the prothrombin level was restored to its baseline value.

Our simulation data are in agreement with previous simulations and in vitro TG studies that demonstrate the crucial role of prothrombin in haemodiluted blood.\textsuperscript{17–19} Although rFVIIa can be effective for achieving haemostasis in haemophilia patients with inhibitors who have normal prothrombin levels, the response to rFVIIa in haemodiluted blood is proportionally improved by increasing the prothrombin level.\textsuperscript{20} Variations in plasma concentration of FVII, FIX, and FX do not affect TG until levels decrease well below normal. Only 5, 20, and 30% of FVII, FIX, and FX, respectively, are required to maintain normal TG.\textsuperscript{21} However, reduced TG becomes evident when FII decreases below 75–100% of normal.\textsuperscript{19,21} Our simulation data indicate the potential utility of 3F-PCC, by increasing FII, in restoring decreased TG in post-CPB neonates.

In the second part of our study, we investigated whether similar effects of 3F-PCC and rFVIIa are observed ex vivo in post-CPB neonatal plasma. Our standard protocol for blood product replacement resulted in a significant increase in thrombin activity relative to pre-CPB neonatal plasma; however, the lag time remained prolonged compared with baseline. We speculate that transfused platelets and cryoprecipitate did not provide sufficient extrinsic pathway factors (i.e. vitamin K-dependent factors) to affect lag time, but were able to improve TG by increasing FVIII levels via cryoprecipitate.\textsuperscript{22} The addition of rFVIIa to our standard haemostatic therapy of platelet and cryoprecipitate did not further enhance the rate or peak amount of thrombin but significantly decreased the lag time. Conversely, the addition of 3F-PCC significantly elevated peak, increased rate, and shortened lag time. Our ex vivo measurements of TG were supported by our computer-simulation data and are similar to the results of Mitrophanov and colleagues\textsuperscript{23} regarding rFVIIa. These authors also used a computational model to determine the effects of rFVIIa administration on TG parameters to show that rFVIIa acts primarily to accelerate the onset of TG with a minimal effect on rate and peak.

Neonates especially are prone to the haemostatic alterations of CPB and often require significant transfusion of blood products to achieve haemostasis.\textsuperscript{1,2} Traditionally, transfusion of packed RBCs, platelets, cryoprecipitate (i.e. fibrinogen-rich component), and FFP are essential to control post-CPB bleeding in these patients. We demonstrated that peak TG surpasses baseline values with the initial transfusion of platelets and cryoprecipitate. Despite this increase, almost half of the subjects received additional blood products for continued post-CPB bleeding. Median postoperative bleeding was clinically significant (equivalent to \textasciitilde 1 litre loss in a 70 kg adult in 24 h), in the range of reported values in infants\textsuperscript{24} and adults.\textsuperscript{25} Given the safety concerns and effects on the immune system associated with blood product administration,\textsuperscript{26} these findings emphasize that the search for other effective options to treat post-CPB bleeding in neonates remains an important challenge.

Both rFVIIa and 3F-PCC have been studied in animal models. In a porcine trauma model of haemodilution after spleen injury,\textsuperscript{27} both rFVIIa and PCC significantly shortened prothrombin time. However, PCC alone reduced blood loss and augmented TG. In a second porcine model mimicking post-CPB bleeding,\textsuperscript{17} PCC was effective in reducing diffuse bleeding and increasing TG from post-CPB levels. In the latter study, the authors did not compare PCC with rFVIIa.
Improvement of TG parameters with 3F-PCC in our study also suggests that it could be useful in the treatment of microvascular bleeding after CPB.

Any procoagulant intervention carries a potential risk of thrombosis; it is impossible to estimate the risk with 3F-PCC based on our study. The TG assay is a static test and thus does not fully reflect in vivo based on our study. The TG assay is a static test and thus does not reflect in vivo coagulation processes that are affected by rheological and other factors (blood flow, blood viscosity, lack of endothelium, etc.). In the previously mentioned porcine model of post-CPB coagulopathy,17 peak TG after administering PCC (30 IU kg \(^{-1}\)) was well above baseline, but the animals presumably did not incur acute thromboembolic events underscoring that the procoagulant activity of PCC might be limited by in vivo blood flow and localized platelet activation.28 Threshold levels of peak TG for thrombosis and haemostasis have not been defined in either adults or children, although elevated levels in adults have been associated with increased risk of venous thrombosis.29 In children, there is a paucity of data on the risk of venous thrombosis, although paediatric patients undergoing cardiac surgery do appear to be at increased risk compared with other hospitalized children.30 The risk of arterial thrombosis also cannot be estimated from plasma TG patterns which are devoid of platelet activity.

In general, locally generated thrombin is more likely to be washed away by high flowing arterial blood.31 Furthermore, the risk of thrombosis is significantly influenced by underlying clinical conditions, including heart failure, vascular damage, infection, and inflammation. Nevertheless, the risk of thrombosis must be taken seriously and should be evaluated in a dose escalation study of PCC. Current data can help determine an appropriate starting dose, probably <0.3 IU ml \(^{-1}\).

Our study has several limitations. First, it is unclear whether ex vivo results can be accurately applied to the in vivo state. Our ex vivo experiments were performed under static conditions and do not take into account rheological effects on coagulation encountered under physiologic conditions. Also, they do not account for contributions of the endothelial system to coagulation. A second limitation is that we did not assess 4F-PCC in this study; however, our primary focus was to demonstrate the key role of prothrombin in TG and prothrombin content is similar between 3F-PCC and 4F-PCC.13 In addition, our previous ex vivo study demonstrated that TG patterns were similar between 3F-PCC and 4F-PCC when the international normalized ratio (INR) is <4.0.31 A final limitation is the potential inaccuracy of commercially available TG assays in neonates. Most TG assays rely on fluorogenic substrates that are cleaved by both free and \(\alpha_2\)-macroglobulin-bound thrombin. Software accounts for this by using a mathematical algorithm that assumes a likely contribution of \(\alpha_2\)-macroglobulin-bound thrombin to the overall thrombin produced. However, in populations where \(\alpha_2\)-macroglobulin is elevated, such as neonates, it is possible to overestimate the total amount of thrombin

### Table 2 Subject characteristics and CPB-related data

<table>
<thead>
<tr>
<th>Age (day)</th>
<th>9 (1–20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>3.2 (2.6 – 3.8)</td>
</tr>
<tr>
<td>BSA (m(^2))</td>
<td>0.20 (0.18 – 0.24)</td>
</tr>
<tr>
<td>Gender: M (%)/F (%)</td>
<td>9 (82)/2 (18)</td>
</tr>
<tr>
<td>PT (s)</td>
<td>14.8 (13 – 20.7)</td>
</tr>
<tr>
<td>INR</td>
<td>1.1 (0.9 – 1.7)</td>
</tr>
<tr>
<td>aPTT (s)</td>
<td>37.3 (28.6 – 43.3)</td>
</tr>
<tr>
<td>Fibrinogen (mg dl (^{-1}); n = 9)</td>
<td>296 (158 – 447)</td>
</tr>
<tr>
<td>RACHS – 1 score</td>
<td>4 (2 – 6)</td>
</tr>
</tbody>
</table>

**Diagnosis**
- Hypoplastic AA
- TAPVR
- Truncus arteriosus
- TOF
- PA/VSD
- Hypoplastic AA

**CPB time (min)** 98 (53 – 160)
**AXC time (min)** 55 (24 – 96)
**Lowest temperature (°C)** 31 (18 – 34)
**Regional perfusion (min; n = 2)** 0 (0 – 67)
**DHCA (min; n = 2)** 0 (0 – 2)

Values expressed as median (range).

BSA, body surface area; M, male; F, female; PT, prothrombin time; INR, international normalized ratio; aPTT, activated partial thromboplastin time; RACHS – 1, risk adjustment for congenital heart surgery; HLHS, hypoplastic left heart syndrome; TAPVR, total anomalous pulmonary venous return; PS, pulmonary stenosis; dTGA/IVS, d-transposition of the great arteries with intact ventricular septum; TOF, tetralogy of Fallot; PA/VSD, pulmonary atresia with ventricular septal defect; AA, aortic aneurysm; CPB, cardiopulmonary bypass; AXC, aortic cross clamp; DHCA, deep hypothermic circulatory arrest.

### Table 3 Ex vivo TG with and without added haemostatic agents

<table>
<thead>
<tr>
<th></th>
<th>Pre-CPB</th>
<th>After CPB + plts/cryo</th>
<th>After CPB + plts/cryo + rFVIIa</th>
<th>After CPB + plts/cryo + 3F-PCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log time (min)</td>
<td>1.5 (1 – 3.5)</td>
<td>3* (1.7 – 4)</td>
<td>2.3*** (1.3 – 4.2)</td>
<td>2.5*** (1.8 – 3.2)</td>
</tr>
<tr>
<td>Rate (nM min (^{-1}))</td>
<td>60.5 (14.2 – 112)</td>
<td>79.9* (22.4 – 155)</td>
<td>76.9* (26.7 – 140)</td>
<td>225* (108 – 351)</td>
</tr>
<tr>
<td>Peak (nM)</td>
<td>128 (49.8 – 262)</td>
<td>194* (111 – 271)</td>
<td>200* (103 – 140)</td>
<td>487* (344 – 626)</td>
</tr>
</tbody>
</table>

Values expressed as median (range).

*P < 0.05 vs baseline.

**P < 0.05 vs after plts/cryo.

CPB, cardiopulmonary bypass; plts, platelets; cryo, cryoprecipitate; rFVIIa, recombinant activated factor VII; 3F-PCC, three-factor prothrombin complex concentrate.
produced. However, our experiments depend less on the absolute amount of thrombin generated and more on the comparison of peak levels between differing conditions.

In conclusion, we demonstrate that the transfusion of platelets and cryoprecipitate post-CPB effectively restores the rate and the peak amount of TG in neonates undergoing cardiac surgery requiring CPB. However, these transfusion products do not return the lag time to baseline values and many neonates in our study required further blood product administration to control clinical bleeding. The addition of rFVIIa did not further enhance the rate or peak of TG, but did significantly shorten the lag time. On the other hand, 3F-PCC improved all three TG parameters. We conclude that the greater effect of 3F-PCC is a result of its ability to augment FII, which was confirmed by both the computer-simulation and ex vivo experimental results. However, caution is warranted with the use of 3F-PCC because its prothrombotic risk is unclear. A greater understanding of the procoagulant effects of 3F-PCC in neonates undergoing cardiac surgery with CPB is needed so that well-designed randomized controlled trials can be performed to evaluate its efficacy and safety.

Authors’ contributions

N.A.G.: study design; development of methodology; collection of data; analysis, interpretation of data, or both; and writing and revising of the manuscript; F.S.: development of methodology; collection of data; analysis, interpretation of data, or both; and writing and revising all or sections of the manuscript; K.A.T.: study design; development of methodology; collection of data; analysis, interpretation of data, or both; and writing and revising all or sections of the manuscript.

Declaration of interest

K.A.T. has a consulting relationship with Grifols, which manufactures Profilnine; however, the consultation is unrelated to Profilnine. Grifols had no role in the planning, conduct, and analysis of this study or in the preparation of this manuscript.

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References


Table 4 Ex vivo prothrombin and AT activity with and without added haemostatic agents

<table>
<thead>
<tr>
<th></th>
<th>Before CPB</th>
<th>After CPB + plts/cryo</th>
<th>After CPB + plts/cryo + rFVIIa</th>
<th>After CPB + plts/cryo + 3F-PCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>FII (% activity)</td>
<td>55 (49–97)</td>
<td>54 (44–68)</td>
<td>70*** (57–87)</td>
<td>95*** (81–99)</td>
</tr>
<tr>
<td>AT (% activity)</td>
<td>57 (49–72)</td>
<td>53.5* (38–64)</td>
<td>53.5* (42–61)</td>
<td>52* (39–60)</td>
</tr>
</tbody>
</table>

Values expressed as median (range).

FII, factor II (prothrombin); AT, antithrombin; CPB, cardiopulmonary bypass; plts, platelets; cryo, cryoprecipitate; rFVIIa, recombinant activated factor VII; 3F-PCC, three-factor prothrombin complex concentrate.

†n = 10 for AT.

*P < 0.05 vs baseline.

**P < 0.05 vs after plts/cryo.

Table 5 Postoperative bleeding data

<table>
<thead>
<tr>
<th>Total (n = 11)</th>
<th>No postoperative transfusion (n = 6)</th>
<th>Postoperative transfusion* (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chest tube output (ml kg⁻¹)</td>
<td>19.5 (11.8–141)</td>
<td>25.1 (11.9–57.3)</td>
</tr>
</tbody>
</table>

Values expressed as median (range).

*Postoperative transfusion is defined as transfusion in those neonates who received additional blood products after the initial protocol. Data collected for the first 24 h after operation.
20 Allen GA, Hoffman M, Roberts HR, Monroe DM. Manipulation of procoagulant concentration on rate, peak and time to total thrombin generation in plasma anticoagulated with warfarin. Thromb Res 2010; 125: 432 – 7

Appendix

The mass balance equations were written around each protein (e.g. FXa) or protein complex (e.g. FXa-FVa) yielding the set of differential equations (vector form):
\[ \frac{dx}{dt} = f(S, r(x, k)) \]
\[ x(t_0) = x_0 \]
where \( S \) denotes the stoichiometric matrix \((193 \times 301)\), \( x \) denotes the concentration vector of proteins or protein complexes \((193 \times 1)\), \( k \) denotes the vector of kinetic parameters \((301 \times 1)\), and \( r(x, k) \) denotes the vector of reaction rates \((301 \times 1)\). Each row in \( S \) describes a particular protein or protein complex, while each column describes the stoichiometry associated with a specific interaction in the network. Thus, the \((i, j)\) element of \( S \), denoted by \( s_{ij} \), describes how protein \( i \) is connected to rate process \( j \). If \( s_{ij} < 0 \), then protein \( i \) is consumed in \( r_j \); conversely, if \( s_{ij} > 0 \), then \( i \) is produced by \( r_j \); and if \( s_{ij} = 0 \), there is no connection between protein \( i \) and rate process \( j \). We have assumed mass action kinetics for each interaction, and the rate expression for the general reaction \( q \):
\[ \sum_{j \in \{R_q\}} s_{ij}x_j \rightarrow \sum_{k \in \{P_q\}} s_{jk}x_k \]

is given by:
\[ r_q(x, k_q) \rightarrow k_q I x^{k_q - 1} \]
\[ j \in \{R_q\} \]
where \( \{R_q\} \) denotes the set of reactants for reaction \( q \), \( \{P_q\} \) denotes the product set for reaction \( q \), \( k_q \) denotes the rate
constant governing the qth reaction, and $\sigma_{pq}, \sigma_{sq}$ denote stoichiometric coefficients (elements of the matrix $S$). We have treated every rate as nonnegative; all reversible reactions in the data source were split into two irreversible reaction steps. Thus, every element of the reaction rate vector $r(x,k)$ takes the form shown in equation (3).

The model equations were solved using the Livermore solver for ordinary differential equations (LSODE) routine of the OCTAVE programming environment (http://www.octave.org; version 2.1.71) on an Apple Computer MacOSX (http://www.apple.com; v10.5.3).  

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