Thrombin regulation in neonates undergoing cardiopulmonary bypass

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Dear Sir,

Cardiopulmonary bypass (CPB) is associated with activation of coagulation leading to enhanced formation of the key enzyme of this pathway, thrombin. Thrombin formation during CPB is an unsteady process in adults and a substantial, up to 20-fold thrombin escalation occurs within minutes after the opening of the aortic cross-clamp (1–3). This specific thrombin burst results in increased soluble fibrin formation (1). The reperfusion-induced thrombin burst was recently shown to be associated with postoperative myocardial damage in adults (2). Furthermore, the use of hirudin, a thrombin inhibitor, was associated with better postoperative haemodynamic outcome in a porcine model (4). Thus, thrombin regulation during reperfusion of the heart may have clinical significance. Given that this aspect has not been studied in the context of neonatal cardiac surgery, we now characterized thrombin generation in newborns during CPB, with a special focus on the early reperfusion period.

The study was approved by the ethics committee of the hospital. After informed consent of the parents, 15 neonates, aged four to 18 days (median 10 days) and scheduled to undergo cardiac surgery with CPB, were enrolled into the study. Twelve patients were operated on due to transposition of the great arteries (4 with and 8 without additional defects) and three patients due to a hypoplastic aortic arch.

Standardized surgical and CPB techniques were used. The volume of CPB circuit (Lilliput 1, D901, Dideco, Mirandola, Italy) was 400 ml with phosphorylcholine-coated tubes. The circuit was primed with reconstituted blood and/or 4% albumin with a target haematocrit value of 30%. Thus, three patients received only albumin for priming the circuit; in the remaining 12 patients the mean volume of reconstituted blood in the prime was 133 ± 23 ml. All patients received reconstituted blood during CPB (365 ± 33 ml). Veno-venous continuous hemofiltration was carried out by Ultraflux AV 400 (Fresenius, Oberusel, Germany). CPB lasted 126–265 minutes (min) (median 155 min). A standard dose of aprotinin (30,000 IU/kg to the prime, 30,000 IU/kg as a bolus to the patient 1 hour [h] after the induction of anesthesia, and a steady infusion of 8,000 IU/kg/h during CPB) was given to all patients. Anticoagulation by heparin was monitored with an activated clotting time, maintained for over 480 seconds.

Blood samples were collected 1) preoperatively (baseline), 2) at the beginning of CPB, 3) before the release of the aortic cross-clamp, and 4) 15 min after the release of the crossclamp. Prothrombin fragment F1+2 and thrombin-antithrombin complex (TAT) were analyzed as previously described (5). D-dimer was measured by Tina-quant D-dimer (Hoffmann-La Roche Ltd., Basel, Switzerland).

Due to substantial haemodilution, F1+2, TAT, and D-dimer were corrected for haemodilution. The measured marker levels were multiplied by the correction factor F, calculated by a formula described earlier (6): $F = \frac{(100 – Hc_{\text{sample}}) \times Hc_{\text{preCPB}}}{(100 – Hc_{\text{preCPB}}) \times Hc_{\text{sample}}}$, where Hct (as %) is the haematocrit.

The increase in F1+2 across the opening of the aortic cross-clamp has been shown to be on average 50% in adults (1, 2). The current study had 80% power at the significance level of 0.05 to detect a 13% difference in F1+2 across the aortic declamping. The Mann-Whitney U test and the Wilcoxon test were used for non-paired and paired comparisons, respectively.

Haemodilution-corrected levels of F1+2 (Fig. 1) and TAT increased markedly during CPB. Median F1+2 increased four-fold and TAT nine-fold prior to opening of the aortic crossclamp as compared to the baseline levels. Opening of the aortic crossclamp was not associated with any further increase in either F1+2 or TAT (Fig. 1). In contrast, when the haemodilution-corrected levels were analyzed, decreases in F1+2 (p=0.03) and TAT (p=0.002) were observed during this period. D-dimer was not increased during CPB (Fig 1).

In newborn infants, coagulation was invariably activated during CPB in accordance with previous reports (7, 8). The profile of neonatal thrombin generation, however, significantly differed from that in adults (1, 2). In the newborns, no thrombin burst was observed during the first 15 min of reperfusion, whereas in adults the enhanced thrombin formation becomes measurable al-
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ready at 1 min after opening of the aortic cross-clamp, and increases up to at least 15 min after clamp release (2, 9).

Multiple mechanisms including 1) the exposure of blood to non-endothelial surfaces and the consequent inflammatory response, 2) surgical trauma to the blood vessels inducing tissue factor (TF) expression, and 3) the re-infusion of blood aspirated from the operational field (10), very likely contributed to coagulation activation in our patients. Additionally, infusion of adult reconstituted blood activates coagulation in neonates (5). The lower potential of newborn plasma to generate thrombin (5) might in part down-regulate thrombin markers during CPB. This, however, does not seem significant enough to completely prevent the subsequent reperfusion-induced thrombin peak. Rather, in neonates the activating factors of coagulation would probably lead to enhanced thrombin generation due to e.g. proportionally larger non-endothelial surfaces and surgical trauma.

The origin of the reperfusion-induced thrombin in adults remains incompletely characterized (1, 2). Three major vascular regions, namely splanchnic microcirculation (11), pulmonary vascular bed (12), and ischemic coronary circulation (9) are likely to be involved. Whether neonates and adults differ regarding prothrombin activation in these vascular beds is currently not clear. One confounding factor in the current study might be the attenuation of thrombin generation by aprotinin. In one study involving neonates and older infants, Mössinger et al. demonstrated that aprotinin downregulates F1+2 one hour after CPB, after protamine, but no such effect could be seen in any samples during heparinization, including samples at 5 min after aortic declamping (8). Similarly, in a study by Flaujac et al., aprotinin did not affect F1+2 levels at 5 min after CPB (13). It thus seems unlikely that aprotinin is a confounder, and the lack of reperfusion-induced thrombin burst may, indeed, be a specific feature of the neonatal haemostatic system and vasculature. This suggestion is supported by the fact that neonatal physiology is remarkably adaptive to the changing conditions in oxygenation and cardiac output redistribution in various vascular beds at and after birth. The central effects of thrombin on coagulation and inflammatory systems in clinically significant ischemia-reperfusion situations in newborn infants still remain to be revealed.

References