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Letter to the Editors-in-Chief

Global impairments in the haemostasis systems after cardiopulmonary bypass☆

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CABG

Dear Editors,

Approximately 10% of cardiac surgery performed with cardiopulmonary bypass (CPB) leads to excessive postoperative bleeding, accompanied by significant increase in morbidity and mortality [1]. Thrombin generation is an important regulator that alters the haemostatic potential after trauma and surgical procedures. Thrombin generation and fibrin formation are triggered by CPB, but attenuated by systemic heparinization [2].

Low plasma levels of fibrinogen are associated with increased perioperative blood loss [3], but the relationship with transfusion of blood products is less clear. Notably, CPB-related hyper-fibrinolysis has been reported. In one study, a 100-fold increase in plasmin generation immediately after the start of CPB was observed, along with increasing rate of fibrin formation and removal of fibrin, paralleling each another throughout the pump run [4]. Thus, despite known alterations in coagulation and fibrinolysis during the perioperative phase of cardiac surgery, there are still uncertainties as to how various pro- and *anti*-thrombotic perturbations proceed in combination. The aim of the present study was to determine the global haemostatic alterations during cardiac surgery and in the early postoperative phase.

Eleven patients undergoing CABG with CPB were included in a prospective observational study after informed written consent. Patient characteristics are presented in Supplementary Table 1. Study procedures were approved by the regional ethics committee, and were performed in accordance with the Declaration of Helsinki. Before CPB, patients received 350 IU of unfractionated heparin per kg body weight. Anti-coagulation on CPB was monitored with standard activated clotting time (ACT) and target ACT of >480 s was aimed for. After CPB, heparin was reversed with protamine sulphate, 1 mg per 100 U of initial heparin dose. No patients received extra heparin or protamine during or after CPB. All patients received 2 g tranexamic acid (TA) intravenously before and after surgery. Postoperative autotransfusion was not used. Blood samples of 80 or 20 ml were collected at four time points: before surgery and 10 min, 2 h, and 24 h after CPB. Haemoglobin, haematocrit, heparin effect (*anti*-FXa), thrombin generation capacity (calibrated automated thrombogram, CAT), fibrinogen activity/antigen, viscoelasticity (thromboelastometry/ROTEM), fibrin permeability, fibrin network images by scanning electron microscopy (SEM), and clot lysis time (CLT) were tested.

For detailed description of methods and laboratory data, see Supplementary materials. Anti-FXa levels increased significantly 10 min ($p = 0.001$) and 2 h after CPB ($p = 0.004$) relative to baseline, and returned to baseline levels after 24 h. CAT showed significant decrease in thrombin peak and endogenous thrombin potential (ETP) 10 min and 2 h after CPB (Fig. 1A and B). Other CAT variables, such as lag phase time and time to peak, were also significantly prolonged (Supplementary Table 2).

Fibrinogen concentration declined 10 min and 2 h after CPB ($p = 0.001$, both methods) relative to baseline, and increased after 24 h ($p = 0.002$ for activity and $p = 0.004$ for antigen) (Fig. 1C and D). There was a significant correlation between fibrinogen activity and fibrinogen antigen ($r = 0.94$, $p < 0.0001$).

ROTEM EXTEM clotting time (CT) was prolonged 10 min after CPB relative to baseline but returned to baseline after 2 h (Fig. 2A). EXTEM maximum clot firmness (MCF) was reduced 10 min and 2 h after CPB and returned to baseline levels after 24 h (Fig. 2B). FIBTEM MCF was significantly reduced at 10 min and 2 h after CPB ($p = 0.024$ and $p = 0.039$, respectively), and at 24 h, FIBTEM MCF was significantly higher than at baseline ($p = 0.0029$) (data not shown). There was a significant correlation between FIBTEM MCF and fibrinogen activity and antigen ($r = 0.85$ and 0.81, respectively; $p < 0.001$ for both).

Fibrin permeability (Ks, Fig. 2C) increased 10 min and 2 h after CPB relative to baseline ($p = 0.001$ and $p = 0.002$, respectively), and returned to baseline levels after 24 h. There were significant inverse correlations between Ks and fibrinogen activity and antigen ($r = -0.70$ and $r = -0.61$, respectively; $p < 0.001$ for both) and between Ks and FIBTEM MCF ($r = 0.61$; $p < 0.001$). SEM was performed in two patients with representable levels of fibrinogen, thrombin generation, and Ks (images from one case are given in Fig. 2D). Fibrin network permeability increased 10 min and 2 h after CPB, and then decreased at 24 h.

In the majority of patients, clot lysis time (CLT) remained unaltered or increased at all postoperative time points. There was, however, one important exception: a patient with excessive bleeding who later underwent re-exploration. This patient had normal CLT 10 min after CPB (138% of control) but markedly shorter CLT (33% of control) at 2 h after CPB, clearly indicating a hyperfibrinolytic state. All other haemostatic variables at this time point were within range of those in the remaining non-bleeding patients, except higher fibrinogen concentration and lower permeability (Supplementary Table 3). No surgical bleeding could be detected at re-exploration, and 24 h after surgery the patient's CLT was within range again (Supplementary Fig. 1).

CPB induces massive coagulation activation due to exposure of blood to artificial surfaces and surgical trauma [5]. In the present study, however, CAT data revealed a decrease in thrombin generation, which was already significant10 min and 2 h after CPB. As CAT measurements are sensitive to presence of heparin, one might suspect that the poor

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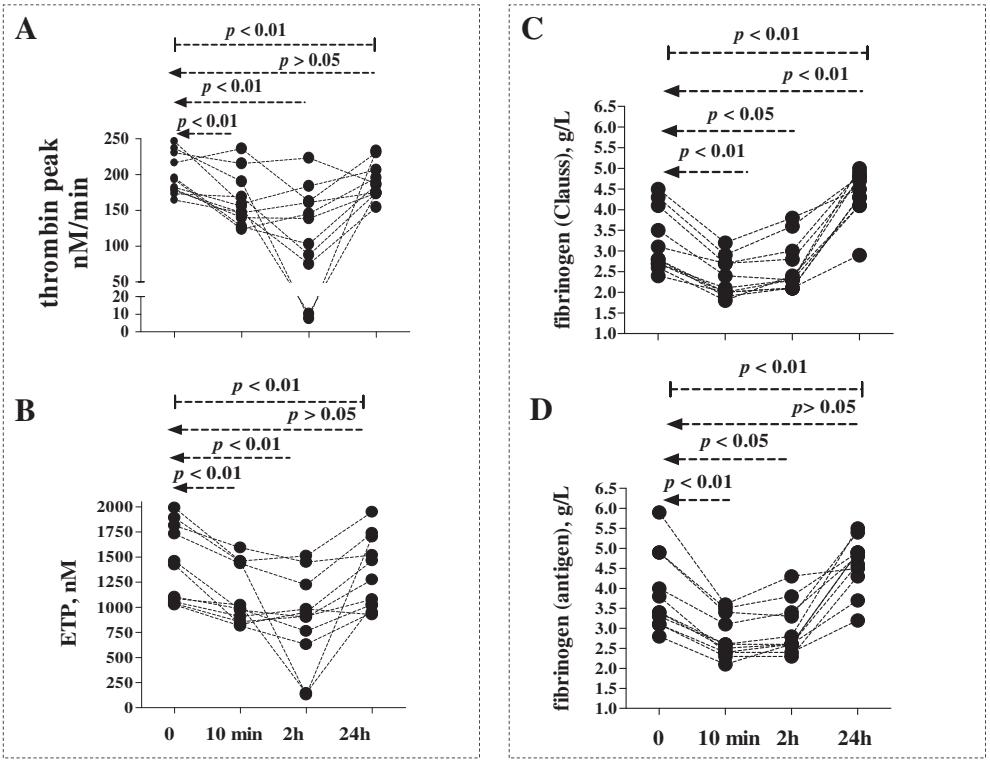

Fig. 1. A. Calibrated automated thrombogram (CAT) thrombin peak. Maximum concentration of thrombin generated. B. Calibrated automated thrombogram (CAT) ETP Endogenous thrombin potential, calculated from the area under the curve during the whole registration time. C. Fibrinogen activity (Clauss). Data displayed as g/L. D. Fibrinogen antigen. Data displayed as g/L. Overall significance was tested with Friedman non-parametric ANOVA; *p*-value shown above the arrows. Arrow = Individual comparisons were made between time point 0 and "10 min", "2 h", or "24 h" using Wilcoxon signed-rank test. All *p*-values are indicated.

thrombin generation capacity observed was at least partly due to residual effect of heparin. In fact, sustained heparin effect (increased *anti*-FXa activity) was demonstrated at 10 min and 2 h after CPB. In addition, there were significant inverse correlations between anti-FXa and ETP ($r = -0.45$; $p = 0.006$) and between anti-FXa and thrombin peak values ($r = -0.47$; $p = 0.004$). This observation agrees with Radulovic et al. [6], and one interpretation may be that thrombin generation is more affected by heparin than by dilution of coagulants/anti-coagulants. To prevent bleeding, full neutralization of heparin by protamine may help, e.g. by improving algorithms. It should, however, be noted that overdosing of protamine may cause increased bleeding [7].

Haematocrit and haemoglobin concentration are plasma volume-dependent and therefore markers of haemodilution: low levels reflect a greater degree of plasma dilution. In the present study, haemodilution was apparent 10 min and 2 h after CPB—most likely a combined result of blood loss and infusion of crystalloid fluids. According to data published by our group [8], plasma dilution does not attenuate ETP to a critical extent, possibly due to diluted protein S concentration and therefore weakened protein C pathway. Dilution of other proteins such as antithrombin may also contribute.

Thrombin generation capacity and fibrinogen concentration serve as the main regulators of fibrin clot properties such as fibrin viscoelasticity and fibrin network permeability. In the present study, reductions were observed postoperatively in both thrombin generation and fibrinogen quantity. These two changes restrain fibrin formation and together may lead to the observed alterations in fibrin network architecture.

The changes in fibrin network permeability and prolongations in both EXTEM CT time and CAT lag phase reflect a reduction in the rates of fibrinogen activation by thrombin. The decline in EXTEM MCF reflects haemodilution with reduced fibrin formation and fibrin viscoelasticity. Moreover, the highly significant correlation between the immunological, functional fibrinogen levels as well with FIBTEM values, strongly indicate that fibrinogen function per se is not altered. Interestingly, it has previously been shown that clots with a reduced quantity of fibrin and weakened fibrin firmness are more susceptible to fibrinolysis [9].

In order to protect patients from hyper-fibrinolysis, intravenous tranexamic acid (TA) was administered before and after surgery. In 10 of 11 patients investigated, no postoperative haemorrhage was seen, despite impairments in thrombin generation and fibrin formation. This may partly be due to use of TA, which resulted in the unchanged/prolonged CLT in patient samples containing exogenous t-PA.

Interestingly, excessive postoperative bleeding occurred in one patient who had a critically shortened CLT at 2 h after surgery, whereas thrombin generation and fibrin formation were not different from that in the other 10 patients. Our bleeding patient was obese, which is generally associated with increased risk of thrombotic complications, which is partly explainable by altered fibrinolysis and increased levels of PAI-1 [10]. In this one bleeding patient, however, we cannot rule out an increase in plasminogen level or a reduced α_2-antiplasmin concentration. However, the observation that CPB patients may bleed due to hyperfibrinolysis—a finding of others also [4]—is interesting and deserves further investigation.

The present study had important limitations, but also strengths. The number of patients included was limited, and the regulated blood volume limited the number of analyses. The strengths included the fact that CAT, fibrin permeability, thromboelastometry, and CLT used the same reagent (rTF, please see Supplementary materials for details) as the coagulation trigger. This unified the clotting mechanisms in the different assays, which may simplify comparisons.

In summary, our results suggest that cardiac surgery with CPB leads to global impairments of balance between thrombin generation and fibrin formation, as well as coagulation and fibrinolysis. The induced bleeding risk may - at least, when tranexamic acid is used - be associated with residual heparin effect, plasma dilution, and possibly