Changes in specific markers of haemostasis during reduction mammoplasty

J.-F. PAYEN, M. BARUCH, E. HORVILLEUR, M. RICHARD, T. GARIOD AND B. POLACK

Summary
We have investigated the time course of the coagulation and fibrinolytic changes during moderate surgical trauma (elective reduction mammoplasty) in the absence of other confounding factors that could affect haemostasis. Specific markers for coagulation (prothrombin fragment 1.2 (F1.2), thrombin–antithrombin III complex (TAT)) and fibrinolysis (plasmin–antiplasmin complex (PAP) and D-dimer) were examined. Blood samples were obtained in 20 ASA I anaesthetized female patients at T0 (before operation), T75 (during operation) and T150 (before the end of operation). There was a progressive increase in blood loss during operation: mean 110 (SD 80) ml at T75 and 470 (180) ml at T150. This was associated with a significant increase in plasma concentrations of F1.2, PAP and D-dimer at T150 only (P<0.05 vs T0). We conclude that moderate surgical trauma with blood losses greater than 300 ml can activate thrombin generation and fibrinolysis during operation. (Br. J. Anaesth. 1998; 80: 464–466)

Keywords: blood, coagulation; blood, loss; surgery, haemostatic response; surgery, plastic

Several clinical studies have suggested that the postoperative hypercoagulable state probably begins during operation, reflected by a decrease in antithrombin III, protein C and plasminogen activities,1–3 and by an increase in thrombin formation.4–7 In most cases, the intraoperative increase in coagulation activity was observed during extensive surgery, in the presence of haemodilution or blood transfusion, which are known to affect haemostasis.8–10 In addition, other changes in intraoperative coagulation and fibrinolytic activity have been documented during brain and cardiac procedures, but these tissues can selectively express tissue factor.11 Finally, these haemostatic changes were found after various operation times, and duration of surgery may influence the postoperative hypercoagulable state.12 It is not known if moderate surgical trauma and accompanying blood loss could activate intraoperative thrombin generation per se without these confounding factors acting on intraoperative haemostasis.

Our aim was to investigate the time course of intraoperative haemostatic response to a surgical procedure with estimated moderate blood loss, in the absence of blood transfusion or haemodilution. We studied patients undergoing reduction mammoplasty because of the homogeneous healthy population, expected moderate intraoperative blood loss13 and the fact that expression of tissue factor in mammary tissue has not been reported. The intraoperative time course of specific markers for coagulation (prothrombin fragment 1.2 (F1.2) and thrombin–antithrombin III complex (TAT)) and fibrinolysis (plasmin–antiplasmin complex (PAP) and D-dimer) was determined together with assays for factor VIII coagulant activity (VIII:C) and von Willebrand factor antigen (vWF).

Materials and methods
The study was approved by the Hospital Committee for the Protection of Human Subjects. After obtaining informed consent, we studied 26 adult ASA I female patients undergoing elective reduction mammoplasty. Exclusion criteria were recent therapy with salicylates and non-steroidal anti-inflammatory drugs, oral contraceptives, preoperative haemoglobin concentration less than 90 g litre⁻¹, or abnormal blood coagulation by clinical history or by preoperative laboratory screening (platelet count <100 × 10⁹ litre⁻¹, prothrombin time <80%, activated partial thromboplastin time >38 s).

After oral premedication with hydroxyzine 100 mg, anaesthesia was induced with propofol 2.5 mg kg⁻¹ i.v. and alfentanil 30 μg kg⁻¹ i.v. After administration of atracurium 0.5 mg kg⁻¹ i.v. and tracheal intubation, patients underwent mechanical ventilation (tidal volume 10 ml kg⁻¹ at 12 bpm) with 65% nitrous oxide in oxygen, maintaining end-expired P_{CO₂} at 4.0–4.6 kPa. The operating table was adjusted so that the patient was in a semi-sitting position. Anaesthesia was maintained with continuous i.v. infusion of alfentanil 30 μg kg⁻¹ h⁻¹ and inspired isoflurane at an alveolar concentration required to maintain mean arterial pressure (MAP) at 60–80 mm Hg (Dynanap, Critikon Inc., Tampa, FL, USA). Rectal temperature was maintained at 36–36.5°C using a heated mattress. Over the whole operation time, patients received Ringer’s solution at a rate of 4–6 ml kg⁻¹ h⁻¹. If infusion of colloid,

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autologous blood or plasma, or administration of vasoactive drugs were necessary before the study ended, the patient was excluded. Blood loss was determined by weighing sponges and operative drapes. Anatomical specimens were weighed and subjected to histological examination.

The study started in anaesthetized haemodynamically stable patients (T0). Sequential venous blood samples were obtained directly from peripheral foot vein punctures at T0 (before operation), T75 (during operation) and T150 (before the end of operation) while the patient was still anaesthetized in the semi-sitting position. For each period, blood samples were obtained into Vacutainer tubes in the following order: haemoglobin concentration and platelet count into a tube containing lithium heparin (14.3 u. ml⁻¹ blood (United States Pharmacopeia units)), and plasma coagulation factors into Diatube H tubes containing CTAD mixture (9:1 vol/vol). All tubes were purchased from Becton Dickinson (Meylan, France). Plasma was separated by centrifugation at 3500 g for 20 min at 18 °C, and stored in 250-ml aliquots at −80 °C until assayed.

Standard laboratory techniques were used for measurement of osmolality and electrolyte concentration (Hitachi 717, Boehringer Mannheim, Meylan, France). Haemoglobin concentration and platelet counts were measured using a Coulter counter STKS (Coultronics, Margency, France). Chromometric tests were performed on a STA coagulometer (Diagnostica Stago, Asnières, France) for fibrinogen (Fibrinomat, Biomérieux, Marcy-l’Etoile, France; normal range 2–4 g litre⁻¹) and VIII:C (F VIII deficient plasma, Immuno, Orly, France; normal range 60–130%). An enzyme-linked immunosorbent assay (ELISA) was used for measurement of vWF, TAT, F1.2, PAP and D-dimer. TAT complex, F1.2=prothrombin fragment 1.2, PAP=plasmin-antiplasmin complex. *P<0.05, **P<0.01 compared with T0.

### Table 1

<table>
<thead>
<tr>
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<th>T0 min</th>
<th>T75 min</th>
<th>T150 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood loss (ml)</td>
<td>0</td>
<td>110 (80)**</td>
<td>470 (180)**</td>
</tr>
<tr>
<td>Haemoglobin (g litre⁻¹)</td>
<td>117 (9)</td>
<td>116 (9)</td>
<td>114 (10)*</td>
</tr>
<tr>
<td>Packed cell volume (%)</td>
<td>33.6 (2.4)</td>
<td>33.7 (2.3)</td>
<td>32.9 (2.8)</td>
</tr>
<tr>
<td>Platelet count (x10⁹ litre⁻¹)</td>
<td>240 (43)</td>
<td>250 (47)</td>
<td>242 (49)</td>
</tr>
<tr>
<td>Fibrinogen (g litre⁻¹)</td>
<td>2.9 (0.7)</td>
<td>2.8 (0.5)</td>
<td>2.6 (0.7)**</td>
</tr>
<tr>
<td>VIII:C (%)</td>
<td>81 (24)</td>
<td>80 (30)</td>
<td>77 (22)</td>
</tr>
<tr>
<td>vWF (%)</td>
<td>79 (24)</td>
<td>77 (20)</td>
<td>76 (22)</td>
</tr>
<tr>
<td>TAT (µg litre⁻¹)</td>
<td>3.2 (3.2)</td>
<td>4.2 (4.3)</td>
<td>4.3 (5.2)</td>
</tr>
<tr>
<td>F1.2 (nmol litre⁻¹)</td>
<td>0.97 (0.40)</td>
<td>1.07 (0.31)</td>
<td>1.25 (0.40)**</td>
</tr>
<tr>
<td>PAP (µg litre⁻¹)</td>
<td>168 (50)</td>
<td>171 (65)</td>
<td>212 (97)*</td>
</tr>
<tr>
<td>D-dimer (µg litre⁻¹)</td>
<td>156 (52)</td>
<td>159 (58)</td>
<td>173 (67)**</td>
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</table>

### Statistical Analysis

Results are expressed as mean (sd). Analysis of the data was performed using one-way analysis of variance (ANOVA) for repeated measurements (StatView SE program, Abacus Concepts Inc., Berkeley, CA). Each value was compared with that obtained at control (T0) using the Scheffé F test, with a significance level of 0.05.

### Results

Of the 26 patients enrolled in the investigation, six did not complete the study because of technical problems in blood sampling in five, and requirement for autologous blood transfusion in one patient. The study population consisted of 20 female patients, mean age 28 (range 19–49) yr and mean weight 62 (sd 9) kg. Mean operation time was 200 (50) min. The anatomic specimens weighed 830 (380) g (range 260–1760 g), and showed no signs of malignancy on histological examination.

There was progressive intraoperative blood loss, resulting in a small decrease in haemoglobin concentration at T150 while packed cell volume remained unchanged (table 1). At T75, there was no significant change in the plasma values of the haemostatic variables. However, at T150 the plasma concentration of fibrinogen decreased significantly (P<0.01), while plasma concentrations of F1.2, PAP and D-dimer increased significantly (P<0.05). These haemostatic changes corresponded to a mean increase of 34% for F1.2, 25% for PAP and 10% for D-dimer from their values at T0. There were no changes in platelet count or plasma VIII:C and vWF concentrations. There was no significant change in the values of plasma sodium and osmolality throughout the study (data not shown). No patient had clinical evidence of deep vein thrombosis in the postoperative period.

### Discussion

Thrombin formation is a key regulatory step in haemostasis. Trapse plasma proteins, activated on exposure to tissue factor, initiate a series of reactions culminating in the conversion of prothrombin to thrombin. During this process, the amino terminal of the prothrombin molecule is cleaved to generate the inactive F1.2 fragment. The short half-life of active thrombin, primarily caused by the inhibitory effect of antithrombin III, precludes direct measurement of
plasma thrombin concentration. Therefore, the F1.2 assay provides a direct immunochemical measurement of prothrombin activation and thrombin generation in vitro. As the selective cellular expression of tissue factor in humans is restricted to the perivascular cells,13 activation of blood coagulation via contact of tissue factor with blood during surgical trauma is expected. However, no such change has been found in plasma TAT concentrations, mainly because of intra-individual variability of TAT measurements. F1.2 is then a sensitive marker of prothrombin activation, as noted previously in patients anticoagulated with warfarin.

Intraoperative thrombin generation was associated with increased fibrinolytic activity, indicated by an increase in plasma values of PAP and D-dimer at T150. When plasmin acts on cross-linked fibrin, E fragments and D-dimers are generated. Through the serine of its activation site, plasmin bonds with α2 plasmin inhibitor to form PAP complex. Therefore, PAP is a direct indicator of plasmin activity, while D-dimers reflect degradation of fibrin. Increased plasma concentrations of PAP and D-dimers have been reported after trauma10 and during major orthopaedic surgery.6 Fibrinolysis is activated mainly by tissue plasminogen activator (tPA). Although concentrations of tPA were not measured in this study, mammary tissue is not known to contain high concentrations of tPA. However, thrombin is one of the stimuli for secretion of tPA by the endothelial cells.3 This suggests that enhanced fibrinolysis was secondary to thrombin generation in our study.

Although we did not measure plasma concentrations of catecholamines, intraoperative plasma values of vWF and VIII:C, which may be related to the endocrine response to anaesthesia,18 remained unchanged. Thus prothrombin activation was related to surgical trauma during non-stress anaesthetic conditions.

We have isolated surgical trauma and accompanying blood loss (>300 ml) as determinants of intraoperative activation of thrombin generation and fibrinolysis, in the absence of confounding factors acting on intraoperative haemostasis. However, these haemostatic changes are small compared with those observed during major surgical procedures where F1.2 concentrations were >3 nmol litre−1.16 In both studies, the highest concentrations of F1.2 were observed in the postoperative period. Conversely, no significant intraoperative change in F1.2 was found during elective craniotomy with blood losses <250 ml.7 No change in haemostatic response was found during ophthalmic surgery whether the anaesthetic technique.19 This indicates that associated factors such as blood transfusion or major blood losses could have a prominent role in intraoperative activation of coagulation.

Increased plasma concentrations of TAT, F1.2 and D-dimer have been found to correlate with thrombotic tendency.15,20 The moderate changes in these markers in our study make it difficult to translate these results into a real risk of venous thromboembolism or recommend a form of prophylaxis for this type of surgical procedure. Postoperative assessment of these specific markers of haemostasis coupled with limb venography would be needed to establish guidance. Nevertheless, this study confirmed that activation of thrombin generation begins during operation, even during moderate surgical trauma.

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References