Additive roles of platelets and fibrinogen in whole-blood fibrin clot formation upon dilution as assessed by thromboelastometry

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Summary

Blood dilution after transfusion fluids leads to diminished coagulant activity monitored by rotational thromboelastometry, assessing elastic fibrin clot formation, or by thrombin generation testing. We aimed to determine the contributions of blood cells (platelets, red blood cells) and plasma factors (fibrinogen, prothrombin complex concentrate) to fibrin clot formation under conditions of haemodilution in vitro or in vivo. Whole blood or plasma diluted in vitro was supplemented with platelets, red cells, fibrinogen or prothrombin complex concentrate (PCC). Thromboelastometry was measured in whole blood as well as plasma; thrombin generation was determined in parallel. Similar tests were performed with blood from 48 patients, obtained before and after massive fluid infusion during cardiothoracic surgery. Addition of platelets or fibrinogen, in additive and independent ways, reversed the impaired fibrin clot formation (thromboelastometry) in diluted whole blood. In contrast, supplementation of red blood cells or prothrombin complex concentrate was ineffective. Platelets and fibrinogen independently restored clot formation in diluted plasma, resulting in thromboelastometry curves approaching those in whole blood. In whole blood from patients undergoing dilution during surgery, elastic clot formation was determined by both the platelet count and the fibrinogen level. Thrombin generation in diluted (patient) plasma was not changed by fibrinogen, but improved markedly by prothrombin complex concentrate. In conclusion, in dilutional coagulopathy, platelets and fibrinogen, but not red blood cells or vitamin K-dependent coagulation factors, independently determine thromboelastometry parameters measured in whole blood and plasma. Clinical decisions for transfusion based on thromboelastometry should take into account the platelet concentration.

Keywords

Blood dilution, coagulation, fibrin clot formation, thrombin generation, platelets

Introduction

It is still a matter of debate how insufficiencies of the haemostatic system lead to bleeding under conditions of massive fluid infusion during cardiothoracic surgery. Fluid infusion diminishes a variety of processes, including platelet activity, coagulation factor levels, thrombin generation and fibrin clot formation. The latter process is monitored by rotational thromboelastometry or thromboelastography, which are frequently used to monitor patients during surgery (1-3). Elastic fibrin clot formation can be measured in whole blood samples, thus providing a rapid point-of-care method for goal-directed coagulation management of surgery or trauma patients with, for example, fibrinogen concentrate (4).

Recently, we have shown that combined plasma measurement of thrombin generation and fibrin clot formation results in a better prediction of the bleeding risk after major surgery than the use of either test alone (5). The rationale is that thrombin generation monitors the rate and extent of formed thrombin, as a central controlling enzyme of the coagulation cascade (6). On the other hand, thromboelastometry, being more sensitive for fibrinogen (7, 8) and platelets (8, 9), reports on the more advanced stage of elastic fibrin clot formation. In patients with bleeding during major surgery or experiencing trauma, impaired haemostasis can be restored by administration of fibrinogen (10-12) or vitamin K-dependent coagulation factors (prothrombin complex concentrate, PCC) (10, 13, 14). Both types of concentrates are also effective in large-animal models of injury-induced bleeding (15-17), suggesting that normalisation of part of the coagulation factors helps to stop bleeding after dilution.
Vitamin K-dependent coagulation factors, platelets and fibrinogen all contribute to the process of elastic clot formation by providing enzymatic activity, strength and mass of a clot, respectively. However, it is still unresolved how, under conditions of dilutional coagulopathy, reduced levels of these blood components interact to limit fibrin clot formation, nor is it clear how red blood cells are involved in this process. In the present paper we aimed to determine these interactions. Using thromboelastometry, we measured fibrin clot formation in whole blood and plasma under conditions of dilution in vitro or in vivo, and assessed the effects of reconstitution of blood cells (platelets, red blood cells) and plasma coagulation factors (fibrinogen, PCC). The results show that the clotting process is controlled by platelets and fibrinogen in additive and independent ways, implicating that the platelet count is an important functional variable, independently of fibrinogen, in whole-blood thromboelastometry under condition of in vivo dilution in patients undergoing cardiothoracic surgery.

Materials and methods

Materials

Bovine serum albumin (BSA) and apyrase were obtained from Sigma (St. Louis, MO, USA). Human thrombin calibrator and thrombogram software were from ThrombinoScope (Maastricht, The Netherlands), thrombin substrate Z-Gly-Gly-Arg aminoethyl coumarin (Z-GGR-AMC) was from Bachem (Bubendorf, Switzerland). Thromboelastometry International (Munich, Germany) supplied the corresponding hardware, software and cuvettes.

Fibrinogen concentrate (Haemocomplettan P) and prothrombin complex concentrate (PCC, Beriplex) were from CSL Behring (Marburg, Germany). PCC contains the vitamin K-dependent coagulation factors, prothrombin and factor VII, IX and X, and the anticoagulant factors, protein C and S, and antithrombin/heparin; 1 U/ml PCC refers to 100% prothrombin. Recombinant tissue factor (Innovin) was from Dade Behring (Deerfield IL, USA). Phospholipid vesicles containing phosphatidylserine, phosphatidylcholine and phosphatidylethanolamine (20:60:20) were purchased from Avanti (Alabaster, AL, USA) and prepared by ultrasonication (18). All other reagents came from sources described before (5).

Blood donors

The studies were approved by the local medical ethics committee (MEC 07-2-114). Healthy donors and patients gave full informed consent for blood donation, according to the Helsinki declaration. Healthy subjects had not taken antithrombotic medication for at least two weeks. For in vitro dilution experiments, blood was obtained from 15 healthy subjects by venipuncture using a 1.2 mm needle, allowing the blood to drip freely into open tubes (first 2-3 ml were discarded). The collection tubes contained either 1/10 volume of trisodium citrate (0.129 M) for the preparation of platelet-free plasma; or 1/6 volume of acidic citrate dextrose (ACD: 80 mM trisodium citrate, 52 mM citric acid and 180 mM glucose) for the isolation of washed platelets.

Blood samples were obtained from 48 patients before and after a cardiopulmonary bypass (CPB) procedure. This sample size is based on the results of an earlier study, where the effects of in vivo dilution on elastic fibrin clot formation in only plasma were determined (19). Patients were admitted in the hospital in the period of October to December 2010. Patients had stopped taking anticoagulant drugs at least one week before the procedure. Blood samples were collected into 0.129 M trisodium citrate (1:10) Vacuett tubes (Greiner, Alphen a/d Rijn, The Netherlands) at two time points: (i) after induction of anesthesia, but prior to the CPB procedure and heparin administration; (ii) after surgery and infusion of protamine to neutralise heparin, when the activated clotting time (ACT) was normalised. In samples from four patients coagulation times pointed to the presence of residual traces of heparin. In these cases, assays were repeated in the presence of 10 µg/ml polybrene, i.e. a concentration not influencing the assay parameters (data not shown). None of the patients developed surgical complications other than bleeding during the time span of blood sampling. Transfusion of blood products during surgery was guided on the basis of low blood cell counts, prolonged aPTT, and/or clinical observation of bleeding. Whole blood thromboelastometry was measured immediately after collection; plasma samples were stored for later measurements.

Preparation of plasma, washed platelets and red blood cells

Platelet-free plasma was obtained from citrate-anticoagulated blood, by centrifuging twice at 2,630 g for 10 minutes (min) (20). Plasma samples were immediately snap-frozen into liquid nitrogen, and stored at -80°C until further use. Washed platelets were prepared from ACD-anticoagulated blood, and suspended in Hepes buffer pH 7.45 (10 mM Hepes, 136 mM NaCl, 2.7 mM KCl, 2 mM MgCl₂, 0.1% glucose and 0.1% BSA), as described (19). Platelet count was determined with a thrombocounter (Coulter Electronics, Luton, UK). For the isolation of red blood cells, citrate-anticoagulated blood was centrifuged at 240 g for 15 min, after which the red cell layer was supplemented with Hepes buffer pH 7.45 in a 1:2 volume ratio. Red cells were then centrifuged at 2,630 g for 10 min, suspended with Hepes buffer pH 7.45 (2:1), and washed again, which yielded a highly purified suspension of red cells (99.9%).

Dilution of whole blood or plasma and reconstitution experiments

Whole blood or plasma was diluted in vitro with saline medium, consisting of 137 mM NaCl, 12.9 mM trisodium citrate, 2 mM CaCl₂ and 2 mM MgCl₂, in order to keep equal concentrations of free Ca²⁺ and Mg²⁺ in all diluted samples. Coagulation factor levels in plasma were determined, as described (5). Percentage values of plasma are given as final concentrations (relative to citrate-anticoagulated plasma taken as 100%). In all reconstitution experiments,
replacement of plasma by added supplements was taken into ac-
count when calculating the final extent of dilution. Red blood cells
were added at 1:10 volume ratio. Where indicated, factor concen-
trates in saline were added to plasma samples from healthy sub-
jects or patients, and incubated for 10 min before starting the ex-
periment.

**Rotational thromboelastometry**

In samples from the same donors, elastic fibrin clot formation was
measured in whole blood and plasma (citrate-anticoagulated) by
thromboelastometry, using equipment and cuvettes from TEM In-
ternational. Coagulation was triggered with 10 pM tissue factor
and a surplus of CaCl$_2$ (19). Plasma samples were supplemented
with phospholipid vesicles (4 μM, final concentration) or washed
platelets from one healthy donor (100-250 × 10$^9$/l, final count).

Runs were performed in duplicate or, when >5% variation was ob-
served, in triplicate. Curves were analysed for slope of elastic clot
formation (α-angle), maximum strength of the clot (maximal clot
firmness, MCF) and the time to the onset of clot formation (clot-
ting time, CT).

**Thrombin generation**

Thrombin generation in plasma was measured, using the cali-
brated automated thrombogram (CAT) method (19). Plasma
samples were supplemented with either phospholipid vesicles (4
μM) or washed platelets from a single healthy donor (100-250 ×
10$^9$/l). Assays were run in 96-well U-bottom plates (Milford, MA,
USA) in the presence of fluorogenic substrate Z-GGR-AMC (2.5
mM) and CaCl$_2$ (16.7 mM, final concentrations). Coagulation was
triggered with 10 pM tissue factor. Measurements were performed
to 40% was supplemented (final concentrations) with prothrombin complex
concentrate (PCC 1 U/ml, equivalent to 100% prothrombin), red blood cells
(RBC, 37% haematocrit), autologous platelets (PLT, 250 × 10$^9$/l) and/or fibri-
nogen concentrate (FC, 3.0 g/l). Values of MCF, α-angle and CT are given in
comparison to the control condition with undiluted blood (100%). Means ±
SD (n = 5–8), *P < 0.05 vs no addition.
in triplicate. First-derivate curves of thrombin generation were obtained by using Thrombogram software (20). Curves were analysed for maximal rate of thrombin generation (thrombin peak height), thrombin-generating capacity (endogenous thrombin potential, ETP) and the time until thrombin formation (lag time). Samples containing added PCC were analysed for 10 min after triggering coagulation, to ensure adequate curve calibration.

**Statistical analysis**

Data are given as means ± SD. The Shapiro-Wilk test was used to test for normal distribution of the data. Statistical analysis was performed, as appropriate, using the Mann-Whitney U test. Patient data were not normally distributed and are given as medians with interquartile ranges. The Wilcoxon matched pairs signed ranked test was used for comparing pre- and post-surgical values. Determinants of thromboelastometry curves were identified by multiple linear regression analysis. P-values <0.05 were considered statistically significant. The program GraphPad Prism 6.0 (GraphPad Software, San Diego, CA, USA) was used for graphical purposes and relevant statistics; the SPSS 20 (IBM, Armonk, NY, USA) package was used for regression analysis and statistical testing.

**Results**

**Determinants of thromboelastometry and thrombin generation curves for whole blood and plasma upon in vitro dilution**

Blood samples from healthy subjects were diluted in vitro, constituted with various blood components, and analysed for elastic fibrin clot formation by thromboelastometry. Coagulation was triggered via the extrinsic pathway with recombinant human tissue factor, to ensure adequate curve calibration. Determinants of thromboelastometry curves were identified by multiple linear regression analysis. P-values <0.05 were considered statistically significant. The program GraphPad Prism 6.0 (GraphPad Software, San Diego, CA, USA) was used for graphical purposes and relevant statistics; the SPSS 20 (IBM, Armonk, NY, USA) package was used for regression analysis and statistical testing.

**Figure 2:** Determinants of thromboelastometry and thrombin generation in diluted plasma. Normal plasma (fibrinogen 3.2 g/l, prothrombin 100%) was diluted with saline medium. Samples were replenished (final concentrations) with fibrinogen concentrate (FC, 3.0 g/l) or prothrombin complex concentrate (PCC, 100% prothrombin). Final plasma dilution of all samples was 40%. Coagulation was triggered with 10 pM tissue factor in the presence of either procoagulant phospholipid vesicles (PV, 4 µM) or platelets (PLT, 100 x 10^9/l). A, C) Representative thromboelastometry curves with phospholipid vesicles or platelets. B, D) Representative thrombin generation curves with phospholipid vesicles or platelets. E-H) Effect of added fibrinogen or PCC on parameters of thromboelastometry (E) and thrombin generation (F-H). This analysis underscored the fibrinogen level as a major variable (independently of platelets) in elastic clot formation. In the presence of platelets (100 x 10^9/l), the rate and maximal amplitude of elastic clot formation were markedly higher and reached the maximal levels detected in whole blood (Figure 2C, E), thus pointing to an additive effect of platelets to the thromboelastometry curves. In this case, plasma dilution to 40% only moderately diminished the thromboelastometry curves. In measurements of thrombin generation with platelets, plasma dilution was of limited effect on thrombin peak height, total amount of thrombin (ETP) or lag time (Figure 2F-H).

Dose-response studies were performed to better define the effects of fibrinogen and PCC in diluted plasma. For thromboelastometry curves, added fibrinogen up to 3–4 g/l (final concentration) caused a dose-dependent increase in MCF and α-angle, regardless of whether PCC was added or not (Figure 3A, B). Added fibrinogen did not affect the CT, while only the highest concentration of PCC caused a slight prolongation in CT (Figure 3C). Addition of PCC resulted in a dose-dependent increase in thrombin generation, reaching 300% of the normal thrombin peak height and ETP level. At none of the doses, fibrinogen influenced parameters of thrombin generation (Figure 3D, E). However, PCC at high doses prolonged the lag time to thrombin formation (Figure 3F), which can be explained by the presence of anticoagulant proteins in this concentrate. Together, these data indicate that, in diluted whole blood and plasma, fibrin clot formation relies on the amounts of platelets and fibrinogen, whereas the thrombin generation process is regulated by the levels of vitamin K-dependent coagulation factors, as present in PCC.
In thromboelastometry, effects of varying the levels of both platelets and fibrinogen were then compared. These blood components were added in various combinations to diluted plasma, while keeping the final extent of dilution constant. The three-dimensional plot of Figure 4A indicates that, up to $250 \times 10^9$ platelets/l and 2.7 g fibrinogen/l, the MCF increased with higher levels of both platelets and fibrinogen (Figure 4A). Even at the highest fibrinogen concentrations, the MCF raised with the platelet count. This is also apparent from a plot of the $\alpha$-angle, although this parameter reached a maximal value at lower fibrinogen and platelet concentrations.

**Figure 3**: Dissimilar effects of fibrinogen and prothrombin complex concentrate on thromboelastometry and thrombin generation in diluted plasma. Normal plasma was diluted with saline medium and replenished with fibrinogen concentrate (FC, 1.4–2.4 g/l) and/or prothrombin complex concentrate (PCC, 0.1–1.6 U/ml). Final plasma dilution in all samples was 40%. Coagulation was triggered with 10 pM tissue factor in the presence of phospholipid vesicles. Effects of addition of different amounts of FC with or without PCC (0.5/1.4 U/ml) on MCF (A), $\alpha$-angle (B) and CT (C) of the thromboelastometry curves. Effects of addition of different amounts of PCC with or without FC (2.4 g/l) on peak height (D), ETP (E) and lag time (F) of the thrombin generation curves. Means ± SD ($n = 3–6$).
levels (▶Figure 4B). Regression analysis revealed that the platelet count contributed more strongly to the MCF than the fibrinogen level; with a standardised regression coefficient \( \beta \) of 0.760 and 0.498, respectively. Furthermore, the relative contribution of fibrinogen to MCF appeared to be independent of the platelet count, i.e. standardised regression coefficients for fibrinogen were >0.89. This was also true for the \( \alpha \)-angle (see Suppl. Table 1, available online at www.thrombosis-online.com).

**Predictive variables of whole blood thromboelastometry and thrombin generation in patients undergoing haemodilution during surgery**

To assess the clinical relevance of these different roles of platelets and fibrinogen in thromboelastometry, blood and plasma samples were studied from patients undergoing cardiothoracic surgery and in vivo dilution by massive fluid infusion. Blood samples were analysed from 48 patients, with mean age of 67 (range 51-75) years, of whom 33 underwent coronary artery bypass grafting and 10 replacement of the aortic or mitral valve (see Suppl. Table 2, available online at www.thrombosis-online.com). Total fluid volume transfused during the surgery procedure was 4.6 ± 1.4 l (mean ± SD). A minority (12 patients) needed transfusion with 2.2 ± 1.5 units packed red cells. Blood samples were obtained from the patients before and after the surgical procedure. All patients received heparin (344 ± 77 mg) after collection of the first blood sample, and the heparin was antagonised with protamine (269 ± 51 mg) before the second blood collection. Plasma samples were checked for absence of residual heparin activity.

In the group of patients, the platelet count dropped with 45%, the haematocrit level reduced with 25%, and the aPTT significantly prolonged from 30.0 (median) to 35.0 seconds (▶Table 1). Plasma levels of prothrombin, antithrombin and factor X reduced with 41-46%, while fibrinogen decreased from 3.50 (median) to 1.90 g/l. In remaining plasma samples from a subgroup of seven patients, levels of other coagulation factors were determined; factors VII, VIII and IX were reduced by 46%, 32% and 38%, respectively. Overall, this pointed to a dilution of ~40% of most blood components due to fluid infusion.

Whole blood samples pre- and post-surgery from all patients were analysed by thromboelastometry upon triggering with tissue factor (▶Table 2). As expected, curves of clot formation narrowed significantly, in that the MCF decreased from median 66.0 (62.0-70.0) to 55.0 (48.0-60.0) mm, while the \( \alpha \)-angle decreased from 76.0 (74.0-79.0) to 70.5 (63.4-71.80) degrees. Both the CT and clot formation time (CFT) significantly prolonged. During the
Table 1: Haematological parameters of patients before and after cardiothoracic surgery. Blood was obtained from 48 patients before and after a CPB procedure. Inactivation of heparin was checked in all post-CPB samples. Mean changes (+/-) due to the surgery procedure are indicated in percentages. Medians with interquartile ranges (n = 48). **P < 0.001 vs pre-surgery. 

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-surgery</th>
<th>Post-surgery</th>
<th>Δ</th>
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<tbody>
<tr>
<td>Platelets (x 10^9/l)</td>
<td>199 (159–227)</td>
<td>110 (83.3–140)**</td>
<td>-44.7%</td>
</tr>
<tr>
<td>Haematocrit (ml/ml)</td>
<td>0.32 (0.30–0.36)</td>
<td>0.24 (0.23–0.26)**</td>
<td>-25.0%</td>
</tr>
<tr>
<td>Haemoglobin (mM)</td>
<td>6.70 (6.10–7.50)</td>
<td>5.00 (4.68–5.53)**</td>
<td>-25.4%</td>
</tr>
<tr>
<td>aPTT (s)</td>
<td>30.0 (28.0–30.0)</td>
<td>35.0 (32.0–38.0)**</td>
<td>+16.7%</td>
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<tr>
<td>Prothrombin (%)</td>
<td>103 (87.0–118)</td>
<td>55.5 (43.5–64.0)**</td>
<td>-46.1%</td>
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<tr>
<td>Antithrombin (%)</td>
<td>98.0 (90.5–109)</td>
<td>57.5 (52.0–65.8)**</td>
<td>-41.3%</td>
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<tr>
<td>Factor X (%)</td>
<td>86.0 (74.5–109)</td>
<td>47.5 (42.3–58.0)**</td>
<td>-44.8%</td>
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<tr>
<td>Fibrinogen (g/l)</td>
<td>3.50 (2.90–4.30)</td>
<td>1.90 (1.50–2.30)**</td>
<td>-45.7%</td>
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</tbody>
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Table 2: Parameters of thromboelastometry in whole blood from patients before and after surgery. Thromboelastometry was determined in whole blood from 48 patients pre- and post-surgery, as described in Methods. Data are medians with interquartile ranges. *P < 0.05, **P < 0.001 vs corresponding pre-surgery.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-surgery</th>
<th>Post-surgery</th>
<th>Δ</th>
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<tbody>
<tr>
<td>MCF (mm)</td>
<td>66.0 (62.0–70.0)</td>
<td>55.0 (48.0–60.0)**</td>
<td>-16.7%</td>
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<tr>
<td>Alpha angle (°)</td>
<td>76.0 (74.0–79.0)</td>
<td>70.5 (63.4–72.8)**</td>
<td>-7.2%</td>
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<tr>
<td>CT (s)</td>
<td>112 (98.0–143)</td>
<td>135 (117–156)*</td>
<td>+20.5%</td>
</tr>
<tr>
<td>CFT (s)</td>
<td>70.0 (57.0–85.0)</td>
<td>104 (88.0–138)**</td>
<td>+48.6%</td>
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</table>

Table 3: Predicting variables of thromboelastometry in whole blood from patients before and after surgery. Fibrin clot formation was measured in whole blood from 48 patients pre- and post-surgery. Predicting variables of thromboelastometry MCF were obtained by linear, multiple regression analysis. b indicates unstandardised regression coefficient; SE b, standard error of b; β, standardised regression coefficient.

<table>
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<td>Pre-surgery</td>
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<tr>
<td>Constant</td>
<td>45.59</td>
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<tr>
<td>Fibrinogen</td>
<td>2.655</td>
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<tr>
<td>Platelets</td>
<td>0.054</td>
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<tr>
<td>Haematocrit</td>
<td>7.989</td>
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<tr>
<td>Prothrombin</td>
<td>-0.042</td>
</tr>
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</table>

30 min of measurement, no signs of fibrin degradation were observed (not shown).

Multiple regression analysis demonstrated that in pre-surgery as well as in post-surgery samples, the platelet count and fibrinogen level were significant predictors of whole blood thromboelastometry. Standardised regression coefficients indicated that platelet count and fibrinogen level contributed similarly to the variation in MCF. In contrast, variation in red blood cell count (haematocrit) or prothrombin level did not significantly add to this variation in either pre-surgery or post-surgery samples (p>0.1).

Thromboelastometry and thrombin generation were also determined in plasma samples prepared from pre- and post-surgery blood. Post-surgery thromboelastometry curves, obtained with plasma containing phospholipid vesicles, showed a consistent reduction in MCF (-46%) and α-angle (-11%) and a prolongation of the CT (+30%). These parameters also changed with platelets present. Analysis of thrombin generation measurements indicated a marked dilution effect post-surgery on thrombin peak height (-28%) and ETP (-7%), particularly when assessed with phospholipids. Exclusion of the data from patients, who had received red blood cells (n=12), resulted in similar changes of thromboelastometry and thrombin generation data (not shown).

To determine the potential for normalisation, post-surgery plasma samples were supplemented with fibrinogen concentrate and PCC. These concentrates were added at amounts corresponding to the expected effect of transfusion of 2 units fresh frozen plasma (5), i.e. 0.4 g/l fibrinogen plus 0.11 U/ml PCC. This addition significantly increased MCF in the presence of phospholipids from 16.5 (12.5-20.0) to 21.0 (17.0-25.0) mm, but left the parameter unchanged in the presence of platelets (Table 4). The same addition caused a 25-33% increase in thrombin generation (thrombin peak height and ETP), both with phospholipids and platelets.

The MCF of whole blood thromboelastometry with pre- and post-surgery samples was dependent on the fibrinogen level (partial correlation coefficients 0.917 and 0.890, respectively, p<0.001). Notably, the MCF from curves obtained with whole blood samples...
correlated significantly with the MCF measured with plasma plus phospholipids \((R = 0.83, \ p < 0.001)\) or with plasma plus platelets \((R = 0.75, \ p < 0.001)\). On the other hand, the various parameters of thromboelastometry curves (whole blood or plasma) did not correlate with those of thrombin generation \((p > 0.2)\). Together, this indicated that the predictive variables of thromboelastometry curves of patient whole blood and plasma were similar, but differed from those predicting thrombin generation curves.

**Discussion**

In this paper, we studied principal sources of variation of the processes of elastic fibrin clot formation (thromboelastometry) and thrombin generation under conditions of *in vitro* or *in vivo* dilution. Coagulation in all cases was fully activated with tissue factor \((21)\). It appeared that, in whole blood or plasma diluted *in vitro* to 40%, both the platelet count and fibrinogen concentration determine the kinetics and extent of fibrin clot formation in additive ways, with no more than limited contributions of red blood cells and vitamin K-dependent coagulation factors. In other words, certain (threshold) values of thromboelastometry parameters could be obtained by supplementation of platelets and fibrinogen at different relative amounts, but not by red blood cells. On the other hand, the best way to restore impaired thrombin generation in diluted plasma was by increasing the levels of vitamin K-dependent coagulation factors.

In the present paper we find that, under conditions of haemodilution, thromboelastometry parameters in whole blood are not improved by raising the haematocrit. A limited contribution of red blood cells is also apparent from the observation that curve parameters were similar in whole blood and in platelet-containing plasma (without red blood cells). Other authors, using undiluted blood have reported a negative contribution of the haematocrit on thromboelastometry parameters \((22–24)\). Comparing with the present results, this suggests that under high viscous conditions, as in undiluted blood, red blood cells interfere with the formation of an elastic platelet-fibrin clot, while this interference becomes smaller under less viscous conditions.

The recognition that platelets and fibrinogen in additive ways contribute to thromboelastometry parameters sheds new light on our earlier conclusion \((19)\), that platelets can partly compensate for the dilutional effect on elastic clot formation. The apparently independent contribution of platelets and fibrinogen most likely reflects differences in function in this process, i.e. providing elasticity to the fibrin clot by contraction (platelets) and by determining the mass of a fibrin clot (fibrinogen) \((25)\). This suggestion is supported by the finding that also in blood samples from patients subjected to *in vivo* dilution, the platelet count and fibrinogen level are independent variables of the clot-forming process.

Marked differences were found, when comparing the effects of factor concentrates on thromboelastometry and thrombin generation under conditions of dilution *in vitro* or *in vivo*. Supplementation of fibrinogen concentrate, but not of PCC, in a dose-dependent way restored elastic fibrin clot formation, as detected by an increased MCF and α-angle, without affecting thrombin generation. Interestingly, the CT was hardly changed by PCC addition to whole blood or plasma, which agrees with published findings that the CT prolongs when coagulation factors fall below 35% of normal \((26)\). Conversely, supplementation of PCC, but not of fibrinogen concentrate, restored thrombin generation. These data indicate that, although thromboelastometry is often viewed as an inte-

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**Table 4: Comparison of thromboelastometry and thrombin generation tests in patient plasmas before and after surgery.**

Plasma samples from patients, before and after surgery, were supplemented with either 4 μM phospholipid vesicles or 100 x 10^6 platelets/l, as indicated. Coagulation was triggered with 10 pM tissue factor. Parallel plasma samples post-surgery were supplemented with fibrinogen concentrate (FC, 0.4 g/l, f.c.) and prothrombin complex concentrate (PCC, 0.11 U/ml, f.c.). Medians with interquartile ranges \((n = 48)\).

\*P < 0.05, **P < 0.001 vs pre-surgery samples, or vs no addition.

<table>
<thead>
<tr>
<th>Parameter</th>
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<th>Δ</th>
<th>Post + FC/PCC</th>
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<tr>
<td><strong>Thromboelastometry in plasma</strong></td>
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<tr>
<td>Phospholipid vesicles</td>
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</tr>
<tr>
<td>MCF (mm)</td>
<td>30.5 (26.0–37.0)</td>
<td>16.5 (12.5–20.0) **</td>
<td>-46%</td>
<td>21.0 (17.0–25.0) **</td>
</tr>
<tr>
<td>Alpha angle (°)</td>
<td>79.0 (77.0–81.0)</td>
<td>70.0 (63.0–74.0)**</td>
<td>-11%</td>
<td>74.0 (71.0–78.0)**</td>
</tr>
<tr>
<td>CT (s)</td>
<td>89.0 (83.0–103)</td>
<td>116 (100–132) **</td>
<td>+30%</td>
<td>90.5 (77.0–105)**</td>
</tr>
<tr>
<td>Platelets</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCF (mm)</td>
<td>73.0 (71.0–76.0)</td>
<td>65.0 (62.0–69.0) **</td>
<td>-11%</td>
<td>63.5 (59.0–66.0)</td>
</tr>
<tr>
<td>Alpha angle (°)</td>
<td>79.0 (77.0–80.0)</td>
<td>77.0 (75.0–78.0) **</td>
<td>-2.5%</td>
<td>76.5 (74.0–79.0)</td>
</tr>
<tr>
<td>CT (s)</td>
<td>104 (93.0–135)</td>
<td>111 (97.0–127)</td>
<td>+6.7%</td>
<td>95.5 (85.0–112)**</td>
</tr>
<tr>
<td><strong>Thrombin generation in plasma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phospholipid vesicles</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak height (nM)</td>
<td>297.0 (248.9–324.8)</td>
<td>213.6 (179.1–232.2)**</td>
<td>-28%</td>
<td>269.5 (229.7–294.5)**</td>
</tr>
<tr>
<td>ETP (nM x min)</td>
<td>1159 (996.8–1313)</td>
<td>1083 (921.7–1252) **</td>
<td>-6.6%</td>
<td>1415 (1291–1563)**</td>
</tr>
<tr>
<td>Platelets</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak height (nM)</td>
<td>144.5 (113.8–167.8)</td>
<td>133.6 (113.3–151.4)**</td>
<td>-7.5%</td>
<td>167.1 (147.4–191.2)**</td>
</tr>
<tr>
<td>ETP (nM x min)</td>
<td>1282 (1094–1412)</td>
<td>1159 (1041–1358)*</td>
<td>-9.6%</td>
<td>1536 (1423–1805)**</td>
</tr>
</tbody>
</table>
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Figure 5: High correlation of thromboelastometry MCF values in whole blood and plasma samples. Thromboelastometry was measured in whole blood and plasma with phospholipids or platelets (100 × 10^9/l) from 48 patients. MCF was derived from the thromboelastometry curves. A) MCF in whole blood vs plasma/phospholipids; B) MCF in whole blood vs plasma/platelets. Correlation coefficients and p-values are given of combined pre- and post-surgery data.

What is known about this topic?
- Fibrinogen is a key determinant of elastic fibrin clot formation, assessed by whole-blood thromboelastometry or thrombelastography.

What does this paper add?
- Platelets and fibrinogen contribute in additive and independent ways to elastic fibrin clot formation under conditions of in vitro or in vivo dilution.
- In dilutional coagulopathy, platelets and fibrinogen, but not red blood cells or vitamin K-dependent coagulation factors, determine thromboelastometry parameters measured in whole-blood and plasma.
- Clinical decisions for transfusion of fibrinogen or plasma based on thromboelastometry should take into account the platelet concentration.
- In diluted plasma, thrombin generation is more dependent on vitamin K-dependent coagulation factors than elastic clot formation.

What is known about this topic?


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Conflicts of interest
None declared.

References