Finding the optimal concentration range for fibrinogen replacement after severe haemodilution: an in vitro model

D. Bolliger1, F. Szlam1, R. J. Molinaro2, N. Rahe-Meyer3†, J. H. Levy1† and K. A. Tanaka1*†

1Department of Anesthesiology and 2Department of Pathology and Laboratory Medicine, Emory University, School of Medicine, Atlanta, GA, USA. 3Department of Anesthesiology, Hannover Medical School, Hannover, Germany

*Corresponding author: Department of Anesthesiology, Emory University, School of Medicine, 1364 Clifton Road, N.E., Atlanta, GA 30322, USA. E-mail: ktanaka@emory.edu

Background. Replacement of fibrinogen is presumably the key step in managing dilutional coagulopathy. We performed an in vitro study hypothesizing that there is a minimal fibrinogen concentration in diluted whole blood above which the rate of clot formation approaches normal.

Methods. Blood samples from six healthy volunteers were diluted 1:5 v/v with saline keeping haematocrit at 24% using red cell concentrates. We measured coagulation factors and thrombin generation in plasma at baseline and after dilution. Thromboelastometry was used to evaluate (i) speed and quality of clot formation in diluted samples supplemented with fibrinogen 50–300 mg dl\(^{-1}\) and (ii) clot resistance to fibrinolysis. Diluted and undiluted samples with no added fibrinogen served as controls.

Results. Coagulation parameters and platelets were reduced by 74–85% after dilution. Peak thrombin generation was reduced by 56%. Adding fibrinogen led to a concentration-dependent improvement of all thromboelastometric parameters. The half maximal effective concentration (EC50) for fibrinogen replacement in haemodiluted blood was calculated to be 125 mg dl\(^{-1}\). Adding tissue plasminogen activator, 0.15 μg ml\(^{-1}\), led to a decrease of clot firmness and lysis time.

Conclusions. The target plasma concentration for fibrinogen replacement was predicted by these in vitro results to be greater than 200 mg dl\(^{-1}\) as only these concentrations optimized the rate of clot formation. This concentration is twice the level suggested by the current transfusion guidelines. Although improved, clots were prone to fibrinolysis indicating that the efficacy of fibrinogen therapy may be influenced by co-existing fibrinolytic tendency occurring during dilutional coagulopathy.

Br J Anaesth 2009; 102: 793–9

Keywords: blood, coagulation; blood, haemodilution; complication, coagulopathy; measurement technique, coagulation

Accepted for publication: March 31, 2009

Massive haemorrhage in surgical and trauma patients is primarily treated by administration of crystalloid and colloid fluids to maintain normovolaemia. If haemoglobin decreases <6 g litre\(^{-1}\), transfusion of red blood cell (RBC) products to maintain oxygen delivery capacity is recommended.1 However, the administration of crystalloid or colloid fluids and RBC concentrates for managing massive haemorrhage causes clotting factors, including fibrinogen, to decrease. Critically decreased fibrinogen concentrations (<100 mg dl\(^{-1}\)) tend to occur earliest, whereas critical concentrations of the other coagulation factors and also critically low platelet counts are reached later during the course of massive blood loss and fluids substitution.2–3 In addition, various experimental and clinical studies suggest that replacing fibrinogen is presumably the key step in managing dilutional coagulopathy.4–8

† Declaration of interest. Drs N.R.-M., J.H.L., and K.A.T. have served on advisory board meetings on perioperative haemostasis held by CSL Behring.
Thromboelastometric techniques that show the speed and quality of coagulation initiation have proved to be useful in monitoring and managing the effect of impaired coagulation system during haemodilution. Different crystalloid and colloid fluids have previously been used to dilute whole blood to about 20–60% in in vitro and in vivo models. Dosage of fibrinogen used in vivo varies between 30 and 250 mg kg\(^{-1}\). However, the optimal level of fibrinogen remains unclear as most studies were performed using a single concentration of fibrinogen to reverse dilutional coagulopathy. Nielsen and colleagues studied the effect of increasing fibrinogen concentrations in fibrinogen-deficient plasma on thromboelastometric parameters, showing that only addition of >250 mg dl\(^{-1}\) leads to results comparable with control plasma. International guidelines suggest a minimal fibrinogen concentration over 80–100 mg dl\(^{-1}\) but there is paucity of information to support this recommendation.

Therefore, we aimed to evaluate various fibrinogen concentrations using a new in vitro model, in which we simulated massive haemodilution to about 80% with crystalloid fluids and RBC concentrates. We hypothesized that there is a minimal fibrinogen concentration in diluted blood above which the rate of clot formation approaches normal.

**Methods**

After institutional ethical approval and informed written consent, blood samples were collected into 5 ml Vacutainer tubes (Beckton-Dickinson, Franklin Lakes, NJ, USA) containing 0.5 ml of 3.2% sodium citrate from six healthy volunteers who had not received any drugs in the preceding 2 weeks and had no history of coagulopathy.

Collected whole blood (WB) was either used immediately or was centrifuged for 20 min at 3000\(\times\)g to obtain baseline platelet-poor plasma (PPP). WB was diluted 3:10 v/v with normal saline, and haematocrit was restored to 23–25% by adding allogeneic type-matched RBC concentrate, leading to a total dilution of coagulation factors and platelets to about 1:5 (20%) of baseline. All fibrinogen replacement experiments were performed with this adjusted diluted WB. For thrombin generation and measurement of coagulation factors, diluted WB was centrifuged at 3000\(\times\)g for 20 min to obtain diluted PPP. For the thromboelastometry experiments, 1 ml aliquots of diluted WB were incubated with 0.5, 1.0, 1.5, 2.0, and 3.0 mg of pasteurized fibrinogen concentrate (Haemocomplettan; CSL Behring, Marburg, Germany) corresponding to an increase in fibrinogen concentration of 30–300 mg dl\(^{-1}\). Using a stock solution of 20 mg ml\(^{-1}\), these correspond to volumes of 25–150 \(\mu\)l added to 1 ml diluted WB samples and results in a maximal additional 2.5% dilution with the highest fibrinogen concentration. Additionally, in order to induce fibrinolysis, 1 ml aliquots of undiluted WB and diluted WB without added fibrinogen or with fibrinogen 150 or 300 mg dl\(^{-1}\) were incubated with tissue-type plasminogen activator (tPA; Alteplase; Genentech, South San Francisco, CA, USA), 0.15 \(\mu\)g (corresponding to an additional 0.3–1.5% dilution), to a final concentration of 0.15 \(\mu\)g ml\(^{-1}\).

**Effects of dilution on platelet counts and coagulation factors**

We determined the levels of the following coagulation markers and factors at baseline and in diluted PPP: prothrombin time (PT), standardized international ratio (INR), fibrinogen, antithrombin III (ATIII), coagulation factor II (FII), VII (FVII), IX (FIX), and X (FX). Fibrinogen concentrations were determined using a modified Clauss method on the BCS system (Dade Behring, Deerfield, IL, USA). Concentrations of coagulation factor were determined using the one-stage clotting assay in individual factor deficient plasma (R² Diagnostics, South Bend, IN, USA) according to prothrombin time (for factor II, VII, and X measurements) and activated partial thromboplastin time (for factor IX measurement) on the BCS system. ATIII was determined using a chromogenic method in the presence of thrombin excess on the BCS system. Haematocrit and platelet count were measured by Coulter® A’T Series Analyser (Coulter Corporation, Miami, FL, USA).

**Effects of dilution on thrombin generation**

The calibrated automated thrombin generation (Thrombinscope®) assay was used to evaluate thrombin generation in diluted and undiluted whole blood. Thrombin generation experiments were performed with 80 \(\mu\)l of PPP in each well, 20 \(\mu\)l of 5 pM PPP reagent, and 20 \(\mu\)l of substrate-calcium chloride buffer. Thrombin generation reagents were obtained from Diagnostica Stago (Parsippany, NJ, USA), and were used according to manufacturer’s directions as described previously. Thrombin generation experiments were carried out in duplicate using round-bottom 96-well Microfluor-2 black plates (Thermo Labsystems, Franklin, MA, USA). A thrombin calibrator with a constant, known thrombin-like activity (Thrombinscope, B.V., Maastricht, The Netherlands) was always used to eliminate the signal differences as a result of light absorption characteristics of different plasmas, inner filter effects, and non-linearity of the emission signal. The 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 and the acquired data were automatically processed by commercially available Thrombinscope software (Thrombinscope, B.V.) that displays the progress of reaction and calculates the lag time (in min) and the peak thrombin concentration (in nM) by continuous comparison with the signal generated in thrombin calibrator wells.
Haemodilution and fibrinogen level

**Effects of dilution and fibrinogen replacement on thromboelastometry**

Eleven thromboelastometric measurements were simultaneously conducted using three thromboelastometry analysers (ROTEM™; Pentapharm, Munich, Germany). All measurements were repeated twice at 37°C using 300 μl diluted or undiluted WB after recalcification with 20 μl of 0.2 M CaCl₂ and activation with 10 μl of tissue thromboplastin (EXTEM®, Pentapharm, Munich, Germany). Thromboelastometry measures the viscoelastic development from thrombin-mediated fibrin polymerization and platelet activation. Thromboelastometry is characterized by specific variables described earlier. Using this technique we collected the following parameters (Fig. 1): (i) clot formation time (CFT), which corresponds to coagulation time (in s); (ii) clot formation time (CFT), which corresponds to coagulation time (in s) and reflects the rate of fibrin polymerization; (iv) maximal clot firmness (MCF), which refers to the maximal amplitude (in mm) of the tracing and reflects the tensile strength of thrombus; and (v) A10, which reflects the amplitude at 10 min. In the samples with added tPA additional parameters were evaluated: (i) lysis index at 30 min (LI30), which describes the percentage of clot firmness remaining after 30 min in relation to maximum measured clot firmness; (ii) lysis onset time (LOT) defined as the time needed for clot firmness to decrease by 15% of MCF (in s); and (iii) lysis time (LT) defined as the time needed for clot firmness to decrease by 90% of MCF (in s).

**Data analysis**

All experiments had n=6 per condition, as this number of experiments is typically required to obtain a β≥0.8 and an α≤0.05 for most thromboelastographic variables as demonstrated in previous *in vitro* investigations. Data were expressed as mean (sd) after testing by Kolmogorov–Smirnov statistics for normal distribution.

Haematology and coagulation parameters before and after dilution were compared using paired *t*-test. Serial data for increasing fibrinogen concentration were evaluated by repeated-measures analysis of variance (ANOVA), followed by paired *t*-test with Bonferroni correction (SPSS®, SPSS Inc., Chicago, IL, USA). A *P*-value of <0.05 was considered significant. For the concentration–response curves that saturated, the concentrations of fibrinogen inducing 50 and 90% of the maximum effect (EC50 and EC90, respectively) were calculated using logistic curve fitting equation:

\[
E = E_0 + \left[ (E_{\text{max}} - E_0) \times C^\gamma \right] / (C^\gamma + EC50^\gamma)
\]

where *E* is the response, *E₀* is the initial response, *Eₘₐₓ* is the maximal response, *C* is the fibrinogen concentration, and *γ* is the slope parameter (Sigma Plot®, Systat Software Inc., San Jose, CA, USA).

**Results**

**Effects of dilution on platelet count and coagulation factors**

Baseline values of haematocrit, platelet count, and coagulation factors were within normal ranges. They were all significantly decreased by dilution (each *P*≤0.003). Mean change in platelet count and coagulation factors ranged from 74 to 85% (Table 1).

**Effects of dilution on thrombin generation**

Peak of thrombin generation decreased from 433 (sd 117) nM at baseline to 190 (63) nM in diluted plasma [mean reduction of 56 (7)%; *P*<0.001]. There was no significant change in lag time of thrombin generation, 2.7 (0.4) min in undiluted and 2.5 (0.4) min in diluted plasma [mean reduction of 13 (13)%; *P*=0.525]. A representative

<table>
<thead>
<tr>
<th>Table 1 Measured coagulation parameters in diluted and undiluted whole blood. Values are mean (sd). <em>P</em>-values were obtained by paired <em>t</em>-test. **International normalized ratio (INR) was not available (NA) in diluted whole blood.</th>
<th>Undiluted</th>
<th>Diluted</th>
<th>% Decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (g litre⁻¹)</td>
<td>15.9 (2.4)</td>
<td>9.1 (0.3)*</td>
<td>42 (10)</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>42.2 (7.6)</td>
<td>23.8 (0.8)*</td>
<td>42 (12)</td>
</tr>
<tr>
<td>Platelet count (×10⁹ ml⁻¹)</td>
<td>262 (63)</td>
<td>52 (15)*</td>
<td>80 (4)</td>
</tr>
<tr>
<td>Prothrombin time (s)</td>
<td>12.1 (0.6)</td>
<td>NA**</td>
<td>NA**</td>
</tr>
<tr>
<td>INR</td>
<td>1.0 (0.1)</td>
<td>NA**</td>
<td>NA**</td>
</tr>
<tr>
<td>Fibrinogen (mg dl⁻¹)</td>
<td>384 (75)</td>
<td>70 (11)*</td>
<td>81 (4)</td>
</tr>
<tr>
<td>Antithrombin III (%)</td>
<td>106 (14)</td>
<td>18 (2)*</td>
<td>82 (3)</td>
</tr>
<tr>
<td>Factor II (%)</td>
<td>115 (17)</td>
<td>25 (1)*</td>
<td>78 (4)</td>
</tr>
<tr>
<td>Factor VII (%)</td>
<td>107 (25)</td>
<td>16 (4)*</td>
<td>85 (3)</td>
</tr>
<tr>
<td>Factor IX (%)</td>
<td>90 (18)</td>
<td>23 (5)*</td>
<td>74 (5)</td>
</tr>
<tr>
<td>Factor X (%)</td>
<td>113 (16)</td>
<td>19 (4)*</td>
<td>83 (3)</td>
</tr>
</tbody>
</table>
thrombin generation curve in diluted and undiluted PPP is depicted in Figure 2.

**Effects of dilution and fibrinogen replacement on thromboelastometry**

Adding fibrinogen led to a concentration-dependent improvement of all thromboelastographic parameters ($P<0.001$ by ANOVA, Fig. 3). These improvements were significant in all parameters even with the lowest concentration of fibrinogen ($50 \text{ mg dl}^{-1}$) except for angle ($\alpha$) and A10 requiring $100 \text{ mg dl}^{-1}$.

Angle increased from $42 (6)^\circ$ to $78 (4)^\circ$ and became comparable with undiluted WB sample [$73 (3)^\circ$] when fibrinogen $\geq 150 \text{ mg dl}^{-1}$ was added. Using $\alpha$, EC50 and EC90 were calculated to be $125 (5) \text{ mg dl}^{-1}$ and $182 (7) \text{ mg dl}^{-1}$ fibrinogen, respectively (Fig. 3).

A10 and MCF increased from $23 (3)$ to $42 (3)$ mm and from $31 (3)$ to $52 (3)$ mm, respectively, but never normalized when compared with undiluted WB sample [$54 (5)$ mm and $60 (4)$ mm, respectively], even when fibrinogen was added in the highest concentration (300 mg dl$^{-1}$).

CT and CFT decreased concentration dependently from $121 (24)$ to $66 (13)$ s and from $465 (116)$ to $117 (30)$ s, respectively. Addition of $50 \text{ mg dl}^{-1}$ or more of fibrinogen to diluted WB corrected the CT ($72 (8)$ s), but at least $300 \text{ mg dl}^{-1}$ was required to correct CFT back to undiluted WB values [86 (21) s]. Using CFT, EC50 was calculated to be $135 (40) \text{ mg dl}^{-1}$ fibrinogen.

**Effect of tPA on thromboelastometry**

Addition of tPA ($0.15 \mu\text{g ml}^{-1}$) to diluted WB samples reduced MCF significantly from $31 (8)$ to $8 (2)$ mm, a 75% decrease ($P<0.001$). Similarly, addition of tPA to the samples with replaced fibrinogen, 150 and 300 mg dl$^{-1}$, reduced MCF from $45 (2)$ to $21 (2)$ mm, a 53% decrease ($P<0.001$) and from $52 (3)$ to $31 (5)$ mm, a 40% decrease ($P<0.001$), respectively. On the other hand, addition of tPA to undiluted WB had no effect on MCF values [60 (4) mm vs 57 (3); $P=0.164$]. Also other lysis parameters were improved but not normalized by fibrinogen. LI30, LOT, and LT were 1 (1)%, 224 (64) s, and 442 (221) s ($P<0.001$ vs WB) in diluted WB, 1 (2)%, 413 (71) s, and 616 (142) s ($P<0.001$ vs whole blood) when fibrinogen
Discussion

In this in vitro haemodilution model, which simulates massive transfusion of crystalloid fluids and RBC concentrates, we demonstrate that the replacement of fibrinogen concentration dependently improved thromboelastometry-derived clotting parameters. Our data suggested that the rate of clot formation is optimized only after increasing fibrinogen concentration to over 200 mg dl$^{-1}$. Nevertheless, in the presence of fibrinolytic stimuli (tPA), fibrinogen-supplemented clot remained more prone to fibrinolysis when compared with the (pre-dilution) baseline.

Dilutional coagulopathy occurs after massive haemorrhage in trauma and surgery, and it adversely affects morbidity and mortality. Conventional haemostatic therapy consists of allogeneic blood products [e.g. fresh frozen plasma (FFP), platelet concentrates], but pasteurized fibrinogen concentrate has been reported to be efficacious for this indication. However, there is no standardized dosage for fibrinogen concentrate as it was previously administered between 30 and 250 mg kg$^{-1}$ (2.0–17.5 g for 70 kg person) in a porcine model of dilutional coagulopathy, different major surgical procedures, and after massive haemorrhage. Therefore, we decided to evaluate various fibrinogen concentrations in diluted whole blood samples after volume replacement with normal saline and RBC concentrates (target haematocrit, 23–25%). The latter reflects the common practice of using RBCs as a first-line therapy in major bleeding, and a potential influence of RBCs on coagulation. After haemodilution, concentrations of platelet, antithrombin, FII, FIX, and FX were decreased to approximately one-fifth of the baseline. Resultant coagulopathic state is reflected in out-of-range PT values, delayed clot formation ($\alpha$<45º) and reduced clot strength (MCF<40 mm) on thromboelastometry. The $\alpha$-angle represents the rate of thrombin-dependent fibrin polymerization. This parameter would be useful in determining the optimal concentration of fibrinogen (substrate) when thrombin (enzyme) activity is presumably limited by low factor concentrations. The concentration–response relationship of fibrinogen and $\alpha$ angle demonstrated that EC50 and EC90 of added fibrinogen were 125 and 182 mg dl$^{-1}$, respectively. Taking into account the pre-treatment value of 70 mg dl$^{-1}$ and EC50, we suggest that minimal fibrinogen concentrations should be about 200 mg dl$^{-1}$ to improve clot formation and about 250 mg dl$^{-1}$ to fully optimize coagulation. Our finding is in agreement with a previous in vitro study in fibrinogen-deficient plasma, demonstrating that thromboelastometric parameters were normalized only after fibrinogen concentration was increased to more than 250 mg dl$^{-1}$. However, our present model is more reflective of clinical coagulopathy because it takes into account the physiological patterns of fibrin polymerization, and its interaction with residual platelets in diluted whole blood. The recommended fibrinogen concentration for isolated (congenital) fibrinogen deficiency is 100 mg dl$^{-1}$, and similar concentrations of 80–100 mg dl$^{-1}$ are adapted in the general guidelines for fibrinogen replacement. Previous studies suggested that even a lower concentration (50 mg dl$^{-1}$) would be sufficient for prevention of microvascular bleeding in patients after massive transfusion or undergoing invasive procedure. However, recent clinical studies indicated that plasma fibrinogen concentrations only higher than 200 mg dl$^{-1}$ were beneficial for haemostasis in patients undergoing cardiac surgery and in patients with severe postpartum haemorrhage. Furthermore, the importance of replacing fibrinogen is supported by two other studies that included patients undergoing normovolaemic haemodilution or surgery with high perioperative blood loss, showing that fibrinogen was

Fig 4 Representative traces of fibrinolytic effects of tissue plasminogen activator (tPA) on clot firmness. (A) Effect of increasing fibrinogen replacement in diluted whole blood (WB) on thromboelastometric traces. (B) Effects of adding tPA in the same sample. Adding fibrinogen weakens but does not normalize the fibrinolytic effect of tPA on clot firmness.

150 mg dl$^{-1}$ was added, 5 (8%), 565 (153) s, and 983 (589) s ($P<0.001$ VS WB) with replacement of fibrinogen 300 mg dl$^{-1}$, and 65 (37%), 1805 (440) s, and 2565 (688) s for undiluted WB. Representative thromboelastometry traces with and without tPA are depicted in Figure 4.
the first clotting factor that reaches critical plasma concentration only after a blood loss of about 150%. On the other hand, critical concentrations of clotting factors II, VII, IX, and X (≤20% of normal) were only reached after exchange of 75% of blood volume in a porcine model, and after massive transfusion beyond 2 to 2.5 times the blood volume in a human.3

The concentration-dependent recovery of all thromboelastometric variables in our study supports that fibrinogen deficiency is the primary problem associated with haemodilution, and fibrinogen replacement should be considered as a first therapy step in dilutional coagulopathy to optimize the activity of locally produced thrombin by replacing its substrate (e.g. fibrinogen).6,7 Severely decreased fibrin formation and massive reduction of coagulation factors as a result of the extent of haemodilution observed in this study correspond to the loss of about two blood volumes in vivo.2,3 Despite low procoagulant factor concentrations (16–25%), endogenous thrombin generation was only modestly decreased (56% reduction). Therefore, sufficient thrombin is available for the enzymatic conversion of fibrinogen to fibrin, thus explaining why replacing fibrinogen is effective in restoring haemostasis in dilutional coagulopathy. The only modest decrease in thrombin generation is presumably because of reduced ATIII (i.e. prolonged thrombin activity). In agreement, we have previously demonstrated that thrombin activity is prolonged after haemodilution in patients undergoing cardiac surgery because of critically low ATIII concentrations.26 Low anticoagulant function might even render coagulopathic patients more susceptible to systemic thrombotic complications from any procoagulant intervention. Although chronic elevation of fibrinogen (>400 mg dl⁻¹) is considered to be a risk factor for increased cardiovascular events, fibrinogen, an acute phase reactant, appears to be an important marker (rather than a cause) of severe atherosclerosis and inflammatory state.23 Replacement of up to 300 mg dl⁻¹ fibrinogen (corresponding to about 8–9 g in a patient weighing 70 kg) did not result in supra-normal (e.g. hypercoagulable) thromboelastometric parameters in this study. In agreement, no hypercoagulability on thromboelastometry or in vivo thromboembolic events were reported after high-dose fibrinogen replacement (250 mg kg⁻¹) in a porcine haemodilution model.6 In addition, elevated fibrinogen concentration per se does not seem to increase the risk of thrombotic occlusion of anastomosed vessels (0.5–1.5 mm in diameter) in a rodent model.28 In various surgical procedures with dilutional coagulopathy, fibrinogen doses up to 200 mg kg⁻¹ have been clinically used.4,5,20,21 The risk of overdosing fibrinogen may be lowered by increased consumption of fibrinogen during haemorrhage and haemodilution.29

In previous studies, thromboelastometric variables, particularly MCF, were viewed as the evidence of recovered haemostasis.6,7,11 However, endogenous fibrinolytic response needs to be considered for the stability of fibrin clot.12,30,31 The release of tPA from endothelium can be triggered during and after surgery by thrombin, bradykinin, complement C5a, epinephrine, vasopressin, and other stimuli.32 We demonstrated that fibrin clot became more prone to tPA-induced fibrinolysis after haemodilution, and antifibrinolytic function of clot was not fully restored even after the highest concentration of fibrinogen. Several mechanisms contribute to the profibrinolytic state in haemodilution. The activity of tPA is not antagonized by reduced plasminogen activator inhibitor-1 (PAI-1) as a result of haemodilution. Fibrin is rendered more susceptible to fibrinolysis by decreases in FXIII, α₂-antiplasmin, and thrombin activable fibrinolysis inhibitor (TAFI). Because thrombin-activated FXIIIa fibrin cross-links reduced α₂-antiplasmin and TAFI to fibrin, reduced concentrations of these factors may increase local plasmin activity, activation or both.33,34 In addition, thrombocytopenia exacerbates fibrinolytic tendency because platelets contain FXIII and PAI-1 in their cytoplasm.34 Taken together, antifibrinolytic therapy, in addition to fibrinogen, may be necessary in stabilizing haemostatic fibrin clot after major haemorrhage and haemodilution.

After major blood loss, FFP is frequently administered with an intention to improve proposed ‘haemostasis’. However, the recommended FFP dosage1 of 10–15 ml kg⁻¹ only increases fibrinogen by 40 mg dl⁻¹, whereas 30 ml kg⁻¹ of FFP increases fibrinogen by 100 mg dl⁻¹.35 Thus, it is extremely difficult to increase fibrinogen concentrations without volume overload (i.e. further dilutional coagulopathy). Cryoprecipitate provides more concentrated fibrinogen (about 150–250 mg per 10 ml), but it is not available in a pathogen-inactivated form. In this regard, pasteurized fibrinogen concentrate is more preferred for low volume requirement (2 g in 50 ml), and low infectious risks.

In conclusion, our in vitro model demonstrated that plasma fibrinogen concentration above 200 mg dl⁻¹ would be necessary to improve the rate of fibrin polymerization (i.e. α angle) on thromboelastometry. Furthermore, the fibrin clot remains prone to fibrinolysis even when fibrinogen is supplemented after haemodilution. Although our conclusion based on in vitro haemodilution cannot be directly inferred to clinical situations, our dilution model closely simulates coagulopathy after acute loss of two blood volumes.7 Future clinical studies are required to prospectively evaluate the optimal concentration of fibrinogen in vivo, and the safety of combination with antifibrinolytic agents in the management of major haemorrhage and severe haemodilution.

Funding
This study was supported in part by a Myron B. Laver grant, Department of Anesthesia, University of Basel, Switzerland (D.B.) and by CSL Behring, Marburg, Germany (K.A.T.).
References


14 Eisenberg JM, Clarke JR, Sussman SA. Prothrombin and partial thromboplastin times as preoperative screening tests. *Arch Surg* 1982; 117: 48–51


27 Reinhart WH. Fibrinogen-marker or mediator of vascular disease? *Vasc Med* 2003; 8: 211–6


33 Valnickova Z, Enghild JJ. Human procarboxypeptidase U, or thromboplastin activator, is a substrate for transglutaminase-catalyzed cross-linking to fibrin. *J Biol Chem* 1998; 273: 72720–4
