# Increased thrombin generation and fibrinogen level after therapeutic plasma transfusion: Relation to bleeding

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**Summary**

In a clinical setting, fresh frozen plasma (FFP) is transfused to diluted patients with complicated surgery or trauma, as guided by prolonged conventional coagulation times or low fibrinogen levels. However, the limited sensitivity of these coagulation tests may restrict their use in measuring the effect of transfusion and hence predicting the risk of perioperative bleeding. We used the more sensitive, calibrated automated thrombogram (CAT) method to evaluate the result of therapeutic FFP transfusion to 51 patients with dilutional coagulopathy. Thrombin generation was measured in pre- and post-transfusion plasma samples in the presence of either platelets or phospholipids. For all patients, the transfusion led to higher plasma coagulation factor levels, a shortened activated partial thromboplastin time, and a significant increase in thrombin generation (peak height and endogenous thrombin potential). Interestingly, thrombin generation parameters and fibrinogen levels were higher in post-transfusion plasmas from patients who stopped bleeding (n=32) than for patients with ongoing bleeding (n=19). Plasmas from 15 of the 19 patients with ongoing bleeding were markedly low in either thrombin generation or fibrinogen level. We conclude that the thrombin generation method detects improved haemostatic activity after plasma transfusion. Furthermore, the data suggest that thrombin generation and fibrinogen are independent determinants of the risk of perioperative bleeding in this patient group.

**Keywords**

Bleeding, dilutional coagulopathy, fibrinogen, platelets, thrombin generation

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## Introduction

Patients with severe blood loss due to complicated surgery or trauma are commonly transfused with citrate-diluted fresh frozen plasma (FFP). This intervention, usually in combination with red cell and platelet transfusions, aims to effectively replace the loss of coagulation factors in a diluted patient and then to improve the haemostasis. Hence, the FFP serves to counteract the (surgical) dilutional coagulopathy and to contribute to the halt of bleeding. However, recent papers have debated the efficacy of prophylactic FFP transfusion upon surgery and trauma (1, 2). Several practical and theoretical restrictions have emerged. One of these is that the coagulant factors in plasma are likely to loose function during the freezing, storage, transportation and defrosting procedures. Another limitation is that FFP preparations are themselves diluted with citrate, implicating that transfusion may even increase dilution of patients (3). Furthermore, FFP transfusion may cause adverse side reactions such as immune responses, fluid overload and citrate toxicity (4).

Current guidelines recommend the (prophylactic) transfusion of FFP to patients treated with crystalloids or colloids, who show overt bleeding and/or an activated thromboplastin time (aPTT) or prothrombin time (PT) that is prolonged by >1.5 times the normal value (5). The efficacy of the transfusion reaction is evaluated from the same conventional coagulation tests. Unfortunately, these tests are notoriously insensitive at hypocoagulant conditions, which limits their suitability for this particular group of patients (6). The practical consequence is that current decisions on the employment of FFP to treat patients with perioperative bleeding are to a large extent based on expert opinion and personal experience, rather than on precise test outcome. It is therefore important to search for alternative, more

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sensitive methods to determine the need and efficacy of transfusion, and to predict the risk of ongoing bleeding in patients with dilutional coagulopathy.

One of such tests may be provided by rotational thromboelastography, which measures the extent and quality of fibrin clot formation. The test outcome is partly but not exclusively determined by the plasma fibrinogen level (7, 8). Another sensitive and perhaps suitable method is the calibrated automated thrombogram (CAT) test, in which the rate and extent of thrombin generation are continuously measured, following triggering of plasma with tissue factor. Earlier work shows that this method can detect reduced coagulant activity under conditions as diverse as hemophilia, treatment with anticoagulant medication, and dietary intervention with fish oil (9–11).

For this report, we analyzed plasma samples from 51 patients with dilutional coagulopathy upon surgery, who received FFP to prevent ongoing bleeding. We hypothesized that bleeding in these patients can be a consequence of impaired haemostasis due to (partial) deficiency in one or two different processes: the rate/amount of thrombin generation and the extent of fibrin clot formation. To investigate this, we measured the levels of coagulation factors including fibrinogen and used the CAT test to measure thrombin generation (in the presence of phospholipids or platelets) both before and after the transfusion. By comparing the test results with the clinical outcome of the intervention (stopped or ongoing bleeding), we subsequently searched for threshold levels of these processes above which bleeding is less likely to occur.

## Materials and methods

### Patients and blood collection

Plasma samples were collected from 51 patients, who received transfusion with FFP in the academic hospital within a 12-months period (Table 1). The patients were transfused either during major surgery (abdominal or spinal bone surgery) or postoperatively in the intensive care unit (after cardiothoracic or aortic surgery). During or prior to surgery, all patients had previously been transfused with crystalloids and erythrocytes (at least 2 bags of each). None of the patients had multiple-organ failure, sepsis, liver or renal dysfunction. Patients had not received medication that interfered with platelet activation or coagulation.

Most of the patients were transfused with two bags of 300 ml FFP. Only those with severe bleeding received four bags (14 patients), as considered appropriate by the anesthesiologist. The donated FFP was prepared from normal pool plasma of healthy volunteers according to the European guidelines for quality of blood components (12). Based on international guidelines (13, 14), the criteria for transfusion were: aPTT ≥40 seconds (s) (31 patients), plasma fibrinogen concentration <2.0 g/l (32 patients), and/or massive bleeding during or after surgery (29 patients). Nineteen of the 51 patients had ongoing bleeding after FFP transfusion. Within 1 hour, these subjects were transfused again with FFP, red cell or platelet concentrates, as considered appropriate by the anesthesiologist.

Blood (10 ml) was collected from the patients within 30 minutes (min) before and within 30 min after the transfusion with FFP; this blood was used for the clinical assessment of haemostasis. Remnant blood, anticoagulated with sodium citrate (1/10 volume, 129 mM), was used in the research laboratory to prepare platelet-free plasma by centrifugation (twice at 2,630 g for 10 min). Patient plasma was snap-frozen at –80°C, and was later used for analysis. The study was approved by the local Medical Ethics Committee.

**Table 1: Characteristics of patients and conditions of the FFP transfusion.** Means ± SD.

| Patient characteristic | Variable |
|---|---|
| Male / female(n) | 26 / 25 |
| Age (years) | 62 ± 13 |
| Transfusion during surgery (n=29) | |
| abdominal surgery | 24 |
| spinal back bone surgery | 5 |
| Transfusion in intensive care unit (n=22) | |
| Cardiothoracic/aortic surgery | 22 |
| Number of FFP bags transfused | 2.6 ± 0.9 |

### Materials

Apyrase and bovine serum albumin (BSA) were from Sigma (St. Louis, MO, USA). Innovin was from Dade Behring (Marburg, Germany); Z-Gly-Gly-Arg aminomethyl coumarin (Z-GGR-AMC) from Bachem (Bubendorf, Switzerland). Human thrombin calibrator and thrombogram software were supplied by Synapse (Maastricht, the Netherlands) (15). Phospholipid vesicles (phosphatidylserine : phosphatidylcholine : phosphatidylethanolamine, 1 : 3 : 1, mol/mol) were prepared as described (16). The PT was measured using Innovin as trigger, and the aPTT was measured with the actin FSL kit (Dade Behring). Fibrinogen levels were measured as described (11). The Behring coagulation system (Dade Behring) was used to measure prothrombin levels in a one-stage clotting assay; and in this system antithrombin levels were measured with a chromogenic assay. Heparin in plasma was determined as anti factor Xa activity using the Coamatic heparin test (Chromogenics, Mölndal, Sweden).

### Isolation of donor platelets

Blood from healthy donors was drawn with a 1.2-mm needle, dripping freely into open tubes and collected into 1/6 volume of acid-glucose solution (ACD, 80 mM trisodium citrate, 52 mM citric acid and 180 mM glucose). Donors gave full informed consent. Blood samples were centrifuged at 240 g for 15 min to obtain platelet-rich plasma (PRP). After addition of 10% ACD (acid-citrate-dextrose) solution and apyrase (0.1 unit/ml ADPase), PRP was centrifuged at 2,100 g for 2 min, and platelets were collected. Platelets were resuspended in Hepes buffer pH 7.45 (10 mM Hepes, 136 mM NaCl, 2.7 mM KCl, 2 mM $MgCl_2$, 0.1% glucose and 0.1% BSA) (17). Platelet count was determined with a thrombocounter (Coulter Electronics, Luton, UK). Normal pooled plasma was obtained from 40 healthy donors, following guidelines of the University Hospital Maastricht.

**Table 2: Haematological variables of 51 patients before and after FFP transfusion.** Data are means ± SD; ***p<0.001 compared to pre-transfusion.

| | Before transfusion | After transfusion | Normal range |
|---|---|---|---|
| Platelets (× $10^9$ $L^{-1}$) | 84 ± 43 | 85 ± 29 | 130–350 |
| Haematocrit | 0.26 ± 0.06 | 0.26 ± 0.05 | 0.36–0.52 |
| aPTT (s) | 62 ± 32 | 41 ± 12 *** | 23–32 |
| PT (s) | 16 ± 3 | 13 ± 2 *** | 10–13 |
| Prothrombin (% control plasma) | 37 ± 13 | 48 ± 14 *** | 100 |
| Antithrombin (% control plasma) | 37 ± 10 | 49 ± 13 *** | 100 |
| Fibrinogen (g $L^{-1}$) | 1.3 ± 0.6 | 1.8 ± 0.9*** | 1.7–4.0 |

## Thrombin generation measurements

Patient platelet-free plasma was reconstituted with donor platelets (100 × $10^9$ platelets/l) or with phospholipid vesicles (4 μM), and then preincubated with tissue factor (10 pM, all final concentrations). After an incubation time of 10 min, coagulation was initiated with $CaCl_2$ and thrombin substrate Z-GGR-AMC. Thrombin generation was continuously measured at 37°C, as described (15, 17). Briefly, 80 μl of plasma containing donor platelets or phospholipids were pipetted into the wells of a polystyrene 96-wells plate. Coagulation was started by adding 20 μl of Z-GGR-AMC (2.5 mM), dissolved in Hepes buffer pH 7.35 (20 mM Hepes, 140 mM NaCl, 100 mM $CaCl_2$ and 60 mg/ml BSA). After shaking for 10 s, fluorescence from cleaved AMC was measured at excitation and emission wavelengths of 368 and 460 nm, respectively. In separate wells, human thrombin calibrator was added to each type of plasma to obtain reference thrombin curves. Samples were run at least in triplicate. First-de-

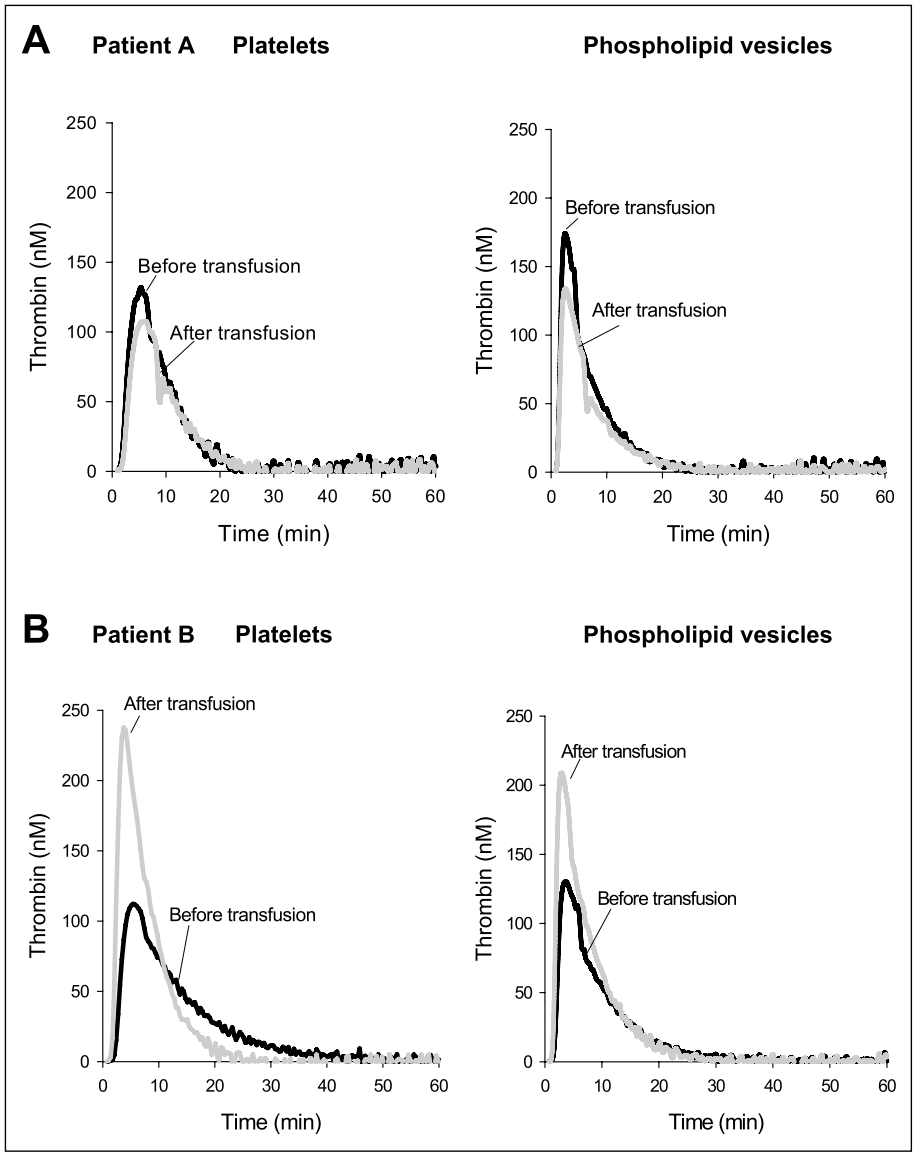


**Figure 1: Inter-patient difference in effect of FFP transfusion on thrombin generation.** Thrombin generation was measured in plasmas of two patients, who were transfused with two bags of FFP during surgery. Pre- and post-transfusion plasma samples were supplemented with donor platelets (100 × $10^9$/l) or phospholipids (4 μM). Coagulation was induced with 10 pM tissue factor plus 16.6 mM $CaCl_2$ (final concentrations). Thrombin generation was measured using the CAT method.
A) Thrombogram of plasma from patient *A* with ongoing bleeding after transfusion. Traces are given before (black) and after (grey) transfusion; aPTT was 150 and 51 seconds (s), and fibrinogen concentration was 0.6 and 1.1 g/, respectively. B) Thrombogram of plasma from patient *B*, who stopped bleeding after the transfusion. Traces are given before (black) and after (grey) transfusion; aPTT was 73 and 41 s, and fibrinogen concentration was 1.3 and 1.9 g/l, respectively.

**Table 3: Effect of FFP transfusion on thrombin generation parameters. Thrombin generation was measured in patient plasmas in the presence of donor platelets or phospholipids (Fig. 1).** Subjects were divided in patients transfused during surgery or when recovering in the intensive care unit (ICU). Normal values of thrombogram parameters were determined in parallel using pooled plasma from 40 healthy volunteers: thrombin peak height of 225 (platelets) and 352 (phospholipids) nM; ETP of 1,470 (platelets) and 1,245 (phospholipids) nM × min. Data are means ± SD; transfusion effect (%) is given within brackets. *p<0.05, **p<0.01, ***p<0.001 compared to pre-transfusion.

| | All patients (n=51) | Surgery (n=29) | ICU (n=22) |
|---|---|---|---|
| **Thrombogram parameters before and after transfusion** | | | |
| Peak height with platelets (nM) | | | |
| Before transfusion | 112 ± 37 | 112 ± 36 | 111 ± 38 |
| After transfusion | 131 ± 43 *** *(+17%)* | 130 ± 45 * *(+16%)* | 132 ± 41 ** *(+19%)* |
| ETP with platelets (nM × min) | | | |
| Before transfusion | 1,136 ± 326 | 1,208 ± 394 | 1,196 ± 435 |
| After transfusion | 1,312 ± 377 ** *(+15%)* | 1,323 ± 459 *(+10%)* | 1,361 ± 364 * *(+14%)* |
| **Thrombogram parameters before and after transfusion** | | | |
| Peak height with phospholipids (nM) | | | |
| Before transfusion | 157 ± 42 | 151 ± 40 | 166 ± 44 |
| After transfusion | 190 ± 53 *** *(+21%)* | 182 ± 48 ** *(+21%)* | 201 ± 58 *** *(+21%)* |
| ETP with phospholipids (nM × min) | | | |
| Before transfusion | 994 ± 248 | 952 ± 235 | 914 ± 164 |
| After transfusion | 1,126 ± 267 ** *(+13%)* | 1,086 ± 231 *(+14%)* | 1,035 ± 134 *(+13%)* |

rivative traces were directly converted into nanomolar concentrations of thrombin, as described (15). In the resulting thrombograms, thrombin peak height (a measure of the rate of thrombin formation) and endogenous thrombin potential (ETP, i.e. area-under-the-curve [AUC] representing the integrated amount of thrombin activity) were used as principal parameters (18). Control experiments showed that triggering with 10 instead of 5 pM resulted in a 15% higher thrombin peak height. The assay variation coefficient was 5–7%.

### Statistics

A paired sample t-test was used to compare transfusion effects; the independent sample t-test was used for comparing patients groups (SPSS 11.0 software). Data are presented as means ± SD.

## Results

The effect was studied of plasma transfusion (FFP) to 51 patients, who were bleeding or at risk of bleeding (Table 1). Of the patients, 29 were transfused during abdominal or spinal back bone surgery. The 22 other patients were subjected to cardiothoracic/aortic surgery, and they received FFP while staying in the intensive care unit. Nineteen of the 51 patients experienced ongoing bleeding after the transfusion. Platelet count and haematocrit in the patient's blood were below normal, as a consequence of earlier surgery and dilution with crystalloids; these values did not increase upon FFP transfusion (Table 2). Further measurements were performed with isolated plasma samples. When comparing the plasmas from all 51 patients, transfusion led to shortening of the conventional coagulation times from 62 ± 32 to 41 ± 12 s (aPTT) and from 16 ± 3 to 13 ± 2 s (PT), which agrees well with published observations (19). The levels of prothrombin and antithrombin before transfusion were 37% of normal values (Table 2), demonstrating the hypocoagulant state of the patients. After transfusion, these levels increased to about 50%. Similarly, the low fibrinogen levels (mean 1.3 g/l) increased with 0.5 g/l following FFP transfusion.

Thrombogram curves, using optimized concentrations of either procoagulant phospholipids (4 μM) or platelets derived from one healthy donor ($100 \times 10^9$ platelets/l), were measured to assess the net transfusion effect on thrombin generation. The coagulation was triggered with optimal doses of tissue factor (10 pM) and $CaCl_2$ (16.6 mM). Typically, the plasmas from some patients failed to increase in thrombin generation after transfusion, regardless of whether phospholipids or platelets were present (Fig. 1A), whilst the plasmas from other patients were markedly increased in thrombin formed (Fig. 1B). This suggested considerable inter-patient variation to the intervention.

To obtain normal values, thrombin generation was also measured using pooled plasma from 40 healthy donors, in the presence of the same batches of tissue factor and phospholipids and with platelets from the same donor as for patient plasmas. In normal pooled plasma, thrombin peak heights were 225 (platelets) and 352 (phospholipids) nM, while ETP values were 1,470 (platelets) and 1,245 (phospholipids) nM × min. Comparison of the 40 individual normal plasmas gave variation coefficients (SD/mean) of 14% (peak height) and 11% (ETP).

Analyzing the pre-transfusion thrombograms from all 51 patients (with platelets or phospholipids), we found mean peak heights amounting to 45–50% of the normal value, and ETP values of 77–80% of normal (Table 3). After transfusion, thrombin peak heights were significantly increased in the presence of both platelets (+17%) and phospholipids (+21%); the transfusion effects on peak height with platelets and phospholipids were strongly correlated (Fig. 2). Post-transfusion samples showed also higher ETP values with platelets (+15%) and phospholipids (+13%) (Table 3). The effects on thrombin generation were similar for the patients who were transfused during surgery and those recovering in the intensive care unit (Table 3). Typically, in post-transfusion plasmas, the aPTT correlated with neither the thrombin peak height nor the ETP (p<0.37). Thus, in these patients with dilutional coagulopathy, the thrombin generation method detected transfusion-mediated changes in coagu-

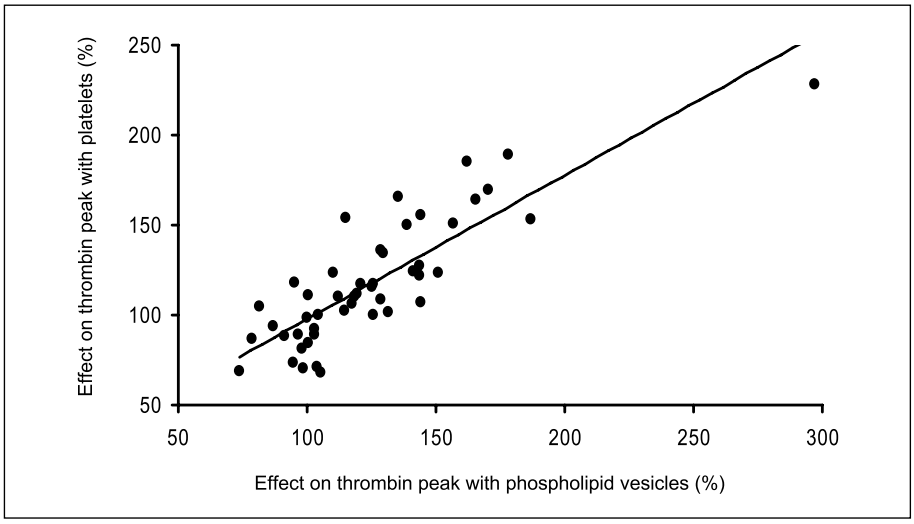


**Figure 2: Correlation of effects of FFP transfusion on thrombin generation in the presence of platelets or phospholipids.** Plasma samples from 51 patients were supplemented with platelets from one healthy donor ($100 \times 10^9$/l) or with phospholipids (4 μM). Thrombin generation was measured as in Figure 1. Shown is correlation plot of the transfusion effect on thrombin peak height in the presence of platelets or phospholipids (n=51, $R^2$=0.72, p<0.01).

lation in a more sensitive way than the aPTT. This in spite of the increase in procoagulant and anticoagulant factors.

Based on the relatively large changes in thrombin peak height relative to ETP, the former parameter was used for further analysis. Thrombogram data were compared for patients with stopped (n=32) and ongoing bleeding (n=19) after the FFP transfusion (Fig. 3). In post-transfusion plasma samples from only patients with ongoing bleeding (white dots), thrombin peak heights remained relatively low in 13/19 of the cases. Using an arbitrary cut-off point of ≤100 nM thrombin (i.e. 45% of the normal value), 8/12 samples below this cut-off were patients with ongoing bleeding (Fig. 3). Control measurements showed that only three plasma samples contained heparin traces (0.23–0.47 anti-factor Xa U/ml), i.e. 1 pre-transfusion and 2 post-transfusion samples (Fig. 3).

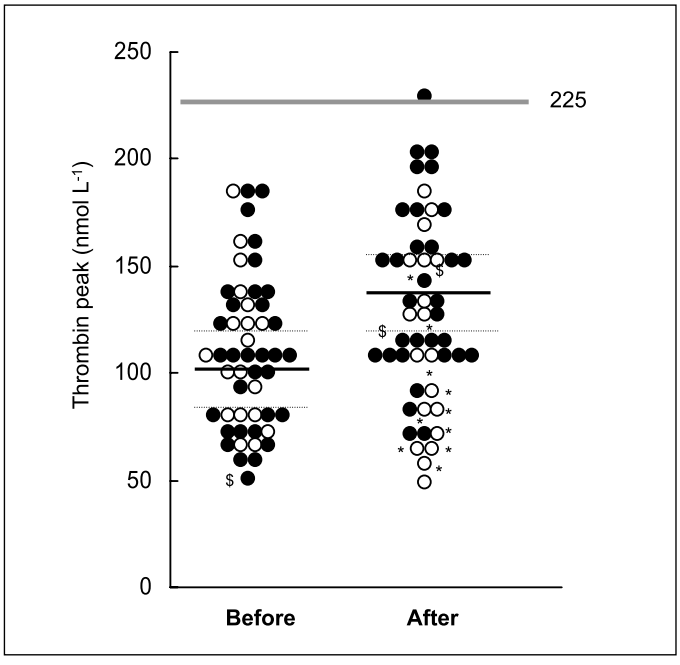


**Figure 3: Thrombin peak height in patient plasmas before and after FFP transfusion.** Thrombin generation was measured in plasma from 51 patients before and after transfusion with FFP. Donor platelets were present, as in Figure 1. Patients were characterized as experiencing stopped (black dots) or ongoing (white dots) bleeding after transfusion. Data are thrombin peak heights; reference value of normal pooled plasma is given as a horizontal bar. Dotted lines represent means ± SD. *Plasma with post-transfusion fibrinogen level ≥1.05 g/l; $plasma with heparin trace.

In comparison to the patients with stopped bleeding, thrombin peak heights were significantly lower for the bleeding patients (Fig. 4A, p<0.05). In addition, the transfusion-mediated increases in peak height were smaller (Fig. 4B, p<0.01). In contrast, conventional coagulation times were not different for the two groups: the post-transfusion aPTT was 41 ± 12 s and 49 ± 12 s for patients with stopped and ongoing bleeding, respectively (p=0.14).

Low plasma fibrinogen is a known risk factor for bleeding in dilutional coagulopathy (21). Theoretically, low fibrinogen levels may impair fibrin clot formation even if sufficient amounts of thrombin are generated. We found that the fibrinogen concentration after transfusion was relatively low in patients with ongoing bleeding (Fig. 5A). Similarly, the increase in fibrinogen upon transfusion was lower in this patient group (Fig. 5B). By setting an arbitrary cut-off of ≤1.1 g fibrinogen/l (i.e. 40% of the mean normal value), seven of the 19 bleeding patients remained below this level. Strikingly, these seven patients were non-identical to the eight bleeding patients with low thrombin generation (Fig. 3). These eight patients had a plasma fibrinogen level of 1.7 ± 0.6 g/, whereas the 11 other bleeding patients had a level of 0.9 ± 0.2 g/l (p=0.005). Taking together, the data indicate that ongoing bleeding in 15 out of 19 patients was accompanied by either a low thrombin generation or a low fibrinogen concentration.

In none of the patient groups, fibrinogen did correlate with the thrombin generation parameters (p>0.26). In agreement with earlier results (11), in-vitro tests using diluted plasma samples, also showed that the fibrinogen level was of only little effect on thrombin generation; thrombin peak height increased with 10% at a 1.5 g/l increase in fibrinogen. Thus, the outcome of the thrombin generation test was no more than moderately influenced by the fibrinogen content. Further regression analysis

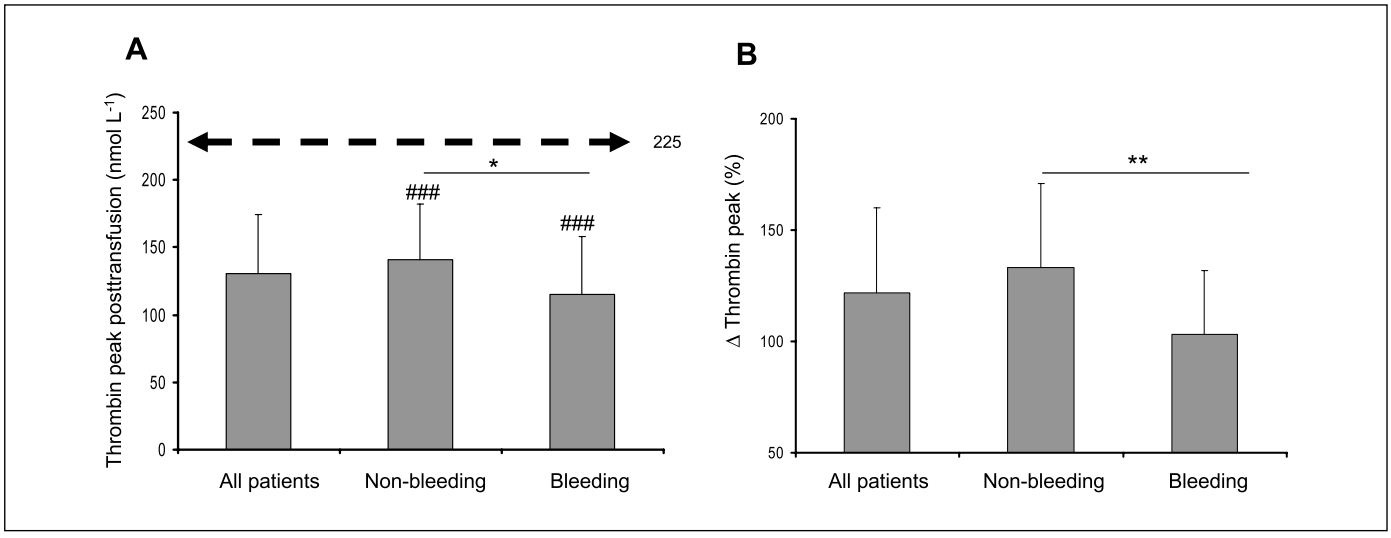


**Figure 4: Transfusion effect on thrombin peak height of patients with stopped or ongoing bleeding.** Thrombin generation was measured in patient plasmas supplemented with platelets from one healthy donor as in Figure 1. A) Thrombin peak height of post-transfusion plasmas from patients with stopped (n=32) or ongoing (n=19) bleeding. B) Change in thrombin peak height after transfusion. First bar shows value for normal pool plasma and the same platelet preparation is given separately. Difference between patient groups: ###p<0.001 compared to normal pool plasma; *p<0.05, **p<0.01 inter-group comparison. Dotted line represents normal value of thrombin generation.

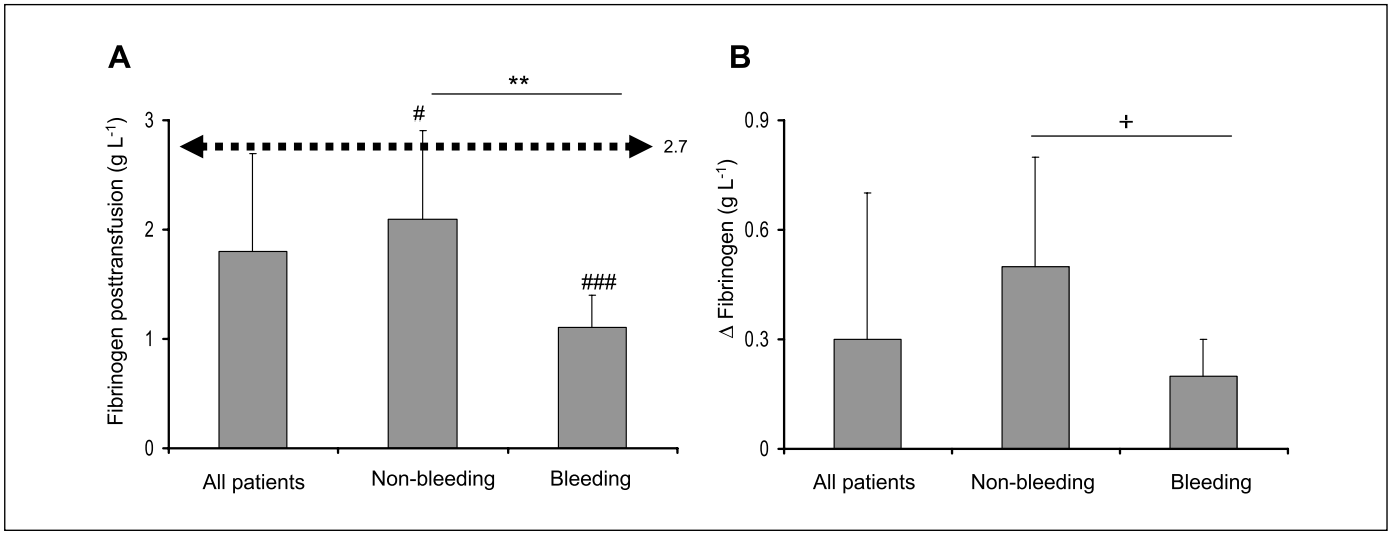


**Figure 5: Transfusion effect on fibrinogen concentration of patients with stopped or ongoing bleeding.** A) Fibrinogen levels in post-transfusion plasmas per patient group. B) Effect of transfusion on the fibrinogen level. First bar shows value for normal pool plasma. #p<0.05, ###p<0.001 compared to normal pool plasma; +p=0.05, **p<0.01inter-group comparison. Dotted line represents normal value of fibrin level.

showed that, for all 51 patients, the fibrinogen concentration correlated, but only poorly, with the aPTT ($R^2 = 0.15$, $p<0.013$). In plasmas from nine of the 32 patients with stopped bleeding and from five of the 19 patients with ongoing bleeding, the aPTT was >40 s, which confirmed that this test is of limited use in the prediction of ongoing bleeding after transfusion.

## Discussion

In this report, we used conventional and novel assays to evaluate the coagulant effect of FFP transfusion to 51 patients, who experienced dilutional coagulopathy. The transfusion resulted in a rise in procoagulant (fibrinogen, prothrombin) as well as anticoagulant (antithrombin) factors. This was accompanied by a shortening of the conventional coagulation times, particularly the aPTT, and increased thrombin generation curves. The increased thrombin generation was detected with either phospholipids or donor platelets as lipid surface; and assessed as a higher thrombin peak height and a higher ETP (AUC). For normal undiluted plasma it is known that prothrombin and antithrombin per-se have antagonistic effects on the coagulation process in general, and on the thrombin generation profile in par-

ticular (11, 20). The present results therefore point to an overriding effect of the positive contribution of procoagulant factors (prothrombin) relative to the negative contribution of anticoagulant factors (antithrombin). The biochemical explanation for this net procoagulant transfusion effect is unclear, since only little is known of the kinetics of the coagulation system in the diluted plasma samples.

When comparing the patients with stopped or ongoing bleeding after transfusion, we found marked differences in thrombin generation parameters. The thrombin peak height was significantly lower in plasma samples from the patients with ongoing bleeding. The same was true for the fibrinogen level, which also increased to a lesser degree in the bleeding patients. In contrast, the aPTT was not statistically different for the two groups, suggesting this assay lacks sensitivity compared to the other tests. The marked changes in thrombin peak height are well compatible with conclusions that this thrombogram parameter is an adequate sensor of the coagulant state of plasma (22, 23).

An intriguing observation was that eight out of 19 plasma samples from patients with ongoing bleeding were markedly low in thrombin peak height (≤100 nM), but not in fibrinogen level (>1.2 g/). Conversely, seven out of 19 plasma samples from different bleeding patients had higher thrombin peak heights (> 100 nM), but were lower in fibrinogen (≤1.1 g/l). These cases were well segregated from patients where bleeding stopped, who all had thrombograms and fibrinogen levels above these cut-off levels. Jointly, 15/19 (79%) of the patients with ongoing bleeding after transfusion showed either low thrombin generation or low fibrinogen. This analysis raises the suggestion that in these patients either insufficient thrombin generation or deficient fibrinogen, likely in an independent way, contributes to the haemorrhage. However, since also the surgery in these patients has contributed to the bleeding, one needs to be careful with drawing far-reaching conclusions. Also, the examined number of patients is still limited, which points to a need for larger-scale follow-up studies.

Even when taking this into account, our results do support the hypothesis that certain amounts of thrombin generation and, independently of this, certain levels of fibrinogen are required to suppress the bleeding in dilutional coagulopathy. This is further supported by recent animal studies, where supplementation of either prothrombin or fibrinogen concentrates were capable of preventing blood loss in experimental coagulopathy (24). Other support comes from thromboelastographic measurements, where fibrinogen was a key predicting variable in the assessment of clot formation in bleeding patients, independently of the outcome of the aPTT (25). This lets us conclude that both thrombin generation and fibrinogen clot formation need to be sufficiently active to ensure normal haemostasis and reduce the bleeding risk, under conditions of dilutional coagulopathy.

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