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Fibrinogen supplementation *ex vivo* increases clot firmness comparable to platelet transfusion in thrombocytopenia†

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Abstract

Background: Fibrinogen concentrate can improve clot firmness and offers a better safety profile than platelet concentrates. Reduction or avoidance of blood transfusions represents a strategy to reduce associated risks. We investigated whether supplementation of fibrinogen concentrate *ex vivo* can compensate for clot strength as compared with platelet transfusion *in vivo*.
Methods: One hundred patients in need of platelet transfusion (PT) were enrolled. Blood samples were collected immediately before PT and at 1 h and 24 h after PT. Fibrinogen concentrate was added to these citrated whole blood samples at concentrations of 50, 100, 200 and 400 mg kg^{-1} and the maximum clot firmness (MCF) was analysed using ROTEM thromboelastometry.
Results: Fibrinogen supplementation increased MCF significantly and dose-dependently before and after PT. The effect of fibrinogen concentrate (equivalent to doses of 100 and 200 mg kg^{-1}) *ex vivo* was comparable to that of PT *in vivo*, whereas 400 mg kg^{-1} fibrinogen significantly improved MCF compared with PT ($P < 0.001$).
Conclusions: Fibrinogen concentrate can match the effect of PT on MCF in thrombocytopenia. This potential alternative haemostatic intervention should be evaluated in clinical trials.

Key words: deficiency; fibrinogen; platelet transfusion; thrombocytopenia; thromboelastography

Thrombocytopenia is generally defined as a platelet count $<150 \times 10^9$ litre^{-1} and occurs frequently in hospitalized patients. Thrombocytopenia in patients in the intensive care unit (ICU) is a risk factor for major bleeding.[1] Bleeding can be quantified by a

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Editor's key points

- Fibrinogen supplementation can improve clot firmness as measured by thromboelastometry.
- The effect of *ex vivo* fibrinogen supplementation on clot firmness was measured in whole blood from thrombocytopenic patients before and after platelet transfusion.
- Fibrinogen supplementation increased clot firmness comparable to platelet transfusion, which might provide a safer adjunct to platelet transfusion in thrombocytopenic patients.

bleeding score, which significantly correlates with clot firmness assessed by thromboelastometry.[2] In cardiac surgery, thrombocytopenia as well as platelet dysfunction are the main reasons for coagulopathic (re)bleeding.

Platelet transfusion (PT) is usually the first-line haemostatic therapy in patients with clinically relevant thrombocytopenia or thrombocytopathy (disorder of platelet function), although the therapeutic efficiency is variable.[3 4] Beside the risk of therapy failure, PT has a notable risk for bacterial or viral infection as a consequence of storage at room temperature, febrile non-haemolytic and allergic reactions, iron overload (in case of chronic transfusions), and transfusion-related lung injury (TRALI).[5–8] Another challenge is the stability of platelet concentrates (PCs): the short storage life of 5 days limits availability and increases costs. Particularly in remote hospitals, PCs may not be available at all. Moreover, platelet transfusions are often not effective in sepsis- and chemotherapy-induced thrombocytopenia, and are contraindicated in certain conditions (e.g. thrombotic thrombocytopenic purpura/haemolytic uremic syndrome) and in the case of antibody formation (e.g. heparin-induced thrombocytopenia type 2).[9]

A diminished platelet count primarily reduces clot firmness,[10 11] which is also influenced by fibrinogen plasma levels.[12–14] Interestingly, patients with high fibrinogen values experience fewer bleeding complications than patients with low fibrinogen values.[15–17] Fibrinogen plays an important role in coagulation and clot stabilization and enhances binding of factor XIII to glycoprotein IIb/IIIa (GPIIb/IIIa), an integrin complex found on platelets.[18 19] In addition, it plays a central role in platelet activation and aggregation by binding to the platelet glycoprotein receptor GPIIb/IIIa. Moreover, the effect of platelet-blocking substances like eptifibatid and tirofiban can be antagonized by increasing fibrinogen concentration.[20] Experimental data in an animal model of thrombocytopenia show that human fibrinogen concentrate administration can compensate clot firmness better than PT.[21] Moreover, fibrinogen concentrate is safe and well tolerated in patients, including children (≥6 years of age), and provides haemostatic efficacy in various patient groups.[22–25]

Fibrinogen concentrate might be a useful haemostatic tool in thrombocytopenia and thus further investigation to clarify the potential effect of fibrinogen supplementation in thrombocytopenia is needed. Therefore, the aim of this study was to investigate whether the increase in clot firmness following PT *in vivo* was comparable to that observed after supplementation of fibrinogen concentrate *ex vivo*.

Methods

Ethics approval

This study was approved by the human subjects review board of the Medical University of Innsbruck, Austria (ref. no. UN4984_LEK) and by the national authority (Bundesamt für Sicherheit im Gesundheitswesen, Vienna, Austria). Written informed consent was obtained from all study participants. If patients were critically ill, they were enrolled according to Austrian law and written informed consent was obtained as soon as the patient regained the capacity to understand and make a decision. The study was performed in compliance with the Declaration of Helsinki and followed the Good Clinical Practice guidelines as defined by the International Conference on Harmonization (ICH-GCP).

Patient population

One hundred patients, ages 18–85 years, in clinical need of platelet transfusion (as assessed by the treating physician) were enrolled at the Medical University of Innsbruck, Department of Anaesthesiology and General Intensive Care Medicine, mostly after heart surgery (platelet concentrates were administered only postoperatively after cardiopulmonary bypass). Exclusion criteria were pregnancy, nursing or active participation in another clinical trial.

Prior medication

Prior medication [within 7 days before Visit 1 (V1)] was recorded if patients received antiplatelet medication (65%), procoagulatory medication (92%) or anticoagulatory medication (91%). Most patients received heparin (85%) and tranexamic acid (85%). Other drugs included aspirin (47%), clopidogrel bisulphate (22%), fibrinogen (22%), enoxaparin (11%), antithrombin III (9%), ticagrelor (8%), fresh frozen plasma (8%), ticagrelor (7%), prothrombin complex concentrate (6%), rivaroxaban (4%), vitamin K (4%), acenocoumarol (2%), fibrogammin (2%), and ticlopidine hydrochloride (1%).

Concomitant medication

Concomitant medication during study procedures, with potential influence on platelets, included catecholamines (94%), coagulation factor concentrates (48%), antibiotics (18%), other procoagulatory medication (9%), antifibrinolytics (7%), and anticoagulatory medication (5%): 89 patients received arterenol (94%), 26 fibrinogen concentrate (27%), 16 teicoplanin (18%), 13 fresh frozen plasma (14%), 8 desmopressin acetate (8%), 7 prothrombin complex concentrates (7%), 6 tranexamic acid (6%), 5 antithrombin III (5%), 1 vitamin K (1%), and 1 factor XIII (1%).

Crystalloid solution administered between V1 and V2 was mostly acetate-buffered solution (ELO-MEL isoton); the median administered volume was 400 ml (range 0–2000).

Collection and preparation of blood samples

Blood samples were drawn before PT (V1), 1 h after the end of PT (V2) and 24 h after PT (V3). Blood was collected into Vacutainer tubes containing 1/10 volume of 3.12% trisodium citrate (Sarstedt, Nümbrecht, Germany) for thromboelastometry and fibrinogen measurements. Ethylenediaminetetraacetic acid tubes (Sarstedt, Nümbrecht, Germany) were applied for blood cell count. Blood samples from V1 served as baseline for coagulation tests with ROTEM (thromboelastometry). Blood samples from V2 served as baseline for comparing the efficacy of PT *in vivo* and fibrinogen administration *ex vivo*. V3 was conducted to verify successful PT. Fibrinogen concentrate (FGTW, LFB Biomedicines, Les Ulis, France) was dissolved in distilled water (sterilized water for injections; LFB Biomedicines) to reach concentrations of 40 mg ml^{-1} fibrinogen before the addition to whole blood samples from V1 and V2, providing final concentrations of 0.88 mg ml^{-1}

(corresponding to an *in vivo* dose of 50 mg kg^{-1}), 1.76 mg ml^{-1} (corresponding to a dose of 100 mg kg^{-1}), 3.52 mg ml^{-1} (corresponding to a dose of 200 mg kg^{-1}), and 7.04 mg ml^{-1} fibrinogen (corresponding to a dose of 400 mg kg^{-1}). To exclude diluting effects, Dulbecco's phosphate-buffered saline (BioWhittaker, Lonza, Belgium) was added to blood samples to produce equal volumes.

Dosages of 50 and 100 mg kg^{-1} correspond to 3.5 and 7 g of fibrinogen concentrate administration, respectively, and are used in clinical practice. Higher doses, such as 200 and 400 mg kg^{-1} body weight, were added to simulate the high fibrinogen levels found *in vivo* in patients suffering from acute-phase reaction following infection or sepsis, where fibrinogen levels can increase to >1000 mg kg^{-1} body weight.

Thromboelastometry

Within 4 h after blood draw, untreated baseline and spiked citrated blood samples were analysed using a ROTEM gamma analyser (TEM Innovations, Munich, Germany) with reagents provided by the manufacturer for EXTEM (extrinsically activated assay with tissue factor) and FIBTEM (extrinsically activated assay with tissue factor and the platelet inhibitor cytochalasin D) measurements. Maximum clot firmness (MCF) was used to evaluate clot strength after *in vivo* PT and *ex vivo* fibrinogen supplementation. We also measured clotting time (CT; the time until clot formation starts), clot formation time (CFT; the time from CT until the clot reaches an amplitude of 20 mm), and alpha angle (α; the angle between the middle axis and the tangent to the clotting curve through the 2-mm amplitude point). For reference values[26] please refer to Tables 2 and 3.

Statistics

Data on patients and laboratory results were recorded in case report forms (CRFs) specially developed for this study. Non-normality of data was defined using the Shapiro–Wilk test. The Wilcoxon signed-rank test was used to test for significant differences between paired groups and the Mann–Whitney U test was used for unpaired groups. Statistical analyses were performed using STATISTICA 10 (StatSoft, Hamburg, Germany). P-values $\leq$0.05 were considered statistically significant.

Results

Patient population

Patient characteristics

One hundred patients in clinical need of PT were enrolled at the Medical University of Innsbruck from January 2013 to March 2014. Of these, 21 female and 75 male patients were eligible for analysis. Five patients were excluded because of insufficient or defective ROTEM measurements. Patient characteristics are displayed in Table 1. For information on prior and concomitant medications, please refer to Supplementary data I.

Indication for platelet transfusion

Platelet concentrate(s) (1–2 units) was administered due to coronary artery bypass surgery (54%), surgical valve replacement (26%), surgery in general (8%), abdominal or thoracic aortic aneurysm (6%), and other reasons (6%) including liver failure, extensive burns, sepsis, cholecystitis, and bleeding. The most frequent reason for PT was diffuse (microvascular) bleeding tendency (blood loss very low and not assessable). Patients

Table 1 Patient characteristics and selected laboratory data

	Median	Range	N
Age (years)	70	37–86	95
Weight (kg)	79	47–125	95
Height (cm)	173	150–190	95
BMI	27	16–46	95
Visit 1			
Platelet count ($\times 10^9$ litre^{-1})	86	8–216	92
Erythrocytes ($\times 10^{12}$ litre^{-1})	3.0	2.3–4.4	92
Fibrinogen (mg dl^{-1})	194	53–375	88
Visit 2			
Platelet count ($\times 10^9$ litre^{-1})	110	22–291	95
Erythrocytes ($\times 10^{12}$ litre^{-1})	3.2	2.2–5.1	95
Fibrinogen (mg dl^{-1})	212	69–396	88
Visit 3			
Platelet count ($\times 10^9$ litre^{-1})	92	10–240	32
Erythrocytes ($\times 10^{12}$ litre^{-1})	3.1	2.6–3.8	32

Table 2 ROTEM EXTEM parameters from V1 to V3. Blood samples from patients 1 h after PT (V2) were defined as baseline values. Differences from V2 are indicated as $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ (Wilcoxon signed-rank test). Data presented as mean (SD). N, number of patients; CT, clotting time (s); CFT, clot formation time (s); MCF, maximum clot firmness (mm); α, alpha angle (degrees); F1, human fibrinogen concentrate. Reference intervals are for healthy individuals[26]

	N	CT	CFT	MCF	α
V1 baseline	96	84 (30)***	262 (194)***	44 (10)***	57 (14)***
V1 + 50 mg kg^{-1} F1	30	72 (36)	252 (187)*	45 (11)*	57 (17)
V1 + 100 mg kg^{-1} F1	96	79 (24)*	235 (191)*	48 (11)	62 (13)
V1 + 200 mg kg^{-1} F1	96	76 (19)	238 (282)	49 (10)	65 (12)***
V1 + 400 mg kg^{-1} F1	65	80 (17)	167 (155)***	53 (9)***	72 (7)***
V2 baseline	**94**	**77 (−27)**	**234 (273)**	**49 (−9)**	**61 (13)**
V2 + 50 mg kg^{-1} F1	30	62 (23)	224 (170)	49 (9)***	60 (14)
V2 + 100 mg kg^{-1} F1	95	71 (18)**	187 (147)***	52 (9)***	65 (12)***
V2 + 200 mg kg^{-1} F1	95	72 (23)*	171 (132)***	53 (9)***	68 (11)***
V2 + 400 mg kg^{-1} F1	65	73 (14)***	124 (73)***	57 (8)***	75 (4)***
V3 24-h control	**30**	74 (54)	131 (59)	58 (11)	70 (8)
Reference interval[26]		42–74	46–148	49–71	63–83

were included only if they got a platelet transfusion, of which 88% were thrombocytopenic (platelet count $<150\times10^9$ litre^{-1}) and 65% had received antiplatelet medication (compare below).

Table 3 ROTEM FIBTEM parameters from V1 to V3. Blood samples from patients 1 h after PT (V2) were defined as baseline values. Differences from V2 are indicated as $^{*}P<0.05$, $^{**}p<0.01$, $^{***}P<0.001$ (Wilcoxon signed-rank test). Data presented as mean (SD). N, number of patients; MCF, maximum clot firmness (mm); α, alpha angle (degrees); F1, human fibrinogen concentrate; NA, not applicable. Reference interval is for healthy individuals[26]

	N	MCF	α
V1 baseline	97	12 (6)***	68 (8)
V1 + 50 mg kg^{-1} F1	30	13 (4)	64 (14)
V1 + 100 mg kg^{-1} F1	97	15 (5)***	68 (9)
V1 + 200 mg kg^{-1} F1	95	18 (5)***	72 (8)***
V1 + 400 mg kg^{-1} F1	67	25 (7)***	76 (6)***
V2 baseline	**93**	**14 (5)**	**68 (−8)**
V2 + 50 mg kg^{-1} F1	27	16 (16)*	65 (14)
V2 + 100 mg kg^{-1} F1	92	16 (5)***	69 (9)***
V2 + 200 mg kg^{-1} F1	91	19 (5)***	74 (7)***
V2 + 400 mg kg^{-1} F1	65	27 (8)***	78 (5)***
V3 24-h control	29	20 (10)	71 (5)
Reference interval[26]		9–25	NA

Baseline thromboelastometry

Compared with standard values in healthy humans,[26] EXTEM CT was prolonged at V1, with a mean of 84 (SD 30) s, as was EXTEM CFT, with a mean of 262 (SD 194) s. EXTEM MCF [mean 44 (SD 10) mm] and α angle (mean 57° (SD 14°)] were reduced. FIBTEM MCF was in the normal range at 12 (SD 5) mm at V1 (Tables 2 and 3).

Fibrinogen supplementation is equivalent or superior to platelet transfusion in the restoration of clot firmness

PC significantly increased EXTEM MCF from baseline V1 ($P<0.001$). Fibrinogen supplementation *ex vivo* (equivalent to 100, 200, and 400 mg kg^{-1}) also increased EXTEM MCF significantly from V1 ($P<0.001$). Fibrinogen equivalent to 100 mg kg^{-1} ($P=0.1$) or 200 mg kg^{-1} ($P=0.6$) showed the same effect on EXTEM MCF as PC, whereas 400 mg kg^{-1} fibrinogen increased EXTEM MCF more than PT ($P<0.001$). In contrast, 50 mg kg^{-1} fibrinogen was less effective than PT ($P=0.03$) in increasing EXTEM MCF and did not increase MCF significantly from baseline V1 ($P=0.07$) (Fig. 1, Table 2).

Exploration of data from the subgroup that received no fibrinogen concentrate *in vivo* ($n=73$) revealed that supplementation equivalent to 50 mg kg^{-1} fibrinogen showed the same effect as PT ($P=0.6$), with similar results for other fibrinogen concentrations.

Comparable to EXTEM MCF, PT increased FIBTEM MCF significantly from baseline V1 ($P<0.001$), as did fibrinogen supplementation [50 mg kg^{-1} ($P=0.005$), and 100, 200, and 400 mg kg^{-1}

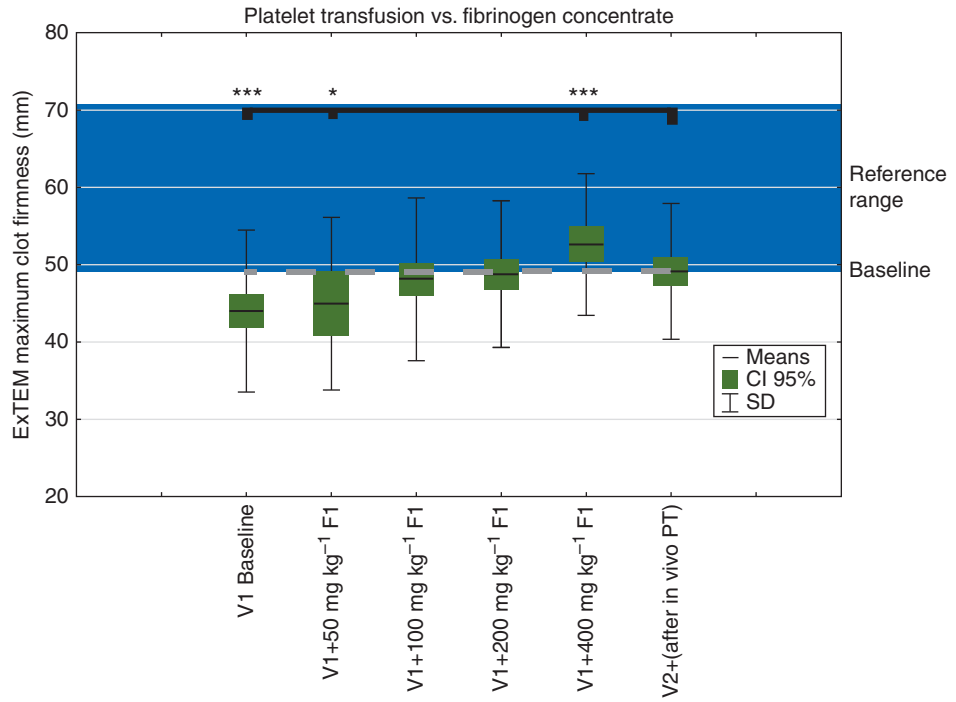

Fig 1 Effect of *ex vivo* fibrinogen supplementation compared with *in vivo* platelet transfusion on EXTEM MCF. EXTEM MCF before (baseline V1) and after platelet transfusion (baseline V2). Samples before PT were spiked with fibrinogen concentrate (equivalent to 50–400 mg kg^{-1} body weight). Blood samples from patients 1 h after PT (V2) were defined as baseline values. Differences from V2 are indicated as $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ (Wilcoxon signed-rank test). Data presented as mean (SD). CI, confidence interval.

($P < 0.001$)]. While supplementation equivalent to 50 mg kg^{-1} fibrinogen ($P = 0.3$) showed no significant difference in FIBTEM MCF, 100, 200, or 400 mg kg^{-1} fibrinogen increased FIBTEM MCF significantly more than PT ($P < 0.001$ for all) (Table 3). In addition to MCF, PT and fibrinogen supplementation (100, 200, and 400 mg kg^{-1}) both significantly improved other ROTEM parameters (CT, CFT, α angle); for details refer to Tables 2 and 3.

Concentration dependence of fibrinogen supplementation

Ex vivo supplementation of fibrinogen in blood samples derived from thrombocytopenic patients before PT significantly and concentration-dependently improved all ROTEM parameters examined (CT, CFT, MCF, α angle); for EXTEM MCF, 100 mg kg^{-1} fibrinogen was more effective than 50 mg kg^{-1} ($P = 0.03$), 200 mg kg^{-1} fibrinogen was more effective than 100 mg kg^{-1} ($P < 0.001$), and 400 mg kg^{-1} was more effective than 200 mg kg^{-1} fibrinogen ($P < 0.001$). An equivalent to 50 mg kg^{-1} fibrinogen showed no significant increase ($P = 0.07$) compared with baseline V1.

Effect of fibrinogen supplementation in addition to platelet transfusion

Ex vivo administration of fibrinogen to blood samples derived from V2 further increased clot firmness following PT. For EXTEM MCF, fibrinogen supplementation significantly ($P < 0.001$) increased MCF concentrations equivalent to 50, 100, 200, and 400 mg kg^{-1} fibrinogen. Again, 200 mg kg^{-1} fibrinogen was more effective than 100 mg kg^{-1} and 400 mg kg^{-1} was more effective than 200 mg kg^{-1} ($P < 0.001$ for both).

Differences in blood cell count

Platelet count and erythrocyte count significantly increased between V1 and V2 ($P < 0.001$). Moreover, a significant decrease in platelet count from V2 to V3 (24 h after PT) was observed ($P = 0.03$) (Table 1). Two units (bags) of PC increased platelet count significantly more than did one unit (mean increase 40×10^9 $litre^{-1}$ compared with 22×10^9 $litre^{-1}$; $P = 0.005$).

Discussion

Clot firmness correlates with bleeding[2] and was significantly increased in thrombocytopenia by administration of fibrinogen concentrate *ex vivo*. PT *in vivo* also significantly and dose-dependently increased clot firmness. Remarkably, *ex vivo* administration of fibrinogen concentrate (equivalent to 100 mg kg^{-1} and 200 mg kg^{-1} body weight) showed effects on clot firmness that were comparable to those of PT (1 or 2 units), whereas 400 mg kg^{-1} fibrinogen significantly improved clot firmness compared with PT.

Besides clot firmness, other ROTEM parameters such as CT, CFT, and α angle also improved, and additional administration of fibrinogen *ex vivo* after PT further improved those parameters. Standard values[26] for MCF partially recovered when using high fibrinogen levels (200 mg kg^{-1} or 400 mg kg^{-1}) and recovered even better with both PT and fibrinogen. In contrast, standard values for CT only recovered with a combination of PT and fibrinogen.

Fibrinogen improves clot firmness dose-dependently and even at low platelet counts.[27–29] Our study also shows a corrective effect of fibrinogen on other ROTEM parameters such as CT or CFT, confirming previous studies using an *ex vivo* model of coagulopathy.[30 31]

A limitation of our study is that fibrinogen addition was performed *ex vivo*. Consequently, the clinical efficacy of fibrinogen substitution could not be evaluated. Therefore, our results must be interpreted with caution, since neither haemodilution occurred nor additional factor concentrates were given. In a previous study we showed improved clot formation during thrombocytopenia in a porcine model by *in vivo* administration of fibrinogen concentrate, which also resulted in reduced blood loss and prolonged survival after liver injury.[21] Whether this holds true in the human model as well must be evaluated in clinical trials.

Another weakness of our study is that a significant proportion of patients (24%) received fibrinogen concentrate in addition to PT, which could influence the results in favour of PT, since we were able to show that a combination of PT and fibrinogen improved ROTEM parameters even better than either alone. Since no clinical endpoints were determined, the results of this study have to be interpreted with caution.

Rotational thromboelastometry (ROTEM) is a very useful tool for predicting, managing and correcting coagulation parameters during surgery.[32–35] Greene and colleagues[2] detected a stronger correlation between clot firmness and bleeding risk than between platelet count and bleeding in patients with immune thrombocytopenia. Petricevic and colleagues[36] also found thromboelastometry to be a useful tool to detect impaired coagulopathy and consequently predict the risk of heavy bleeding after cardiopulmonary bypass surgery. A recent study by Fayed and colleagues[37] revealed that EXTEM MCF can be used as a predictor for PT.

Thrombocytopenic patients with inflammation-induced elevated fibrinogen values in TEG/ROTEM monitoring are often not given platelets, because clot firmness is within the normal range. Interestingly, we observed a significant decrease in platelet count from V2 to V3, whereas MCF further increased from V2 to V3, achieving normal MCF values. These data are in line with recent studies indicating that functional fibrinogen makes a stronger contribution to clot firmness than platelets.[29 38 39] However, other studies indicate a stronger contribution by platelets to clot strength.[40 41] Clearly, both contribute to clot firmness, and we were able to confirm an additive effect of fibrinogen and platelets on clot formation and further reductions in CT and CFT.[42] Lang and colleagues[29] hypothesized that in thrombocytopenia more fibrin fibres connect platelets via GPIIb/IIIa receptors and so restore clot firmness. However, low levels of fibrinogen have previously been found sufficient to saturate the GPIIb/IIIa receptor complexes.[43 44] In the present study, 51% of patients showed fibrinogen deficiency at V1 (compare Table 1), a population that would likely benefit from administration of fibrinogen concentrate.

Tanaka and colleagues[45] compared patients undergoing valve replacement or repair and receiving fibrinogen concentrate (4 g) or 1 unit of apheresis PC. They also concluded that fibrinogen concentrate might reduce PT. Further *in vivo* studies are needed to compare the effects of PT and fibrinogen and to establish doses and the clinical efficacy of fibrinogen concentrates when used as an alternative or supplement to PT.

Our data suggest that administration of fibrinogen concentrate may compensate clot firmness related to thrombocytopenia in a degree similar to that for platelet transfusion. However, these findings must be confirmed in clinical trials. Fibrinogen concentrate may provide an alternative approach when platelet concentrates are not available or platelet transfusion is not effective.

Authors' contributions

B.S., M.B.: study design, application to ethics committee and authorities, data acquisition, laboratory experiments, data analysis and interpretation, writing of the first draft of the manuscript, responsible for data integrity and accuracy of data analysis.

A.K.L, B.T: study design, laboratory experiments, data acquisition, critical revision of the manuscript for important intellectual content.

M.H.: laboratory experiments, data acquisition, critical revision of the manuscript for important intellectual content.

O.H.L., C.F.E.: study concept and design, data analysis and interpretation, critical revision of the manuscript for important intellectual content.

D.W., H.T., C.V.S.: study concept and design, patient recruitment, data acquisition, critical revision of the manuscript for important intellectual content.

D.F.: study concept and design, patient recruitment, data acquisition, critical revision of the manuscript for important intellectual content, application to ethics committee and authorities, data analysis and interpretation.

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Declaration of interests

D.F. has received funding and honoraria for consultancy and board activity from AstraZeneca, AOP Orphan, Baxter, Bayer, B. Braun, Biotest, CSL Behring, Delta Select, Dade Behring, Edwards, Fresenius, Glaxo, Haemoscope, Hemogem, Lilly, LFB, Mitsubishi Pharma, Novo Nordisk, Octapharm, Pfizer, and TEM Innovation. C.F.-E. has received unrestricted grants for research purposes from LFB, CSL Behring, Novo Nordisk, and TEM International.

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