TRANSFUSION MEDICINE
Official Journal of the British Blood Transfusion Society
British Blood Transfusion Society
Transfusion Medicine | ORIGINAL ARTICLE

Fibrinogen concentrate improves clot strength in patients with haematological malignancies requiring platelet transfusion

H. Munk-Andersen,[1] B. Schenk,[2] O.H. Larsen,[3,4] D. Fries[2] & C. Fenger-Eriksen[1,4]

[1]*Department of Anaesthesiology, Aarhus University Hospital, Aarhus, Denmark,* [2]*Department for General and Surgical Intensive Care Medicine, Medical University Innsbruck, Innsbruck, Austria,* [3]*Department of Haematology, Aarhus University Hospital, Aarhus, Denmark, and* [4]*Centre for Haemophilia and Thrombosis, Department of Clinical Biochemistry, Aarhus University Hospital, Aarhus, Denmark*

Received 16 December 2015; accepted for publication 4 May 2016

SUMMARY

Background: Patients with bone marrow failure secondary to chemotherapy often develop thrombocytopenia and require platelet transfusion. Fibrinogen plays an important role in platelet aggregation and the establishment of the primary haemostatic plug.

Objectives: To compare the effects of *in vivo* platelet transfusion on clot firmness in thrombocytopenic patients with *in vitro*-performed fibrinogen concentrate substitution.

Materials and methods: Thirty patients with haematological malignancy admitted for platelet transfusion were included. Haemostatic effects from platelet transfusion and *ex vivo* addition of fibrinogen concentrate at three different doses were evaluated by thromboelastometry, with clot firmness as the primary endpoint (A30 ExTEM assay). Secondary endpoints were other thromboelastometry parameters, thrombin generation parameters, activated partial thromboplastin time (APTT), prothrombin time PT, fibrinogen and factor XIII levels and a clinical bleeding score.

Results: Twenty patients (66%) had clinical bleeding signs by prior to transfusion. Platelets increased from 17 (range, 1–109) to 40 (range 2–139) $\times 10^9$ L^{-1} following transfusion, with a median corrected count increment of 16·7 (range, 0·8–43·5). The A30 value increased significantly by platelet transfusion from 35 ± 11 to 47 ± 10 mm, with no changes in thrombin generation. Fibrinogen concentrate dose-dependently increased A 30 (to 43 ± 10, 49 ± 9 and 50 ± 9 mm, respectively) and reduced parameters of thrombin generation at high doses. Platelet transfusion, together with fibrinogen concentrate, further increased clot firmness. APTT and PT were within normal range, whereas fibrinogen levels were slightly elevated.

Conclusion: Fibrinogen concentrate increased clot firmness to the same degree as platelet transfusion in patients with low platelet count requiring platelet transfusion.

Key words: fibrinogen, haematology, platelet transfusion, thrombelastography, thrombocytopenia.

Platelet transfusions are widely employed to prevent or treat bleeding episodes in patients with thrombocytopenia. Patients with bone marrow failure secondary to haematological malignancy and chemotherapy frequently receive platelet transfusion when platelet level reaches $10-20 \times 10^9$ L^{-1}, with the exact transfusion trigger depending on the clinical situation and local guidelines (Estcourt *et al.*, 2012). Up to two thirds of all platelets transfusions are given to patients with haematological diseases (Pendry & Davies, 2011). Owing to a short storage life, many remote hospitals do not routinely hold platelet concentrates available. Furthermore, a number of potentially adverse transfusion reactions may call for alternatives. Like other blood components, platelet transfusions have achieved a high degree of safety regarding transmission of viral diseases. However, febrile non-haemolytic transfusion reactions and allergic reactions may occur after transfusion with platelet concentrates. Transfusion-related lung injury (TRALI), septic complications induced by bacterial contamination and haemolytic transfusion reactions represent other rare but dangerous side effects of platelet transfusions (Kiefel, 2008; Keller-Stanislawski *et al.*, 2009; Refaai *et al.*, 2011). Furthermore, the haemostatic capacity of platelet concentrates is variable and decreases along with increased storage time of the product (Sarkodee-Adoo *et al.*, 1998; Sahler *et al.*, 2011). During acute blood loss, platelets adhere to the sites of vascular injury, where they get activated and aggregate via binding of fibrinogen. They form an initial clot establishing primary haemostasis. In actively bleeding patients, fibrinogen drops to a critically low level even before significant thrombocytopenia occurs (Hiippala *et al.*, 1995). Consequently, fibrinogen supplementation reduces blood loss in a number

Correspondence: Christian Fenger-Eriksen MD Ph.D., Department of Anaesthesiology, Aarhus University hospital, Nørrebrogade, DK-8000 Aarhus C, Denmark.
Tel: +45 7846 6912; Fax: +45 7846 4455; e-mail: chfen@dadlnet.dk

First published online 30 May 2016
doi: 10.1111/tme.12323

of different clinical settings (Sorensen *et al.*, 2012). Fibrinogen concentrates are commercially available and show several advantages compared to allogeneic blood products in terms of efficacy and safety for treatment of acquired hypofibrinogenemia during active bleeding (Franchini & Lippi, 2012). Patients with chemotherapy-induced low platelet levels often experience normal or even slightly elevated fibrinogen levels because of its role as an acute phase protein. Nevertheless, it may be speculated whether increased fibrinogen levels could compensate for low platelet levels by improvement of clot strength.

Thus, the aim of the present study was to compare the effects of platelet transfusion on clot firmness in thrombocytopenic patients with *in vitro*-performed fibrinogen substitution, hypothesising fibrinogen concentrate and platelet transfusion as equally effective in restoring clot firmness.

MATERIALS AND METHODS

Study subjects

The study was conducted at Aarhus University Hospital between September 2013 and August 2014 upon approval by the Regional Biomedical Human Ethics Committee (reference code 1-16-02-381-13). Following written informed consent, patients between 18 and 85 years of age with clinical need for platelet transfusion were included in the study. Exclusion criteria were intake of platelet inhibitor, acetyl-salicylic acid or non-steroid anti-inflammatory within 7 days prior to blood sampling. All patients were assessed prior to platelet transfusion using a validated thrombocytopenia bleeding score (Page *et al.*, 2007). Transfusion trigger was a platelet count of 10×10^9 L^{-1} or $15-20 \times 10^9$ L^{-1} if patients had a fever or ongoing infection. Corrected count increment was calculated as (Δplatelet count * body surface area, m^2)/platelets transfused ($2{\cdot}5 \times 10^{11}$)

Objectives

The primary objective was to assess the difference in clot firmness (amplitude after 30 min, A30 using ExTEM® assay (A30EX TEM International, Munich, Germany)) comparing *in vitro* fibrinogen spiking with blood samples obtained from the same patients following platelets transfusion). Pre-specified secondary endpoints were remaining thrombelastometric variables, platelet count, thrombin generation, activated partial thromboplastin time (APTT), prothrombin time (PT) and factor XIII (FXIII) levels.

Blood sampling, processing and thromboelastometry

Blood samples were drawn from an antecubital vein using minimum stasis and a 21-gauge butterfly needle, before and (one h) after transfusion of one platelet pool. VenoJect (Terumo Europe, Leuven, Belgium trisodium citrate 3·2%) tubes were applied for ROTEM/thrombin generation analysis and routine coagulation tests, whereas EDTA-coated tubes were applied for analysis of haematological parameters (BD, Franklin Lakes, NJ, USA). Continuous whole blood clot formation profiles were recorded in parallel using thromboelastometry (ROTEM®, TEM International, Munich, Germany); 2500 μL whole blood was gently mixed in four separate aliquots with 440 , 230 , 220 or 0 μL buffer (20 mM HEPES, 150 mM NaCl, pH 7·4), together with 0, 110, 220 or 440 μL fibrinogen concentrate, respectively (FGTW, LFB Biomedicaments, Les Ulis, France). Final fibrinogen concentrations corresponded to the administration of 0, 100, 200 and 400 mg fibrinogen/kg bodyweight to a patient. ROTEM® plastic cups were pre-warmed to 37 °C and loaded with the spiked whole blood using an automated pipette and the commercial available assays, ExTEM® (tissue factor) and FibTEM® (tissue factor + cytochalasin D) (TEM International, Munich, Germany). Remaining blood samples were centrifuged at 2800 *g* for 15 min at 10 °C to obtain platelet-poor plasma.

Routine coagulation tests and platelet count

APTT, fibrinogen and PT were assessed on a Sysmex CS 2100i coagulation analyser (Siemens Healthcare, Erlangen, Germany) using APTT reagent (TriniCLOT, Bray, Tcoag Ireland), Dade Thrombin Reagent (Siemens Healthcare, Marburg, Germany) for determination of fibrinogen levels according to the Clauss method and PT reagent (PT Owren, Medirox AB, Sweden), respectively. FXIII levels were detected by the antigen method on an ACL TOP analyser applying HemosIL reagent (both Instrumentation Laboratory, Bedford, MA, USA)

Thrombin generation

Thrombin generation was measured using a calibrated, automated thrombogram (Thrombinoscope BV, Maastricht, The Netherlands). Frozen platelet-poor plasma was kept at −80 °C for bulk analysis. A 96-well microtiter plastic plate (Immulon 2HB clear 96-well; Thermo Electron Corporation, Vantaa, Finland) was prepared with 80 μL of platelet-poor plasma, followed by 20 μL of activator containing tissue factor and phospholipids with final concentrations at 5 pM and 4 μM (PPP-Reagent low, Thrombinoscope BV, Maastricht, The Netherlands). After a brief incubation, 20 μL of thrombin substrate (Fluo-Substrate; Thrombinoscope BV, Maastricht, The Netherlands) was added automatically. All reagents were pre-warmed to 37 °C. Continuous development of thrombin was recorded on a Fluoroscan Ascent fluorimeter (Thermo Electron Corporation). Measurements were performed in triplicate, with each well calibrated to a parallel with a thrombin calibrator (Thrombin calibrator TS 20.0; Thrombinoscope BV, Maastricht, The Netherlands) with known thrombin-like activity.

Statistics

All statistical analyses were performed using the statistical programme Sigma Stat 4 (Systat Software Inc San Jose, CA, USA). Groups were pre-tested for equal standard deviations using

the method of Bartlett and were estimated to follow a Gaussian distribution based on the Kolmogorov-Smirnov test. Variables before and after platelet transfusion were evaluated by means of a paired *t*-test or Wilcoxon signed-rank sum test as a non-parametric test. One-way repeated measures analysis of variance was used for evaluating the effect from fibrinogen addition. Standard laboratory tests were evaluated by a paired *t*-test or, alternatively, the Wilcoxon signed-rank sum test. A *P* value less than 0·05 ($P < 0{\cdot}05$) was considered statistically significant.

RESULTS

Patient characteristics

Thirty patients were included in the study (13 females and 17 males), with an average age of 57 years (range, 26 – 84 years) and a mean weight of 75 kg (range, 52 – 126 kg). Patients were recruited at Aarhus University Hospital, Department of Haematology. All suffered from haematological malignancy and experienced hypo proliferative thrombocytopenia secondary to chemotherapy. Twenty of 30 patients presented with a clinical bleeding problem prior to platelet transfusion. One patient reported epistaxis by history, one patient metrorrhagia and one patient was suspect to gastrointestinal bleeding. Remaining bleeding problems were skin bruising by clinical examination (11 patients), by history (6 patients), oral bleeds by examination (1 patient) and oral bleeds by history (2 patients).

Haematological parameters

Upon platelet transfusion, platelet count increased from 17×10^9 L^{-1} (range, $1 - 109 \times 10^9$ L^{-1}) to 40×10^9 L^{-1} (range $2 - 139 \times 10^9$ L^{-1}). Median-corrected count increment was 16·7 (range, 0·8 – 43·5). Pre-transfusion haemoglobin levels were 5·6 μmol L^{-1} (range 3·3 – 6·9 μmol L^{-1} and white blood cell count $3{\cdot}6 \times 10^9$ L^{-1} (range, $0{\cdot}01 - 30 \times 10^9$ L^{-1}). Thrombelastometric curves revealed no signs of hyperfibrinolysis.

Effect of platelet transfusion

The primary endpoint of the A 30 EX was significantly ($P = <0{\cdot}001$) increased from 35 ± 11 mm [mean ± standard deviation (SD)] to 47 ± 10 mm after platelet transfusion ($P = <0{\cdot}001$), whereas no changes were observed in parameters of clotting time, clot formation time or thrombin generation (Fig. 1, Table 1).

Effect of fibrinogen concentrate

Fibrinogen concentrate showed a dose-dependent increment of the primary endpoint A30EX from 35 ± 11 to 43 ± 10 mm, 49 ± 9 and 50 ± 9 mm, respectively ($P = <0{\cdot}001$). FibTEM MCF was also significantly and dose-dependently increased ($P = <0{\cdot}001$). Furthermore, fibrinogen concentrate decreased thrombin generation in a dose-dependent manner as demonstrated by prolonged lag time and time to peak level, whereas endogenous thrombin potential and peak levels were reduced. The reduced thrombin generation corresponded to a prolonged whole blood clotting time, whereas clot formation time was shortened (Fig. 1, Table 1). As pilot experiments, samples from six patients were treated with 50 mg kg^{-1}. A30EX increased from 35 ± 11 to 39 ± 7 mm, without reaching statistical significance ($P = 0{\cdot}130$).

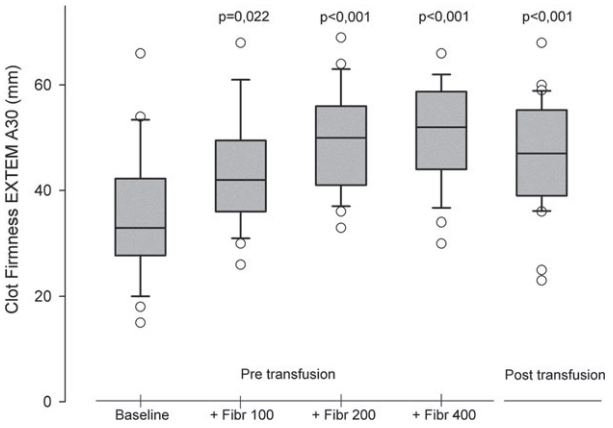

Fig. 1. Clot firmness before (baseline) and after platelet transfusion and after *ex vivo* addition of fibrinogen concentrate (Fibr) at three different concentrations. Data presented as mean ± standard deviation. *Significantly different from baseline.

Effect of platelet transfusion together with fibrinogen concentrate

In vitro fibrinogen concentrate supplementation following *in vivo* platelet transfusion at 100, 200 and 400 mg kg^{-1} further increased A30EX from baseline 47 ± 10 to 51 ± 10, 57 ± 9 and 54 ± 9 mm, respectively ($P = <0{\cdot}001$). Fibrinogen concentrate also, dose-dependently, reduced clot formation time, increased maximum velocity of clot formation and reduced time until maximum velocity of clot formation, whereas clotting time was unaffected (all thrombelastometric variables – data not shown).

Standard coagulation tests

Platelet transfusion did not affect APTT, PT, fibrinogen or FXIII levels. All parameters were within the normal reference interval for healthy individuals, except from slightly elevated fibrinogen levels (Table 2).

DISCUSSION

The hypothesis in the present study was adopted as fibrinogen concentrate improved clot firmness significantly and equal to platelet transfusion in patients with thrombocytopenia.

Even though the thrombocytopenic patients in the present study suffered from clinically relevant thrombocytopenia requiring transfusion, not low fibrinogen levels, we choose to investigate the effect of the addition of fibrinogen. We challenged the

Table 1. Parameters of thromboelastometry and thrombin generation before (baseline) and after platelet transfusion and after *ex vivo* addition of fibrinogen concentrate (Fibr) at three different concentrations

	Before					
	Baseline	+ Fibr 100	+ Fibr 200	+ Fibr 400	After	*P* value
ROTEM-thromboelastometry						
Clotting time, ExTEM (s)	109 ± 57	94 ± 27	106 ± 34	144 ± 42*	95 ± 31	<0·001
Clot formation time, ExTEM (s)	349 ± 374	156 ± 166*	56 ± 58*	49 ± 57*	160 ± 98	<0·001
A 30 clot firmness ExTEM (mm)	35 ± 11	43 ± 10*	49 ± 9*	50 ± 9*	47 ± 10*	<0·001
Max clot firmness ExTEM (mm)	37 ± 12	44 ± 9·7*	51 ± 9*	53 ± 10*	48 ± 9·6*	<0·001
Max clot firmness FibTEM (mm)	24 ± 9·3	36 ± 8·7*	49 ± 8·7*	43 ± 11*	24 ± 9·2	<0·001
Thrombin generation						
Lagtime (s)	3·9 ± 1·1	5·1 ± 2·4	7·1 ± 5·1*	12 ± 8·8*	3·9 ± 1·3	<0·001
ETP (nM × min)	1133 ± 314	1055 ± 277	913 ± 321*	749 ± 385*	1140 ± 336	<0·001
Peak level (nM)	183 ± 85	150 ± 73*	112 ± 63*	61 ± 35*	190 ± 67	<0·001
Time to peak level (s)	7·7 ± 3·8	9·7 ± 5·9	12·7 ± 9·8*	21 ± 13*	7·4 ± 4·3	<0·001

ETP; Endogenous thrombin potential.
Data presented as mean ± standard deviation.
*Significantly different from baseline ($P < 0·001$).

Table 2. Routine coagulation parameters measured before and after platelet transfusion

	Before	After	P-value
Standard parameters			
APTT, s (25 – 38)	32·7 (28·8; 37·5)	31·8 (28; 35·5)	0·20
PT, relative (>0·80)	0·89 (0·6;1·0)	0·85 (0·6;1·1)	0·55
Fibrinogen, g L^{-1} (1·87 – 4·08)	4·5 ± 1·5	4·5 ± 1·4	0·68
FXIII IU mL^{-1} (0·61 – 1·77)	0·83 (0·5;1·01)	0·76 (0·48;0·97)	0·83

APTT, activated partial thromboplastin time.
Tissue factor induced FII, FVII and FX (PT), fibrinogen and FXIII levels. Data presented as median (25; – 75 percentile) or mean ± standard deviation.

idea that increasing fibrinogen concentration would compensate for low platelet levels and improve the overall clot strength as fibrinogen together with platelets forms the primary haemostatic plug. Our results confirm a study by Lang *et al.* reporting improved clot strength after fibrinogen supplementation even at platelet counts as low as 10×10^9 L^{-1} (Lang *et al.*, 2009). Similarly, Velik-Salchner and colleagues succeeded in restoring impaired clot formation due to severe thrombocytopenia by administration of fibrinogen concentrate in a porcine model (Velik-Salchner *et al.*, 2007). The surfaces of platelets hold a vast excess of GPIIb/IIIa receptors. It may be hypothesised that during thrombocytopenia, more fibrinogen could bind to the abundant receptors and improve clot strength in addition to the effect of increased fibrin polymerisation (Wagner *et al.*, 1996). Noteworthy, during pregnancy, a few women develop a relative thrombocytopenia, whereas most pregnant women present an increase in fibrinogen levels. In addition, low fibrinogen has been associated with severe postpartum haemorrhage (Hellgren, 2003; Charbit *et al.*, 2007; Cortet *et al.*, 2012).

In the present study, the transfusion trigger was 10×10^9 or $15 – 20 \times 10^9$ L^{-1} if patients had fever or ongoing infection according to international guidelines (Estcourt *et al.*, 2012). The decision for platelet transfusion was made by the haematologist responsible for the patient; thus, the study investigators had no influence on this.

Most of the *in vivo* thrombin generation takes place on the platelet surface, and it is likely that platelet transfusion, particularly in thrombocytopenic conditions, would improve overall thrombin generation potential. We detected no differences in thrombin generation parameters before and after platelet transfusion, which is most likely explained by the assay being performed in platelet-poor plasma. Addition of fibrinogen concentrate at 200 and 400 mg kg^{-1} reduced thrombin generation significantly, presumably due to the anti-thrombin effect of fibrinogen accentuated by the relatively high doses applied. Despite normal APTT and PT, the clotting time at baseline was significantly prolonged and outside its normal reference interval, possibly due to lack of platelets in the whole blood assay leading to impaired ability to generate thrombin. In a comparable study, patients with platelet counts of around 10×10^9 L^{-1} had a minor prolongation of R-time evaluated by TEG prior to transfusion and experienced no improvement after platelet transfusion (Zhu *et al.*, 2014).

Clinical fibrinogen concentrate dosing in humans is 2 – 8 g depending on the specific clinical situation, although as much as 14 g has been administrated during a clinical trial (Rahe-Meyer *et al.*, 2013). In our study, we investigated 100 – 400 mg kg^{-1} corresponding 7·5 – 30 g for a grown adult. The dose 400 mg kg^{-1} appears inappropriately high as it suppresses thrombin generation without any further improvement of maximum clot firmness as compared to 200 mg kg^{-1}. We performed additional experiments on six patients with fibrinogen 50 mg kg^{-1}

and noted an increased A30EX, which is, however, not significant assumedly due to low number of observations. In clinical practice, a specific platelet count triggers platelet transfusion. However, recently, Greene *et al.* showed that clot firmness measured by thromboelastometry provided a stronger correlation than the platelet count with the same standardised bleeding score as used in this study than the platelet count (Greene *et al.*, 2014).

Thromboelastometry provides a combined evaluation of the haemostatic system, including all cells present in whole blood, which represents an advantage over more traditional coagulation tests like platelet count or PT. A30EX was chosen as the primary endpoint as this variable represents a combined measure of platelets and fibrinogen. Furthermore, the A30EX correlates closely with MCF but has the practical benefit of a shorter run time for the analyses. This study is limited by its small sample size, and results must be interpreted with caution. Firstly, whole blood coagulation profiles were evaluated without the contribution from endothelium or the flow-induced shear stress present *in vivo*. Secondly, we added fibrinogen concentrate *ex vivo* after blood sampling. A majority of patients had minor bleeding history predominately due to bruising and ecchymosis. A few patients experienced mucosal or even gastrointestinal bleeding prior to platelet transfusion. Equivalent alternatives to platelet transfusion could be particularly favourable in patients with contra-indications to platelet transfusions or in situations with lack of platelet availability. Larger clinical trials are needed to clarify whether increased clot strength following fibrinogen supplementation may also reduce clinical bleeding.

In conclusion, our data suggest that administration of fibrinogen concentrate is equal to platelet transfusion regarding improvement of the laboratory parameter clot strength in patients with clinically relevant thrombocytopenia requiring platelet transfusion.

ACKNOWLEDGEMENTS

The authors are grateful for excellent laboratory assistance from Vivi Mogensen and Mai Stenulm Therkelsen, Department of Clinical Biochemistry and the support provided by the entire staff at the Department of Haematology, Aarhus University Hospital, Denmark. The study was supported by an unrestricted research grant from LFB Biomedicaments, France in addition to internal funding. None of the investigators received any personal honoraria for performing the study. Furthermore, the sponsors had no influence on the design of the study or on the analysis and interpretation of the results. D. F., B. S. and C. F. E. planned and designed the study. H. M. A., O. H. L. and C. F. E. recruited and included patients. H. M. A. and C. F. E. wrote the first draft of the manuscript. All authors took part in data analysis and interpretation.

CONFLICT OF INTEREST

D. F. received honoraria for consulting, a lecture fee and sponsoring for academic studies from following companies and institutions: Astra Zeneca, AOP Orphan, Baxter, Bayer, B Braun, Biotest, CSL Behring, Delta Select, Dade Behring, Edwards, Fresenius, Glaxo, Haemoscope, Hemogem, Lilly, LFB, Mitsubishi Pharma, NovoNordisk, Octapharm, Pfizer, TEM-Innovation. C. F. E. received honoraria, a consulting fee or unrestricted grants for academic research from CSL Behring, TEM International, LEO Pharma, LFB and Novo Nordisk. H. M. A., O. H. L. and B. S. have no additional conflict of interest.

REFERENCES

Charbit, B., Mandelbrot, L., Samarin, E. *et al.* (2007) The decrease of fibrinogen is an early predictor of the severity of postpartum hemorrhage. *Journal of Thrombosis and Haemostasis*, **5**, 266–273.

Cortet, M., Deneux-Tharaux, C., Dupont, C. *et al.* (2012) Association between fibrinogen level and severity of postpartum haemorrhage: secondary analysis of a prospective trial. *British Journal of Anaesthesia*, **108**, 984–989.

Estcourt, L., Stanworth, S., Doree, C. *et al.* (2012) Prophylactic platelet transfusion for prevention of bleeding in patients with haematological disorders after chemotherapy and stem cell transplantation. *The Cochrane Database of Systematic Reviews*, **5**, Cd004269.

Franchini, M. & Lippi, G. (2012) Fibrinogen replacement therapy: a critical review of the literature. *Blood Transfusion*, **10**, 23–27.

Greene, L.A., Chen, S., Seery C. *et al.* (2014) Beyond the platelet count: immature platelet fraction and thromboelastometry correlate with bleeding in patients with immune thrombocytopenia. *British Journal of Haematology*, **166**, 592–600.

Hellgren, M. (2003) Hemostasis during normal pregnancy and puerperium. *Seminars in Thrombosis and Hemostasis*, **29**, 125–130.

Hiippala, S.T., Myllyla, G.J. and Vahtera EM. (1995) Hemostatic factors and replacement of major blood loss with plasma-poor red cell concentrates. *Anesthesia and Analgesia*, **81**, 360–365.

Keller-Stanislawski, B., Lohmann, A., Günay, S. *et al.* (2009) The German Haemovigilance System--reports of serious adverse transfusion reactions between 1997 and 2007. *Transfusion Medicine*, **19**, 340–349.

Kiefel, V. (2008) Reactions induced by platelet transfusions. *Transfusion Medicine and Hemotherapy*, **35**, 354–358.

Lang, T., Johanning, K., Metzler. H. *et al.* (2009) The effects of fibrinogen levels on thromboelastometric variables in the presence of thrombocytopenia. *Anesthesia and Analgesia*, **108**, 751–758.

Page, L.K., Psaila, B., Provan, D. *et al.* (2007) The immune thrombocytopenic purpura (ITP) bleeding score: assessment of bleeding in patients with ITP. *British Journal of Haematology*, **138**, 245–248.

Pendry, K. & Davies, T. (2011) An audit of use and wastage in the north west of England and North Wales: where have all the platelets gone? *Blood and Transplant Matters*, **34**, 17–19.

Rahe-Meyer, N., Solomon, C., Hanke, A. *et al.* (2013) Effects of fibrinogen concentrate as first-line therapy during major

aortic replacement surgery: a randomized, placebo-controlled trial. *Anesthesiology*, **118**, 40–50.

Refaai, M.A., Phipps, R.P., Spinelli, S.L. *et al.* (2011) Platelet transfusions: impact on hemostasis, thrombosis, inflammation and clinical outcomes. *Thrombosis Research*, **127**, 287–291.

Sahler, J., Grimshaw, K., Spinelli, S.L. *et al.* (2011) Platelet storage and transfusions: new concerns associated with an old therapy. *Drug Discovery Today: Disease Mechanisms*, **8**, e9–e14.

Sarkodee-Adoo, C.B., Kendall, J.M., Sridhara, R. *et al.* (1998) The relationship between the duration of platelet storage and the development of transfusion reactions. *Transfusion*, **38**, 229–235.

Sorensen, B., Larsen, O.H., Rea, C.J. *et al.* (2012) Fibrinogen as a hemostatic agent. *Seminars in Thrombosis and Hemostasis*, **38**, 268–273.

Velik-Salchner, C., Haas, T., Innerhofer, P. *et al.* (2007) The effect of fibrinogen concentrate on thrombocytopenia. *Journal of Thrombosis and Haemostasis*, **5**, 1019–1025.

Wagner, C.L., Mascelli, M.A., Neblock, D.S. *et al.* (1996) Analysis of GPIIb/IIIa receptor number by quantification of 7E3 binding to human platelets. *Blood*, **88**, 907–914.

Zhu, M., Xu, W., Wang, B.L. *et al.* (2014) Hemostatic function and transfusion efficacy of apheresis platelet concentrates treated with gamma irradiation in use for thrombocytopenic patients. *Transfusion Medicine and Hemotherapy*, **41**, 189–196.