BJA

Relative concentrations of haemostatic factors and cytokines in solvent/detergent-treated and fresh-frozen plasma

O. M. Theusinger [1][*][†], W. Baulig [1][†], B. Seifert [4], M. Y. Emmert [3], D. R. Spahn [1] and L. M. Asmis [2]

[1] Institute of Anaesthesiology, [2] Division of Haematology and [3] Clinic of Cardiac and Vascular Surgery, University Hospital Zurich, Zurich, Switzerland
[4] Biostatistics Unit, Institute of Social and Preventive Medicine, University of Zurich, Switzerland

* Corresponding author. E-mail: oliver.theusinger@usz.ch

Editor's key points

- Relative concentration of haemostatic factors and cytokine could vary between different plasma products.
- These parameters were measured in 25 samples of solvent/detergent-treated plasma and fresh-frozen plasma (FFP).
- Mean concentrations of most factors were similar, but there was more variability in FFP, which also had higher factor (F) V, F X, ADAMTS-13, von Willebrand factor, and cytokine concentrations.

Background. Indications, efficacy, and safety of plasma products are highly debated. We compared the concentrations of haemostatic proteins and cytokines in solvent/detergent-treated plasma (SDP) and fresh-frozen plasma (FFP).

Methods. Concentrations of the following parameters were measured in 25 SDP and FFP samples: fibrinogen (FBG), factor (F) II, F V, F VII, F VIII, F IX, F X, F XIII, von Willebrand factor (vWF), D-Dimers, ADAMTS-13 protease, tumour necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6, IL-8, and IL-10.

Results. Mean FBG concentrations in SDP and FFP were similar, but in FFP, the range was larger than in SDP ($P<0.01$). Mean F II, F VII, F VIII, F IX, and F XIII levels did not differ significantly. Higher concentrations of F V ($P<0.01$), F X ($P<0.05$), vWF ($P<0.01$), and ADAMTS-13 ($P<0.01$) were found in FFP. With the exception of F VIII and F IX, the range of concentrations for all of these factors was smaller ($P<0.05$) in SDP than in FFP. Concentrations of TNF-α, IL-8, and IL-10 (all $P<0.01$) were higher in FFP than in SDP, again with a higher variability and thus larger ranges ($P<0.01$).

Conclusions. Coagulation factor content is similar for SDP and FFP, with notable exceptions of less F V, vWF, and ADAMTS-13 in SDP. Cytokine concentrations (TNFα, IL-8, and IL-10) were significantly higher in FFP. The clinical relevance of these findings needs to be established in outcome studies.

Keywords: coagulation factor; cytokines; haemostasis; plasma; transfusion

Accepted for publication: 3 January 2011

The indications for transfusion of plasma are highly debated,[1] but include perioperative use in actively bleeding patients with multiple coagulation factor deficiencies and prevention of dilutional coagulopathy in patients with major trauma, massive haemorrhage, or both.[2] [3] Other guideline-based indications include single coagulation factor deficiencies whenever a factor concentrate is not available [e.g. factor (F) V deficiency; in some countries, F XI deficiency], multiple coagulation factor deficiency in the presence of bleeding or disseminated intravascular coagulation, and thrombotic thrombocytopenic purpura (TTP).[4] However, most clinical uses of plasma recommended by practice guidelines are not supported by evidence from randomized, prospective, controlled high-quality trials.[5]

Efficacy of plasma infusions depends on the indication for which it is prescribed, the dose and the composition of the plasma being administered. There is very little high-quality data from randomized prospective trials addressing the question of dosing in plasma therapy.[6–8]

Safety issues in plasma transfusion include: (i) allergic reactions, (ii) immunomodulatory effects (with increased risk of subsequent infection), (iii) transmission of infectious agents, (iv) transfusion-associated circulatory overload, and (v) transfusion-related acute lung injury (TRALI).[9–12] Allergic reactions ranging from pyrexia to anaphylaxis are the most frequent adverse effects with a frequency of 0.1–1%.[4] TRALI is a rare but clinically relevant adverse effect as it is associated with a high morbidity and increased mortality. Incidence data on TRALI are scarce but have been reported as 8% for intensive care patients.[13] [14] The UK registry severe hazards of transfusion (SHOT) reported >20 cases yr^{-1} of TRALI but in the 2009 report only nine of the 21 cases could be clearly linked to fresh-frozen plasma (FFP).[4] [15] [16] In 766 trauma patients, Bochicchio and colleagues[17] have shown that transfusion of

---

FFP is associated with an increased incidence of nosocomial infection and ventilator-associated pneumonia, and recently, an increased incidence of multiple organ failure and acute respiratory distress syndrome was found in patients treated with FFP.[18] In particular in patients undergoing surgery without massive transfusion, administration of FFP was associated with a trend towards increased mortality.[19 20]

FFP and solvent/detergent-treated plasma (SDP) are produced differently depending on national regulations. In Switzerland where this study was conducted, each unit of FFP is derived from a single donor. Two techniques exist for the production of plasma (i) by aphaeresis and leucodepletion or (ii) from whole blood filtered to reduce leucocytes. Plasma for transfusion is produced from male donors. Plasma from female donors is used to produce isolated coagulation factors. SDP on the other hand is derived from pools of multiple donors (1500 donors, batch volume 280 litre), which are then treated specifically to reduce the risk of adverse events—particularly the transmission of viral agents. Currently published studies comparing different coagulation factors in FFP and SDP are characterized by relatively small sample sizes in which the authors concluded that the differences between products were without clinical relevance.[21–24] In theory, pooled products such as SDP should have relatively uniform factor content, while FFP should show more variation as it is derived from individuals. To our knowledge, concentrations of cytokines in SDP or FFP have not been reported on a larger scale.

We hypothesized that SDP composition is more consistent compared with FFP, but that due to the production process, the concentration of coagulation and other factors might be lower, and that some adverse events related to plasma transfusion are mediated by cytokines in the plasma. We therefore designed this study to: (i) compare the concentration of coagulation parameters in plasma transfusion in SDP and FFP, (ii) compare the concentration range for these parameters, and (iii) determine the concentrations of cytokines potentially associated with adverse outcomes.

Methods

This study was performed after obtaining authorization by the local ethics committee (Kantonale Ethikkommission, Kanton Zürich, Switzerland, Study number StV 7-2007, amendment 1). In the University Children's Hospital in Zurich, consecutive samples of 9 ml from 25 different units of Octaplas® SDP (Octapharma AG, Lachen, Switzerland) were collected before they were transfused in paediatric cardiac surgery. The Octaplas® units were all delivered by the local blood bank of Zurich. Samples of 9 ml from 25 units of FFP produced by the local blood bank in Zurich were consecutively collected in the University Hospital in Zurich before transfusion to adult cardiac surgery patients. The samples were collected in sterile tubes without addition of citrate or EDTA.

Fibrinogen (FBG), F II, FV, F VII, F VIII, F IX, F X, F XIII, von Willebrand factor (vWF), D-Dimer, ADAMTS-13 protease, interleukin-1β (IL-1), IL-6, IL-8, IL-10, and tumour necrosis

factor-α (TNF-α) concentrations were determined by the institutional laboratory.

Assessment of factor levels was performed in a quality-controlled ISO 17025 accredited institutional laboratory on a Behring coagulation system (Behring Coagulation System, Dade Behring, Düdingen, Switzerland) platform using the manufacturer's reagents (see below) and according to the manufacturer's instructions. Multifibrin® U (fibrinogen), Inovin® (F II, F V, F X), Pathromtin® SL (F VIII, F IX), and Berichrom® F XIII (F XIII) were the respective activators/reagents. The D-Dimer assay was performed using the Tina-quant® reagent [Tina-quant®, Roche Diagnostics (Schweiz) AG, Rotkreuz, Switzerland] on the same platform. The reference range for the assays is as follows: fibrinogen (1.5–4.0 g litre^{-1}), F II (60–150%), F V (50–150%), F VII (60–150%), F VIII (50–200%), F IX (50–200%), F X (60–150%), F XIII (70–140%), vWF 50–200%, and D-Dimer ($<$500 μg litre^{-1}). ADAMTS-13 or vWF-cleaving protease was determined as described by Kostousov and colleagues.[25] The normal range for this assay is 60–130%.

Cytokines were determined using R&D Systems® assays (R&D Systems®, Minneapolis, MN, USA) and the manufacturer's reagents (see below) according to the manufacturer's instructions. Reference range and lot numbers for the kits are as follows: Interleukin 1 Human IL-1 beta Quantikine ELISA Kit (Catalogue no. DLB50, Lot 270960, normal range $<$3.9 pg ml^{-1}), Interleukin 6 Human IL-6 Quantikine ELISA Kit (Catalogue no. S6050, Lot 269425, normal range $<$3.1 pg ml^{-1}), Interleukin 8 Human IL-8 Quantikine ELISA Kit (Catalogue no. D8000C, Lot 270510, normal range $<$0.1 pg ml^{-1}), Interleukin 10 Human IL-10 Quantikine ELISA Kit (Catalogue no. D1000B, Lot 271015, normal range $<$8.0 pg ml^{-1}), TNF-α Human TNF-α Quantikine HS ELISA Kit (Catalogue no. SSTA00D, Lot 270475, normal range $<$6.3 pg ml^{-1}), and Quantikine Immunoassay Control Group 1 (Catalogue no. QC01-1, Lot 208867). Measurements of the ELISA which were less than the limit of detection were registered as zero.

Statistical and graphic analyses

The sample size of 25 per group was determined by power analysis based on the data of Nifong and colleagues[26] to permit determination of a 5% difference in concentration with a power of 90%. Data were transferred from the hospital information system into Microsoft Excel (Microsoft Office 2007, Microsoft Corporation, Redmond, WA, USA) and analysed using PASW Statistic® (version 18, SPSS Inc., Chicago, IL, USA). Factors were expressed as mean (SD), median with minimum and maximum. A Mann–Whitney test and Levene's test for equality of variances were used. $P<0.05$ is considered significant. Sigma Plot for Windows (version 11.0, Systat Software Inc., San Jose, CA, USA) was used to prepare graphs.

Results

Twenty-five plasma samples of SDP and 25 of FFP were analysed for concentrations of coagulation factors and cytokines
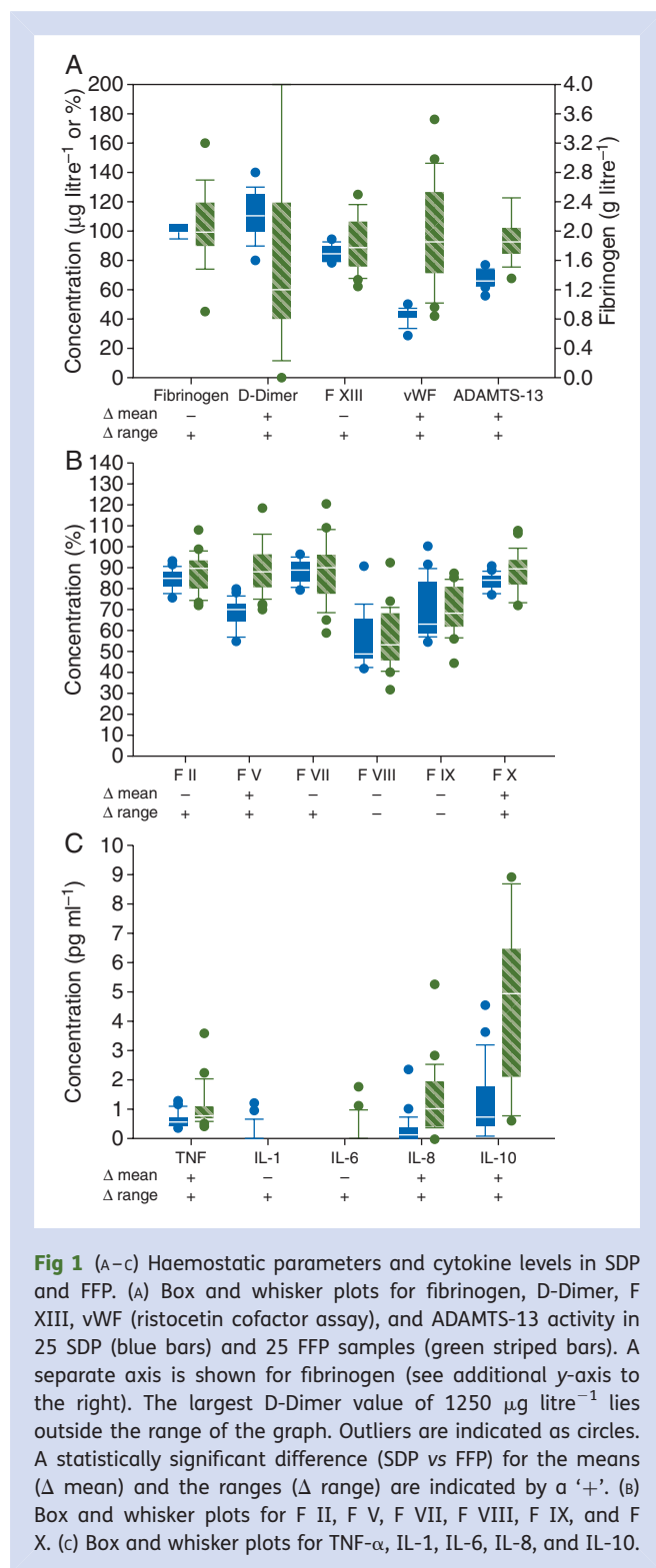
Fig 1 (A–C) Haemostatic parameters and cytokine levels in SDP and FFP. (A) Box and whisker plots for fibrinogen, D-Dimer, F XIII, vWF (ristocetin cofactor assay), and ADAMTS-13 activity in 25 SDP (blue bars) and 25 FFP samples (green striped bars). A separate axis is shown for fibrinogen (see additional y-axis to the right). The largest D-Dimer value of 1250 μg litre^{-1} lies outside the range of the graph. Outliers are indicated as circles. A statistically significant difference (SDP *vs* FFP) for the means (Δ mean) and the ranges (Δ range) are indicated by a '+'. (B) Box and whisker plots for F II, F V, F VII, F VIII, F IX, and F X. (C) Box and whisker plots for TNF-α, IL-1, IL-6, IL-8, and IL-10.

(Fig. 1A–C). Reference ranges for the determined factors are given in Table 1. Mean concentrations of FBG, F XIII, F II, F VII, F VIII, and F IX and also of IL-1 and IL-6 were similar in SDP and FFP. For vWF, ADAMTS-13, F V, and F X, and also for TNF-α, IL-8, and IL-10, significantly higher mean values (*P*<0.01) were found in FFP, whereas the mean value for

D-Dimers was significantly lower in SDP (*P*<0.01). Only in FFP, one sample was found with D-Dimers 2.5-fold higher than the reference range.

With the exception of F VIII and F IX, the range of measured values of other investigated parameters was significantly larger in FFP (*P*<0.01) than in SDP (Table 1, Fig. 1A–C). Whereas IL-1 was not detected in FFP (Fig. 1C), the measured values ranged from zero to 1 pg ml^{-1} in SDP. In contrast, IL-6 was not detected in SDP (Fig. 1C), but showed a large range in FFP. For the five cytokines tested, only two, TNF-α and IL-10, were detected in 25 of 25 samples. For the other three cytokines, samples with undetectable levels were found: IL-1 (SDP 22 of 25, FFP 25 of 25), IL-6 (SDP 25 of 25, FFP 22 of 25), and IL-8 (SDP 12 of 25, FFP one of 25).

For SDP with the exception of F VIII (15 of 25 samples under the reference range) and vWF (24 of 25 samples under the reference range), all other measurements for individual factors were found within the reference range for each test (Table 1). In FFP samples, F VIII (nine of 25 samples), F IX (one of 25 samples), F XIII (four of 25 samples), and vWF (two of 25 samples) were under the reference range (Table 1). However, in SDP, 24 of 25 samples had vWF under the reference range. For F VIII in SDP, 15 of 25 samples had values under the reference range. In 13 of 25 samples of SDP, IL-8 was above the reference range with the highest measured value being 2.00 pg ml^{-1} (Table 1). In contrast, FFP values for F XIII (four of 25 samples), vWF (two of 25 samples), F VIII (nine of 25 samples), and F IX (one of 25 samples) were considerably below the reference range (Table 1). TNF-α and IL-1 were within the normal range in all SDP and FFP samples. However, in FFP, IL-8 (in 24 of 25 samples) and IL-10 (in three of 25 samples) concentrations were detected well above the reference range with values up to 5.19 and 12.80 pg ml^{-1}, respectively (Table 1).

Discussion

The main findings are that (i) the composition of SDP and FFP was similar in most aspects, (ii) the mean value of D-Dimer, vWF, ADAMTS-13, F V, TNF-α, IL-8, and IL-10 was significantly higher in FFP compared with SDP, and (iii) there was a significantly higher variability of the individual values in FFP.

Treatment of congenital or acquired coagulation factor deficiencies requires therapeutic agents with predictable and sufficient concentrations of the respective factors. Unpredictable or insufficient coagulation factor content could preclude efficient and safe patient body weight-guided replacement therapy. SDP and FFP are medical products used for the treatment of coagulation disorders in major bleeding with multiple coagulation factor deficiencies or in deficiency of factors that cannot be replaced by single factor concentrate, such as F V deficiency.[27]

FBG and F XIII are key determinants for clot stability.[28] Both are present in SDP and FFP in similar amounts. The observed ranges are larger in FFP, making the prediction and achievement of a post-transfusional or target level

Table 1 Reference ranges and measured values in SDP and FFP. Values are presented as mean; median (minimum; maximum); and mean and variances of the individual parameters. *P<0.01; **P<0.001

Haemostatic parameters/ cytokines	Number of samples outside the normal range, SDP; FFP	Reference range	SDP	FFP
Fibrinogen (g litre^{-1})	0; 1	1.5–4.0	2.0; 2.0 (1.9; 2.1)	2.0; 2.0 (0.9; 3.2)**
D-Dimer (μg litre^{-1})	0; 1	<500	110; 110 (80; 140)	130*; 60 (0; 1250)*
F XIII (%)	0; 4	70–140	86; 85 (79; 95)	91; 89 (62; 126)**
vWF (%)	24; 2	50–200	43; 44 (29; 50)	97**; 93 (43; 176)**
ADAMTS-13 (%)	1; 0	60–130	68; 66 (56; 78)	94**; 93.0 (68; 123)**
F II (%)	0; 0	60–150	84; 84 (75; 93)	87; 90 (72; 108)*
F V (%)	0; 0	50–150	69; 70 (55; 80)	89**; 88 (70; 108)*
F VII (%)	0; 0	60–150	88; 89 (79; 96)	88; 90 (59; 120)**
F VIII (%)	15; 9	50–200	65; 49 (42; 191)	56; 53 (32; 92)
F IX (%)	0; 1	50–200	74; 63 (55; 134)	70; 68 (45; 87)
F X (%)	0; 0	60–150	84; 84 (77; 91)	89*; 88 (72; 108)*
TNF-α (pg ml^{-1})	0; 0	<6.3	0.65; 0.58 (0.40; 1.23)	1.03**; 0.80 (0.43; 3.55)*
IL-1 (pg ml^{-1})	0; 0	<3.9	0.10; 0.00 (0.00; 1.00)	0.00**
IL-6 (pg ml^{-1})	0; 0	<3.1	0	0.15; 0 (0.00; 2.00)**
IL-8 (pg ml^{-1})	13; 24	<0.1	0.29; 0.11 (0.00; 2.00)	1.28**; 1.01 (0.00; 5.00)*
IL-10 (pg ml^{-1})	0; 3	<8.0	1.20; 0.70 (0.10; 4.50)	4.66**; 4.90 (0.60; 12.80)*

more difficult and uncertain. For both FBG and F XIII, factor concentrates are available in Europe and many other countries. Plasma might thus not represent the treatment of choice for complex deficiency states with low FBG, F XIII concentrations, or both, when the aim of treatment is an isolated increase in FBG or F XIII levels.

There is a potentially relevant difference in the production of SDP and FFP that is illustrated by the D-Dimer levels. In one unit of FFP, we observed D-Dimer levels of 1250 μg litre^{-1}. This value was 2.5-fold higher than the maximum of the reference range. Despite the selection process to assure the recruitment of only healthy donors, some FFP units might contain fibrin split products in concentrations high enough to interfere with fibrin polymerization. In patients with fully activated coagulation, additional administration of D-Dimers by transfusion of FFP could further inhibit fibrin polymerization.[29] Each unit of FFP is generated from a single donor. SDP is generated from a large pool of donor plasma, which overcomes the problem of extreme values in a single unit. This explains why the range of nearly all parameters measured was smaller in SDP than FFP.

Mean vWF concentrations were significantly lower in SDP (43% *vs.* 97% in FFP). This can be of clinical relevance in cases of unknown vWF deficiencies or perioperatively acquired vWF deficiencies. The latter can develop despite the perioperative inflammatory stimulus which results in an increase in vWF levels. As factor concentrates and recombinant vWF formulations are available, these preparations would be preferred over an SDP and FFP for most patients with a known vWF deficiency.

ADAMTS-13 protease mediates degradation of vWF multimers following their release from the endothelium. Severe ADAMTS-13 deficiency with activity below 5–10% is associated with clinically overt TTP.[30] Plasma therapy is directed at restoration of ADAMTS-13 activity to levels above a putative critical threshold and replacement of inhibitor containing plasma in the subset of patients with acquired TTP. The significantly higher amount of ADAMTS-13 protease in FFP found in the current study suggests FFP as the preferred therapeutic option for ADAMTS-13 in TTP or sepsis associated with DIC, where deficiency of ADAMTS-13 might be associated with unfavourable outcome.[31] However, a threshold has not been validated by outcome studies and the lower concentrations of ADAMTS-13 in SDP might in fact be sufficient. UK guidelines for TTP suggest that treatment duration be defined by the patient's clinical response to treatment and not the achievement of a putative target level, and that a pathogen reduced formulation of plasma such as SDP is preferred over FFP.[32]

For combined factor deficiencies in patients with massive bleeding, major indications for plasma transfusion, both SDP and FFP appear adequate based on the measured activities. For F VIII and F IX deficiencies, the two factors in which we observed the lowest concentrations, factor concentrates and recombinant forms are the treatment of choice.

The fact that we observed a greater variability of factor concentrations in FFP than SDP might be clinically relevant when only few plasma products are transfused. In this setting, unexpectedly low post-transfusion levels might result from FFP transfusion, whereas in SDP, the response

will likely be more predictable because of less variation. This aspect can be neglected in massive transfusions with the administration of a high number of plasma units administered where the inter-unit variability is of minor importance since the factor concentrations tend towards the mean, which was not statistically different between FFP and SDP.

There are data suggesting that soluble mediators in blood products could be responsible for immunomodulatory adverse effects associated with transfusion,[33] including TNF-α, IL-1, IL-6, IL-8, and IL-10,[34][35] and also in febrile transfusion reactions[36] and lung dysfunction after cardiopulmonary bypass.[37] On the basis of the hypothesis that transfusion of cytokines into a susceptible host might favour the occurrence of adverse events, we also measured cytokine concentrations in SDP and FFP. FFP contained higher mean concentrations of TNFα, IL-8, and IL-10. IL-1 was only detected in three of 25 preparations of SDP, whereas IL-6 was only found in three FFP preparations. Measurements higher than the reference range were only observed for IL-10 in FFP. These findings support the hypothesis of immunomodulatory effects for 'cell-free' plasma products. *In vitro* data from Schneider and colleagues[38] lend further support to this hypothesis. They reported that blood products (FFP, platelet, and red blood cells) influence spontaneous and stimulated cytokine release. An increase in IL-10 was observed and might be one of the reasons for transfusion-associated immunomodulation leading to higher rates of infections in transfused patients. Another potential adverse effect of plasma products with high IL-10 content is IL-10-dependent development of anaemia in patients with a systemic inflammatory state, that is, Crohn's disease.[39]

Limitations of this pilot study are that no outcome data were assessed, that it is a single institution study, that blood group status was not included, and that coagulation inhibitors were not measured. Strengths of this unsponsored study include a relatively large sample size (25 SDP and FFP units each), and inclusion of cytokine data.

The choice of a plasma product be it SDP, FFP, or factor concentrates necessitates a critical benefit/risk evaluation.[19] On the basis of recent findings that the use of plasma is associated with a higher incidence of nosocomial infections, multiple organ failure, lung injury, and possibly with an increase in mortality,[18] the accepted and mostly non-evidence-based guidelines for plasma transfusion need to be revised. In the context of 'simple' clotting factor deficiencies involving one or few identified coagulation factors, replacement therapy with specific factor concentrates appears more desirable than the use of unfractionated products in view of documented efficiency and lower risk of adverse reactions. Non-infectious complications including volume overload and TRALI and also potential infectious complications need to be integrated into the risk–benefit analysis that should precede every potential transfusion of plasma products. Indeed while TRALI has been reported after FFP use, we are not aware of TRALI after SDP transfusion.[40] A currently undisputed indication for SDP or FFP remains F V deficiency, for which no factor concentrate is currently available.[21]

In conclusion, our data show that coagulation factor composition of SDP and FFP are similar in many regards. Statistically and possibly also clinically relevant differences in individual parameters exist with significantly higher concentrations of F V, vWF, and ADAMTS-13 and more interestingly of D-Dimers, IL-8, and IL-10 in FFP. In other instances where multiple factors need to be replaced simultaneously, SDP might be the preferred agent because of the more uniform distribution of most coagulation factors and lower cytokine levels. However, outcome studies will be necessary to determine the clinical impact of these *in vitro* findings.

Acknowledgements

Conflict of interest

References

1 Snyder CW, Weinberg JA, McGwin G Jr, *et al.* The relationship of blood product ratio to mortality: survival benefit or survival bias? *J Trauma* 2009; **66**: 358–62, discussion 62–4

2 Kor DJ, Stubbs JR, Gajic O. Perioperative coagulation management—fresh frozen plasma. *Best Pract Res Clin Anaesthesiol* 2010; **24**: 51–64

3 Rahe-Meyer N, Pichlmaier M, Haverich A, *et al.* Bleeding management with fibrinogen concentrate targeting a high-normal plasma fibrinogen level: a pilot study. *Br J Anaesth* 2009; **102**: 785–92

4 O'Shaughnessy DF, Atterbury C, Bolton Maggs P, *et al.* Guidelines for the use of fresh-frozen plasma, cryoprecipitate and cryosupernatant. *Br J Haematol* 2004; **126**: 11–28

5 Stanworth SJ, Hyde CJ, Murphy MF. Evidence for indications of fresh frozen plasma. *Transfus Clin Biol* 2007; **14**: 551–6

6 Stanworth SJ, Brunskill SJ, Hyde CJ, McClelland DB, Murphy MF. Is fresh frozen plasma clinically effective? A systematic review of randomized controlled trials. *Br J Haematol* 2004; **126**: 139–52

7 Roback JD, Caldwell S, Carson J, *et al.* Evidence-based practice guidelines for plasma transfusion. *Transfusion* 2010; **50**: 1227–39

8 Drews RE. Critical issues in hematology: anemia, thrombocytopenia, coagulopathy, and blood product transfusions in critically ill patients. *Clin Chest Med* 2003; **24**: 607–22

9 Reutter JC, Sanders KF, Brecher ME, Jones HG, Bandarenko N. Incidence of allergic reactions with fresh frozen plasma or cryosupernatant plasma in the treatment of thrombotic thrombocytopenic purpura. *J Clin Apher* 2001; **16**: 134–8

10 Popovsky MA, Audet AM, Andrzejewski C Jr. Transfusion-associated circulatory overload in orthopedic surgery patients: a multi-institutional study. *Immunohematology* 1996; **12**: 87–9

11 Dara SI, Rana R, Afessa B, Moore SB, Gajic O. Fresh frozen plasma transfusion in critically ill medical patients with coagulopathy. *Crit Care Med* 2005; **33**: 2667–71

12 Chakrabarty P, Rudra S, Hoque MM. Update of pathogen reduction technology for therapeutic plasma. *Mymensingh Med J* 2010; **19**: 308–11

13 Gajic O, Rana R, Winters JL, *et al.* Transfusion-related acute lung injury in the critically ill: prospective nested case–control study. *Am J Respir Crit Care Med* 2007; **176**: 886–91

14 Inaba K, Branco BC, Rhee P, *et al.* Impact of plasma transfusion in trauma patients who do not require massive transfusion. *J Am Coll Surg* 2010; **210**: 957–65

15 Holness L, Knippen MA, Simmons L, Lachenbruch PA. Fatalities caused by TRALI. *Transfus Med Rev* 2004; **18**: 184–8

16 Stainsby D, Jones H, Asher D, *et al.* Serious hazards of transfusion: a decade of hemovigilance in the UK. *Transfus Med Rev* 2006; **20**: 273–82

17 Bochicchio GV, Napolitano L, Joshi M, *et al.* Blood product transfusion and ventilator-associated pneumonia in trauma patients. *Surg Infect (Larchmt)* 2008; **9**: 415–22

18 Watson GA, Sperry JL, Rosengart MR, *et al.* Fresh frozen plasma is independently associated with a higher risk of multiple organ failure and acute respiratory distress syndrome. *J Trauma* 2009; **67**: 221–7, discussion 8–30

19 Murad MH, Stubbs JR, Gandhi MJ, *et al.* The effect of plasma transfusion on morbidity and mortality: a systematic review and meta-analysis. *Transfusion* 2010; **50**: 1370–83

20 Welsby IJ, Troughton M, Phillips-Bute B, *et al.* The relationship of plasma transfusion from female and male donors with outcome after cardiac surgery. *J Thorac Cardiovasc Surg* 2010; **140**: 1353–60

21 Inbal A, Epstein O, Blickstein D, Kornbrot N, Brenner B, Martinowitz U. Evaluation of solvent/detergent treated plasma in the management of patients with hereditary and acquired coagulation disorders. *Blood Coagul Fibrinolysis* 1993; **4**: 599–604

22 Haubelt H, Blome M, Kiessling AH, *et al.* Effects of solvent/detergent-treated plasma and fresh-frozen plasma on haemostasis and fibrinolysis in complex coagulopathy following open-heart surgery. *Vox Sang* 2002; **82**: 9–14

23 Hellstern P. Fresh-frozen plasma, pathogen-reduced single-donor plasma or bio-pharmaceutical plasma? *Transfus Apher Sci* 2008; **39**: 69–74

24 Hellstern P, Haubelt H. Manufacture and composition of fresh frozen plasma and virus-inactivated therapeutic plasma preparations: correlation between composition and therapeutic efficacy. *Thromb Res* 2002; **107**(Suppl 1): S3–8

25 Kostousov V, Fehr J, Bombeli T. Novel, semi-automated, 60-min-assay to determine von Willebrand factor cleaving activity of ADAMTS-13. *Thromb Res* 2006; **118**: 723–31

26 Nifong TP, Light J, Wenk RE. Coagulant stability and sterility of thawed S/D-treated plasma. *Transfusion* 2002; **42**: 1581–4

27 Hoffman R, Benz E, Shattil SJ, eds. *Hoffman: Hematology: Basic Principles and Practice*, 4th Edn. Philadelphia: Elsevier Churchill Livingstone, 2005

28 Theusinger OM, Baulig W, Asmis LM, Seifert B, Spahn DR. In vitro factor XIII supplementation increases clot firmness in Rotation Thromboelastometry (ROTEM). *Thromb Haemost* 2010; **104**: 385–91

29 Nilehn JE, Nilsson IM. Demonstration of fibrinolytic split products in human serum by an immunological method in spontaneous and induced fibrinolytic states. *Scand J Haematol* 1964; **1**: 313–30

30 Moake J. Thrombotic thrombocytopenia purpura (TTP) and other thrombotic microangiopathies. *Best Pract Res Clin Haematol* 2009; **22**: 567–76

31 Kim YK, Lee J, Lee K-A, Kwon HU. Clinical significance of von Will-ebrand factor-cleaving protease (ADAMTS13) deficiency in patients with sepsis-induced disseminated intravascular coagulation. *Infect Chemotherapy* 2009; **41**: 78–81

32 Gibson BE, Todd A, Roberts I, *et al.* Transfusion guidelines for neonates and older children. *Br J Haematol* 2004; **124**: 433–53

33 Ghio M, Contini P, Ubezio G, Mazzei C, Puppo F, Indiveri F. Immunomodulatory effects of blood transfusions: the synergic role of soluble HLA Class I free heavy-chain molecules detectable in blood components. *Transfusion* 2008; **48**: 1591–7

34 Baumgartner JM, Nydam TL, Clarke JH, Banerjee A, Silliman CC, McCarter MD. Red blood cell supernatant potentiates LPS-induced proinflammatory cytokine response from peripheral blood mononuclear cells. *J Interferon Cytokine Res* 2009; **29**: 333–8

35 Klein HG. How safe is blood, really? *Biologicals* 2010; **38**: 100–4

36 Simon TL, Snyder EL, Stowell CP, Strauss RG, Solheim BG, Petrides M. *Rossi's Principles of Transfusion Medicine*, 4th Edn. UK: Wiley-Blackwell, 2009

37 Apostolakis E, Filos KS, Koletsis E, Dougenis D. Lung dysfunction following cardiopulmonary bypass. *J Card Surg* 2010; **25**: 47–55

38 Schneider SO, Rensing H, Graber S, *et al.* Impact of platelets and fresh frozen plasma in contrast to red cell concentrate on unstimulated and stimulated cytokine release in an in vitro model of transfusion. *Scand J Immunol* 2009; **70**: 101–5

39 Tilg H, Ulmer H, Kaser A, Weiss G. Role of IL-10 for induction of anemia during inflammation. *J Immunol* 2002; **169**: 2204–9

40 Triulzi DJ. Transfusion-related acute lung injury: current concepts for the clinician. *Anesth Analg* 2009; **108**: 770–6