Quarantine versus pathogen-reduced plasma-coagulation factor content and rotational thromboelastometry coagulation

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BACKGROUND: Different types of fresh-frozen plasma (FFP) exist, and the concentrations of plasma proteins vary between individuals and blood groups. Furthermore, processing may also influence the content. Quarantine-stored plasma (qFFP) and plasma that was pathogen-reduced using blood-safety (Intercept) technology (piFFP) were analyzed regarding procoagulant and anticoagulant hemostasis proteins, including endogenous thrombin (thrombin-generation) potential (ETP).

MATERIALS AND METHODS: Thirty-five samples of each type of FFP were analyzed using only male Blood Group O donors. FFP units were stored frozen for comparable periods of time before plasma protein content was assessed. Once the units were thawed, all tests were completed within 4 hours. The results are presented as means ± standard deviations or as median (minimum; maximum) and were compared using independent-sample t tests (significance, $p < 0.01$).

RESULTS: Significantly higher concentrations of adintegrin-like and metalloprotease with thrombospondin type-13 motifs (ADAMTS13), fibrinogen, Factor (F)V, FVIII, FXIII, protein S, protein S activity, antithrombin, microvesicle (<900 nm), and α2 antiplasmin were observed in qFFP. The variability of factors was significantly lower in piFFP. Tissue factor (TF) at 1 picomolar (pM) exhibited significantly longer lag time, a lower peak, lower ETP, and a lower velocity index in qFFP compared with piFFP. In TF at 5 pM, significant differences in lag time (longer in qFFP), velocity index (lower in qFFP), and peak (lower in qFFP) were observed. Rotational thromboelastometry revealed a significantly longer ($p = 0.002$) clot-formation time with intrinsic thromboelastometry for piFFP and a significantly shorter clotting time ($p = 0.004$) with thromboelastometry fibrinogen testing for piFFP.

CONCLUSION: Pathogen reduction reduces procoagulant and anticoagulant coagulation factors as well as variability. A thrombin-generation assay showed no reduced ETP and no supraphysiological thrombin generation. None of the FFP preparations is likely to be effective for treating fibrinogen deficiency.

The transfusion of human plasma is part of surgical, intensive care, internal medicine, and pediatric treatment protocols. Because of transfusion safety concerns, current manufacturing regulations require the application of multiple safety steps in

ABBREVIATIONS: ADAMTS13 = adintegrin-like and metalloprotease with thrombospondin type-13 motifs (also known as von Willebrand factor-cleaving protease); CAT = calibrated, automated thrombin-generation; CFT = clot-formation time; CT = clotting time; ETP = endogenous thrombin potential; FIBTEM = thromboelastometry using tissue factor activation plus platelet inhibition (with cytochalasin D) to evaluate the contribution of fibrinogen to clot formation; INTEM = intrinsic thromboelastometry; MCF = maximum clot firmness; piFFP = pathogen-reduced fresh-frozen plasma; pM = picomolar; qFFP = quarantine-stored fresh-frozen plasma; ROTEM = rotational thromboelastometry; TF = tissue factor; VWF = von Willebrand factor.

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the manufacture of fresh-frozen plasma (FFP) to minimize the risk of transmitting infectious agents. By law, these safety measures are prerequisites to the release of products intended for patient treatment.

Clinical-grade FFP is considered a labile blood product for transfusion and must fulfill the standards and quality requirements as stated in the "Vorschriften Blutspende SRK Schweiz,"[1] which relies on the "Guide to the Preparation, Use, and Quality Assurance of Blood Components" by the European Committee on Blood Transfusion.[2] The content of functional plasma proteins is different between individuals[3-9] and varies among donors between the ABO blood groups.[10-13] Therefore, the ABO blood group needs to be considered when carrying out a comparative study of different FFP products by assessing the quantity and activity of plasma proteins as a surrogate for biological effectiveness of the respective FFP product.

In Switzerland, three clinical-grade FFP products are on the market. These are quarantine (qFFP), pathogen-reduced (piFFP), and solvent detergent (SD-FFP) plasma. In this study, conventional qFFP and piFFP using the Intercept procedure (both manufactured by the Regional Blood Transfusion Center of Zurich) were compared regarding their content of procoagulant and anticoagulant factors as well as for adintegrin-like and metalloprotease with thrombospondin type-13 motifs (ADAMTS13) activity, rotational thromboelastometry (ROTEM), and global potency to generate thrombin in vitro.

MATERIALS AND METHODS

Approval of the protocol was waived by the local ethics committee (Kantonale Ethikkommission, Zurich, Switzerland), as no donor data were collected.

Manufacturing qFFP

Whole blood was collected from male donors (Blood Group O; single-unit FFP) using whole blood collection set NGR6449 from Fresenius Kabi AG and was depleted of white blood cells (WBCs) using the integrated filter. The WBC-depleted blood was subsequently centrifuged (for 15 minutes at 20°C and × 4068 *g*; Heraeus Cryofuge 6000i; Thermo Fisher Scientific), and plasma was separated from red blood cells (RBCs) using OptiPress II blood separators (Fresenius Kabi AG). Finally, plasma units (approx. 230 mL per unit of citrated, male, single-donor plasma) were flash frozen (Hof Einfriergerat; Hof Sonderanlagenbau GmbH) and stored at −25°C or lower. The average time from donation to freezing was 10:13 hours (range, 04:09-19:00 hours). The duration of storage was 4 months or more in quarantine, and stored units were assured for infectious safety by confirmed negative test results from testing the respective plasma donor for infectious markers at the end of the quarantine period.

Manufacturing piFFP

Whole blood was collected from male donors (Blood Group O; "mini"-pooled FFP from five identical blood group plasma donors) with whole blood collection set NGR6449 (Fresenius Kabi AG) and was WBC-depleted using the integrated filter. Whole blood was centrifuged (for 15 min at 20°C and × 4068 *g*; Heraeus Cryofuge 6000i; Thermo Fisher Scientific), and plasma was separated from RBCs using OptiPress II blood separators (Fresenius Kabi AG). Plasma units were subsequently stored overnight at 22 ± 2°C. On the next day, 5 to 6 units of plasma were pooled using the Plasmix plasma pooling kit (Grifols, Barcelona, Spain) and a sterile docking device (TSCD II; TerumoBCT, Lakewood, CO), and the units subsequently were split into two plasma components, each containing from 635 to 650 mL of plasma. Each plasma component was then docked onto an Intercept plasma-processing set (INT3103B; Cerus Corporation), which delivered amotosalen-HCl (amotosalen in plasma pool; approx. 136 μM), which inactivates DNA/RNA-containing pathogens while being illuminated with ultraviolet A light (Illuminator; Cerus Corporation). After illumination, unused amotosalen and its photoproducts were almost completely extracted by flow through the compound adsorption device, which is integrated into the plasma-processing set (final amotosalen concentration = ≤2 μM). Subsequently, each treated plasma component was divided into three equal therapeutic units of approximately 200 mL each, flash frozen (Hof Einfriergerat; Hof Sonderanlagenbau GmbH), and stored at −25°C or lower. The average time from donation to freezing was 14:59 hours (range, 09:43-16:48 hr).

Sampling

Thirty-five units of piFFP that had been stored on average for 253 days (range, 181-309 days) and 35 units of qFFP that had been stored on average for 270 days (range, 265-279 days) at −25°C or lower were included in the study. All FFP units were donated by male donors (ABO Blood Group O) and were manufactured by the Zurich Regional Blood Transfusion Service applying validated routine protocols. The FFP units were thawed in a water bath (temperature range, 25-37°C; Type 3044; Koettermann GmbH), and each unit was aliquoted into 16 × 3.5 mL sample tubes (no. 55.484; Sarstedt). After labeling of the tubes with a unique identification code (blinding assessors to which type of FFP the tubes contained), the plasma tubes were refrozen and stored at −25°C or lower until they were shipped on dry ice to the analytical laboratory of the University Hospital Zurich for assessment. The time from

thawing the plasma units to refreezing the aliquots was 62 minutes or less.

Coagulation assays

The blinded sample tubes were stored at −80°C until the coagulation assays were run. Each aliquot was thawed only once. To minimize interassay variation, determination of the coagulation parameters was performed in blocks for each respective parameter; therefore, all aliquots were thawed only once and were not refrozen. All analyses with the exception of ADAMTS13 activity were performed using a BCS XP coagulation analyzer (Siemens Diagnostics).

ADAMTS13 activity

ADAMTS13 activity was determined with an in-house assay using fluorescence resonance energy transfer (FRET) with a synthetic 73-amino-acid von Willebrand factor (VWF) peptide (FRETS-VWF73) as previously described.[14]

Fibrinogen

Fibrinogen was determined with a functional assay according to Clauss[15] using a commercial automated clotting assay (Multifibren U; Siemens Diagnostics). In addition, fibrinogen antigen concentrations were determined using a commercial automated assay (Liaphen fibrinogen; Hyphen Biomed).

Coagulation factors V, VIII, and XIII

Factor (F)V activity was determined using a commercial automated clotting assay (Cryocheck FV-deficient plasma; Precision Biologics), and FVIII, activity was assessed using a commercial automated clotting assay (FVIII-deficient plasma; Siemens Diagnostics). FXIII activity was determined using a commercial automated chromogenic assay (Berichrom FXIII; Siemens Diagnostics).

von Willebrand factor

VWF activity and its ability to interact with platelet glycoprotein Ib/IX were determined with a commercial automated immunoturbidimetric assay using spontaneous binding of VWF to recombinant glycoprotein 1b (GPIb) containing a gain-of-function mutation (VWF:GPIbM; Innovance VWF Ac; Siemens Diagnostics).

Coagulation inhibitors (antithrombin, free protein S antigen, and protein S activity)

Antithrombin activity was determined using a commercial automated chromogenic assay with FXa-inhibition as an endpoint (Berichrom antithrombin III; Siemens Diagnostics). Free protein S antigen concentrations were determined using a commercial automated assay (Coamatic protein S free; Chromogenix by Instrumentation Laboratory). Protein S activity was determined using a commercial automated assay (protein S Ac reagent; Siemens Diagnostics).

Microvesicle levels–flow cytometry

The distribution of extracellular vesicle populations was determined by high-resolution cytometry using BD FACSCanto II (BD Biosciences) and FACS Diva 8.0 Software (BD Biosciences). Cytometer configuration was based on the manufacturer's specifications for the analysis of Nanoparticles ("Megamix" fluorescent beads; Biocytex). Particle sizes were calibrated with 0.5 μm, 0.9 μm, and 3 μm "Megamix" fluorescent beads (Biocytex). Side scatter and forward scatter channel calibrations and settings were used for later detection using the 488-channel (FL2).

Plasma extracellular vesicles were labeled by triple staining with fluorescent antibodies, including antihuman CD41-phycoerythrin/antihuman CD61-PC7 (Beckman Coulter) and antihuman CD41a fluorescein isothiocyanate (BD Biosciences). The analysis time was 180 seconds, and the flow rate was 16 μL per minute (medium available).

α2 Antiplasmin

The activity of α2 antiplasmin (plasmin inhibitor) was determined using a commercial automated chromogenic assay (Berichrom α2-antiplasmin; Siemens Diagnostics).

Thrombin-generation assay with TF 1 pM and TF 5 pM measured by calibrated automated thrombin generation analyses

The calibrated, automated thrombin-generation (CAT) assay was performed using the thrombogram method in a fluorescence plate reader (Fluoroskan Ascent; ThermoLab Systems) in Strasbourg, France. Aliquots (20 μL) of 1 picomolar (pM) tissue factor (TF) and 4 μM phospholipids (PPP reagent low; Stago) or 5 pM TF and 4 μM phospholipids (PPP reagent; Stago) were added to 80 μL of FFP samples in a 96-well plate (Immulon 2 Dynex; Stago) and maintained at 37°C. Thrombin generation was started by the automated addition of fluorogenic thrombin substrate Z-GGR-AMC and $CaCl_2$ (FluoCa kit; Stago). The accumulation of fluorescence from cleaved Z-GGR-AMC was measured continuously at excitation and emission wave lengths of 390 and 460 nm, respectively. First derivative curves of the fluorescence accumulation were converted into thrombin concentrations using a human thrombin calibrator (Stago) and Thrombinoscope BV software (Stago). The area under the curve, also called the endogenous thrombin potential (ETP), corresponds to the total amount of thrombin formed in the sample. The peak height (TP peak) corresponds to the maximum amount of thrombin generated. The lag time (LT) is the moment at which thrombin formation really starts. The time to peak

TABLE 1. Factor concentrations in quarantine-stored and pathogen-reduced fresh-frozen plasma

Factor	Mean ± SD (median [min;max])* qFFP	piFFP	p value
ADAMTS13 activity, %	114 ± 19 (113 [82;149])	95 ± 10 (95 [76;114])	< 0.001
VWF activity, %	71 ± 21 (68 [30;120])	73 ± 12 (68 [44;99])	0.52
FXIII, %	103 ± 19 (104 [71;145])	87 ± 7 (87 [75;107])	< 0.001
Fibrinogen, g/L†	2.3 ± 0.6 (2.3 [1.2;3.4])	1.8 ± 0.2 (1.8 [1.3;2.2])	< 0.001‡
Antigenetic fibrinogen, g/L	2.8 ± 0.6 (2.7 [1.5;4.1])	2.6 ± 0.3 (2.6 [2.2;3.2])	0.20‡
Antithrombin, %	90 ± 8 (91 [67;114])	86 ± 4 (85 [76;95])	0.003
FVIII, %	81 ± 21 (80 [48;128])	53 ± 7 (51 [40;71])	< 0.001
FV, %	102 ± 14 (103 [72;132])	93 ± 10 (93 [72;112])	0.002
Protein S, %	82 ± 23 (80 [30;152])	80 ± 11 (82 [61;105])	0.93
Protein S activity, %	73 ± 14 (69 [46;106])	64 ± 8 (65 [42;82])	0.003
α Antiplasmin, %	102 ± 6 (100 [90;119])	83 ± 3 (83 [73;91])	< 0.001
Microvesicle <900 nm§	523 ± 379 (402 [55;1422])	322 ± 137 (319 [75;629])	0.005

*Data shown are the mean ± standard deviation (median [minimum;maximum]).
†Fibrinogen concentrations were determined using a functional assay (according to Clauss[15]) and an immunogenetic assay (antigen).
‡This p value is for the difference between antigentic and functional fibrinogen content for both plasma types ($p < 0.001$).
§Microvesicle levels were determined by staining with CD41 phycoerythrin-fluorescein isothiocyanate and CD61 phycoerythrin-cyanine 7.

(ttPeak) corresponds to the moment at which the maximum thrombin concentration is attained. The velocity index (velocity index = TP/[ttP-LT]) is the slope between LT and ttPeak.

ROTEM

The ROTEM device (ROTEM delta; TEM International) analyzes the kinetics and quality of clot formation and clot lysis in whole blood and in real time. In the current study, tests were run with plasma only. The following tests were performed: intrinsic thromboelastometry (INTEM) (ellagic acid-activated intrinsic pathway) and FIBTEM (thromboelastometry using tissue factor activation plus platelet inhibition with cytochalasin D to evaluate the contribution of fibrinogen to clot formation). The following variables were determined: clotting time (CT), clot formation time (CFT), maximal clot firmness, and α-angle. Two identical ROTEM devices were used, each of which was equipped with four channels. Both devices underwent complete technical revision by the manufacturer before the study started. Tests ran for exactly 62 minutes. Further technical details of ROTEM coagulation analysis were published previously by Theusinger et al.[16]

Statistical analyses

Considering FV in our earlier publication by Theusinger colleagues,[17] the required number of study samples per plasma preparation was estimated to catch a difference in FV content of 10% or more in piFFP compared with qFFP with statistical power of 80% or more at a significance level of $p = 0.01$.

Data were collected using Microsoft Excel (Microsoft Office 2010; Microsoft Corporation) and analyzed using IBM SPSS Statistics version 22 (IBM Corporation). Continuous variables are summarized as mean ± standard deviation values and are also presented as median [minimum; maximum] values. Plasma types were compared using the independent-sample t test. p values <0.01 are considered statistically significant.

RESULTS

Most procoagulant and anticoagulant factors (ADAMTS13 activity, fibrinogen [using the Clauss method], FV, FVIII, FXIII, antithrombin, and α2 antiplasmin) were significantly ($p < 0.01$) higher in qFFP than in piFFP. There was no significant difference in VWF (VWF:GPIbM) or free protein S antigen concentrations between the two FFP preparations. Protein S activity in qFFP was significantly higher than in piFFP. The content of microvesicle (<900 nm) was significantly higher ($p = 0.005$) in qFFP compared with piFFP. The individual variability of factor concentrations was higher in qFFP compared with piFFP. The detailed results are provided in Table 1.

Fibrinogen concentrations were significantly lower ($p < 0.001$) in piFFP compared with qFFP when determined by functional assay according to Clauss. Determination of the fibrinogen antigen by immunological assay reached no level of significance between the two FFPs, although fibrinogen concentrations were still lower in piFFP. This discrepancy was more pronounced in piFFP than in qFFP (Table 1).

Thrombin generation as measured by CAT using 1 pM TF exhibited a significantly shorter ($p < 0.01$) lag-time for piFFP, a significantly higher TP peak for piFFP, and a significantly higher velocity index for piFFP. Furthermore, the ETP was significantly lower ($p < 0.001$) in qFFP (Table 2). Using 5 pM TF, a significantly longer lag time, lower TP peak, and significantly lower velocity index were observed for qFFP. ttPeak was longer in qFFP compared with piFFP ($p < 0.001$), and ETP was not

TABLE 2. Thrombin-generation measurement using the CAT assay with 1 pM TF

	Mean ± SD (median [min;max])*		
TF 1 pM	qFFP	piFFP	p value
Lag time, min	8.2 ± 2.6 (7.6 [4;16])	5.0 ± 1.1 (4.9 [3;8])	< 0.001
ETP, nmol * min/L	597 ± 176 (583 [302;992])	966 ± 215 (1036 [559;1256])	< 0.001
TP, nmol/L	41.2 ± 13.8 (39.2 [20;70])	75.5 ± 29.7 (78.4 [33;137])	< 0.001
ttPeak, min	17.1 ± 3.1 (16.3 [12;26])	13.1 ± 2.3 (13 [9;17])	< 0.001
Velocity index, nM/min	4.8 ± 2.0 (4.5 [2;9])	10.3 ± 6.0 (10.2 [3;25])	< 0.001

*Data shown are the mean ± standard deviation (median [minimum;maximum]).
TP = thrombin peak; ttPeak = time to thrombin peak.

TABLE 3. Thrombin-generation measurement using the CAT assay with 5 pM TF

	Mean ± SD (median [min;max])*		
TF 5 pM	qFFP	piFFP	p value
Lag time, min	2.9 ± 0.6 (3 [2;4])	2.4 ± 0.4 (2.7 [2;3])	< 0.001
ETP, nmol * min/L	1299 ± 193 (1289 [925;1658])	1282 ± 107 (1282 [925;1445])	0.65
TP, nmol/L	162.2 ± 40.6 (163.07 [82;250])	197.0 ± 47.6 (193.49 [129;291])	0.001
ttPeak, min	7.8 ± 1.8 (7.6 [5;11])	6.0 ± 1.4 (6.3[4;9])	< 0.001
Velocity index, nM/min	37.9 ± 18.1 (34.7 [12;78])	64.0 ± 35.5 (51.4 [24;150])	< 0.001

*Data shown are the mean ± standard deviation (median [minimum;maximum]).
TP = thrombin peak; ttPeak = time to thrombin peak.

TABLE 4. ROTEM results for contact activation and TF activation combined with platelet inhibition

	Mean ± SD (median [min;max])*		
ROTEM results	qFFP	piFFP	p value
INTEM			
CT, sec	342 ± 120 (311 [158;694])	402 ± 144 (405 [188;718])	0.07
CFT, sec	653 ± 1103 (258 [97;4414])	910 ± 873 (614 [118;3849])	0.002
MCF, mm	22 ± 6.0 (21 [11;34])	23 ± 2.6 (23 [17;28])	0.563
α Angle	61 ± 16 (67 [19;83])	59 ± 14 (59 [26;80])	0.397
FIBTEM			
CT, sec	142 ± 39 (127 [77;246])	138 ± 141 (111 [35;929])	0.004
CFT, sec	640 ± 649 (363 [79;2492])	741 ± 517 (565 [81;2231])	0.132
MCF, mm	23 ± 5.7 (23 [11;34])	24 ± 3.0 (24 [16;31])	0.403
α Angle	72 ± 6.2 (74 [56;82])	75 ± 5.0 (76 [62;84])	0.166

*Data shown are the mean ± standard deviation (median [minimum;maximum]).

significantly different between the two FFP preparations (Table 3).

ROTEM measurements revealed a significantly longer ($p = 0.002$) CFT in INTEM for piFFP compared with qFFP and a significantly shorter ($p = 0.004$) CT in FIBTEM for piFFP. No other parameters reached the level of significance (for details, see Table 4).

DISCUSSION

The main findings of this study are: 1) most procoagulant and anticoagulant factor levels are significantly lower in piFFP compared with qFFP except for VWF and protein S, 2) fibrinogen concentration measured using the Clauss method reveals significantly lower results in both plasma preparations compared with using the immunologic method (antigen detection), 3) fibrinogen concentrations in piFPP are lower than in qFFP, 4) piFFP does not generate supraphysiological thrombin, and 5) protein S activity as well as microvesicle (<900 nm) are higher in qFFP.

According to the literature, there is substantial variation in the methods used to prepare plasma and in the final content of coagulation factors.[18] In general, a reduction of up to 20% in coagulation factor concentration from baseline is considered acceptable for pathogen-reduced plasma[19] and does not seem to influence the clinical efficiency of plasma. Physiologically, the coagulation factor reference ranges are large (from 50 to 200%). For, example, the range for fibrinogen is from 1.5 to 4.0 g/L.

In this study, all factors measured in piFFP and qFFP were within the normal in vivo range. Nevertheless, piFFP had significantly lower concentrations of most procoagulant and anticoagulant factors compared with qFFP. When considering FV, to which the powered sample size applies, the average difference between piFFP and qFFP of FV concentration was −9% ($p = 0.01$) in piFFP (Table 1). This difference was −22% for fibrinogen, −35% for FVIII, −16% for FXIII, −17% for ADAMTS13, −4.4% for antithrombin, and −19% for α2 antiplasmin. Protein S and VWF exhibited minor differences of −2.4% and +2.8%, respectively, in piFFP compared with qFFP (neither difference was significant). The treatment of congenital or acquired coagulation factor deficiencies requires agents with a predictable and sufficient content of the respective factor. Although, in most cases, single-factor concentrates are administered, FFP still has a role in the prevention or treatment of bleeding in patients with multiple factor deficiencies or when a single factor concentrate is not available on the market (e.g., FV).[20]

Low protein S activity with normal protein S can lead to thrombosis, as reported by Flamholz and colleagues[21] in SD plasma. However, the SD plasma investigated by Flamholz and colleagues was produced in the United States by VI Technologies (Plas+ SD) and had a different quality than piFFP and the SD plasma currently manufactured in Europe by Octapharma (Octaplas). Although Plas+ SD, Octaplas, and piFFP exhibit significantly reduced protein S activities compared with qiFFP, protein S activities in Octaplas and piFFP are better retained and are still within normal range compared with Plas+ SD.[22] Thus, the clinical safety of large-volume plasma exchange therapy using piFFP or Octaplas remains to be assessed, because the process of pathogen reduction might also lead to that problem, in that our results were similar to those observed with SD plasma. On the other hand, the presence of microvesicle is reduced by the process of pathogen reduction, and this may be an advantage.

Even more important, FFP is the standard replacement fluid used for ADAMTS13 substitution during plasma exchange in patients with thrombotic thrombocytopenic purpura (TTP). ADAMTS13 mediates the degradation of VWF multimers after their release from the endothelium. Severe ADAMTS13 deficiency with activity below 5 to 10% is a strong risk factor for TTP and is identified in the majority (approximately 60%) of patients with the clinical diagnosis of an acute bout of TTP.[23] Plasma exchange therapy is directed at the restoration of ADAMTS13 activity to levels above a putative critical threshold and the removal of ADAMTS13-inhibitory autoantibodies in the subset of patients with acquired TTP. The amount of ADAMTS13 protease identified in both qFFP and piFFP suggests that both types of FFP are a therapeutic option for ADAMTS13 deficiency in TTP or sepsis associated with disseminated intravascular coagulation, in which a reduction in ADAMTS13 may be associated with an unfavorable outcome.[24] In other studies, SD-treated FFP had concentrations as low as 68% of ADAMTS13 but still may be sufficient; and, because it is pathogen-reduced, it may even be preferred to nonpathogen-reduced FFP according to UK guidelines.[17,25,26]

In our study, we found a discrepancy between the fibrinogen concentrations as determined by functional (according to Clauss) and immunologic (fibrinogen antigen) assays. This may indicate an alteration of the fibrinogen molecule by the manufacturing process (dysfibrinogen). The method published by Clauss produced significantly lower concentrations of functional fibrinogen in piFFP compared with the antigenetic determination (1.8 vs 2.6 g/L; $p < 0.001$). The difference between the two methods for measuring fibrinogen in qFFP was not as high (2.3 vs 2.8 g/L; $p < 0.001$) but was still significant. The possibility cannot be excluded that piFFP contains substances that interfere with the optical method of Clauss and thus preclude correct determination of the fibrinogen concentration. Fibrinogen concentrations in piFFP are lower than these measured by Theusinger and colleagues in SD-FFP.[17] Nevertheless, all of these fibrinogen concentrations are too low to adequately achieve levels more than 2.0 g/L in trauma patients, as recommended by the European trauma guidelines.[27]

FXIII is also reduced in piFFP; thus the production of cryoprecipitate out of piFFP will probably lead to a safer product, because its origin is from pathogen-inactivated plasma, but will probably lead to a lower hemostatic quality, because fibrinogen and FXIII will be reduced even more during the production process. However, the hemostatic quality of such cryoprecipitate remains to be evaluated.

Thrombin generation was measured using the CAT assay with two different concentrations of TF (1 pM vs. 5 pM). Actually, there is no standard concentration of TF that suits all analytic needs. The 1-pM assay is considerably more sensitive to changes in the intrinsic system (FVIII, F IX, and FXI) as well as levels of TF pathway inhibitor, whereas the 5-pM assay predominantly reflects changes in FVII, FX, FV, and prothrombin. The 1-pM TF assay is more physiological in the way it reflects the entire coagulation proteome (initiation phase, amplification, and propagation phase), whereas the 5-pM assay predominantly reflects factors involved with the initiation phase of thrombin generation. Our results could indicate that piFFP contains less functional TF pathway inhibitor, although TF pathway inhibitor was not formally measured. Although we observed a lower content of coagulation factors in piFFP compared with qFFP, the thrombin-generation measurements at TF 1 pM and 5 pM did not reflect a lower thrombin-generation potential of piFFP. Importantly, neither FFP source is likely to be effective in substituting fibrinogen deficiency. None of our data raise

concerns that piFFP would generate supraphysiological thrombin levels. Two phenomena also may contribute to these results; first, higher concentrations of microvesicle in qFFP on one side and, second, lower protein S activity with a normal protein S antigen in piFFP.[21]

ROTEM tests were performed in plasma only, leading to different results because of the lack of platelets and RBCs. The purpose was to assess the general coagulation properties using the intrinsic system and fibrinogen. These properties were identical for all measurements regarding fibrinogen in piFFP and qFFP except for CT, which was significantly lower in piFFP ($p = 0.002$). For INTEM, the CFT was significantly longer ($p = 0.004$) in piFFP compared with qFFP, which may be an indication of the lower content of coagulation factors in piFFP. All other parameters in INTEM were identical for both preparations. Furthermore, the lower concentration of microvesicle in piFFP might also lead to prolonged CT and CFT in INTEM. The identical maximal clot firmness for FIBTEM and INTEM, although coagulation factors were lower in piFFP, might be an indication of hypercoagulability or a possible risk of thrombosis due to the imbalance of protein S antigen (normal levels) and protein S activity (reduced levels) in piFFP.[21]

Limitations of this pilot study are the lack of clinical outcome data, which were not intended to be collected, and that it is a single-institution study. However, other groups have demonstrated the comparative clinical effectiveness of piFFP and qFFP for the support of liver transplantation.[28] Strengths of this study include a powered sample size based on FV concentration, the assessment of in vitro thrombin generation, and the exclusive use of male Blood Group O samples manufactured by only one FFP provider.

In conclusion, the process of pathogen inactivation leads to a significant reduction of several procoagulant and anticoagulant factors. Similar findings are reported for FFP that was pathogen-reduced using alternative methods, such as SD, methylene blue, or riboflavin (Mirasol) treatment.[19,29-32] Although factor concentrations are still within the in vivo reference range, in most cases, any kind of FFP is not the first choice for substituting coagulation factors in a bleeding patient because of its unfavorable ratio of factor content to volume. Most important, neither FFP source is likely to be effective in substituting fibrinogen deficiency. However, in other situations of acquired coagulopathies, such as liver disease, major surgery, or trauma, a balanced plasma product with all factors may be preferable to single-factor concentrates. In contrast to single-donor FFP, such as qFFP, pooled FFP preparations like our piFFP and SD-treated FFP have less variability of single-factor concentrations and may harbor a lower risk for transfusion-related acute lung injury complications to the recipient, as has been demonstrated with SD-treated FFP. The imbalance of protein S antigen and protein S activity in piFFP potentially could lead to an increased risk of thrombosis, and countries that are considering the production of cryoprecipitate out of piFFP will need to weigh the benefits of safety against the risk of reduced quality. However, outcome studies will be necessary to determine the clinical impact of these in vitro findings.

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CONFLICT OF INTEREST

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