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ORIGINAL ARTICLE

Fresh frozen plasma transfusion fails to influence the hemostatic balance in critically ill patients with a coagulopathy

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Summary. *Background:* Coagulopathy has a high prevalence in critically ill patients. An increased International Normalized Ratio (INR) is a common trigger to transfuse fresh frozen plasma (FFP), even in the absence of bleeding. Therefore, FFP is frequently administered to these patients. However, the efficacy of FFP in correcting hemostatic disorders in non-bleeding recipients has been questioned. *Objectives:* To assess whether INR prolongation parallels changes in the results of other tests investigating hemostasis, and to evaluate the coagulant effects of a fixed dose of FFP in non-bleeding critically ill patients with a coagulopathy. *Methods:* Markers of coagulation, individual factor levels and levels of natural anticoagulants were measured. Also, thrombin generation and thromboelastometry (ROTEM) assays were performed before and after FFP transfusion (12 mL kg^{-1}) to 38 non-bleeding critically ill patients with an increased INR (1.5–3.0). *Results:* At baseline, levels of factor II, FV, FVII, protein C, protein S and antithrombin were reduced, and thrombin generation was impaired. ROTEM variables were within reference ranges, except for a prolonged INTEM clot formation time. FFP transfusion increased the levels of coagulation factors (FII, 34% [interquartile range (IQR) 26–46] before vs. 44% [IQR 38–52] after; FV, 48% [IQR 28–76] before vs. 58% [IQR 44–90] after; and FVII, 25% [IQR 16–38] before vs. 37% [IQR 28–55] after), and the levels of anticoagulant proteins. Thrombin generation was unaffected by FFP transfusion (endogenous thrombin potential, 72% [IQR 51–88] before vs. 71% [IQR 42–89] after), whereas ROTEM EXTEM clotting time and maximum clot firmness slightly improved in response to FFP. *Conclusion:* In non-bleeding critically ill patients with a coagulopathy, FFP transfusion failed to induce a more procoagulant state.

Keywords: blood coagulation disorders; critical illness; International Normalized Ratio; plasma; thrombelastography.

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Background

Coagulopathy occurs in up to 30% of critically ill patients [1]. Seventy per cent of fresh frozen plasma (FFP) used by critical care facilities is transfused to patients with prothrombin time (PT) prolongation, mostly in the absence of bleeding [2]. To assess bleeding risk and the effectiveness of FFP transfusion, the clinically widely available International Normalized Ratio (INR) is often used. An increased INR indicates that at least one of the vitamin K-dependent coagulation factors is below the hemostatic threshold [3]. Even though the test is designed to monitor therapy with vitamin K antagonists, the INR is a common trigger to administer FFP in clinical practice [4]. However, in acquired coagulopathy in critically ill patients, where multiple clotting factors are deficient, the relationship between clotting factor levels and INR prolongation is complex and not well understood. Indeed, the INR represents only a part of the coagulation cascade, and is insensitive to the activities or concentrations of

anticoagulant and fibrinolytic proteins. In critically ill patients, and particularly in patients with disseminated intravascular coagulation (DIC), levels of anticoagulants are reduced, whereas fibrinolysis is attenuated [5,6].

The hemostatic balance is the net result of the presence of procoagulant, anticoagulant and fibrinolytic factors. Disturbance in the balance of these components can result in variable *in vivo* coagulation profiles, ranging from a hypocoagulable state with an increased bleeding tendency to a procoagulant state with (micro)vascular thrombus formation. Therefore, despite an elevated INR, patients may not necessarily have an increased bleeding tendency. In line with this, the INR poorly reflects the risk of bleeding in critically ill patients [7].

The efficacy of FFP in correcting coagulopathy has only been evaluated in small and heterogeneous studies [8–10]. Notably, the efficacy of FFP in preventing bleeding in coagulopathic critically ill patients has not been demonstrated [11]. In addition, the net effect of FFP transfusion on anticoagulant proteins and the hemostatic balance in critically ill patients is not well known [12].

In a predefined substudy of a multicenter randomized controlled trial on the efficacy of prophylactic FFP transfusion in preventing bleeding in critically ill patients with an increased INR who were scheduled to undergo an invasive procedure [13], we investigated whether INR prolongation parallels changes in the results of other tests investigating hemostasis, and evaluated the effect of a fixed dose of FFP on the hemostatic balance of these patients.

Materials and methods

The original study was approved by the medical ethics committee of the Academic Medical Center, Amsterdam, the Netherlands. Before patients were entered into the study, written informed consent was obtained from the patient or legal representative, in accordance with the Declaration of Helsinki. The study protocol was registered with trial identification numbers NTR2262 (Dutch Trial Register) and NCT01143909 (ClinicalTrials.gov).

Setting and patients

The study was performed between May 2010 and June 2013 in four mixed medical-surgical intensive care units (ICUs) in the Netherlands. Patients were eligible when the INR was 1.5–3.0 and they needed to undergo an intervention [14]. Although the INR was developed to monitor the effects of vitamin K antagonists, in daily practice it is one of the most commonly used tests to assess coagulopathy in critically ill patients, and an increased INR is often a trigger to transfuse FFP [4,15]. Also, use of the INR instead of PT facilitates the combination of results obtained in different centers.

Patients aged < 18 years or with a known bleeding diathesis, treated with vitamin K antagonists, activated protein C, abciximab, tirofiban, ticlopidine, or prothrombin complex concentrates, were excluded. Patients treated with therapeutic doses of heparin or low molecular weight heparin were allowed to participate if medication was discontinued for an appropriate period, which was > 2 h for heparin and > 12 h for low molecular weight heparin. The use of low molecular weight heparin in a prophylactic dose was part of standard care for all patients.

Design

This was a predefined *post hoc* study of a randomized controlled clinical trial on the risk and benefit of FFP transfusion in critically ill patients with a coagulopathy [13,14]. After inclusion, patients were randomized to a single dose of 12 mL kg^{-1} FFP or no FFP transfusion before a scheduled intervention. The FFP was quarantine plasma manufactured by Sanquin, the Dutch National Blood Bank. The dose of FFP was based on clinical practice and a previous study with a target of INR reduction to < 1.5 [9,16]. Patients were observed until 24 h after the intervention for bleeding complications.

Patient data and sample collection

Patient data were collected from the electronic patient data management system, and consisted of the medical history, admission diagnosis, use of anticoagulant medication, and occurrence of bleeding. DIC was assessed according to the ISTH DIC score, which defines overt DIC as a score of ≥ 5 points [17] (Table S1).

Blood samples were drawn from an indwelling arterial catheter at baseline, and the second sample was taken directly after FFP transfusion (but prior to the invasive procedure). Samples were collected in sodium citrate (0.109 M, 3.2%) tubes. Samples were centrifuged within 30 min at 2000 $\times g$ for 15 min at 18 °C, and subsequently for 5 min at 15 000 $\times g$, also at 18 °C. Supernatant was collected and stored at −80 °C until measurements were performed.

Assays

The PT, INR, activated partial thromboplastin time and levels of D-dimer and fibrinogen were all determined immediately after the sample was drawn, with standard assays on an automated coagulation analyzer (Sysmex CA7000 and all reagents: Siemens Healthcare Diagnostics, Marburg, Germany), according to the manufacturer's protocols. After termination of the study, the remaining assays were performed collectively in all patients. Levels of factor II, FV and FVII were determined with a PT-based one-stage clotting assay (ACL TOP 700; Instrumentation Laboratory, Bedford, MA, USA), with recombiplastin and FII-deficient, FV-deficient and FVII-deficient plasma (Instrumentation Laboratory). Antithrombin was assessed with a chromogenic substrate method (Sysmex

CA7000) with reagents and protocols of the manufacturer.

Protein C activity was measured with a kinetic assay (Coamatic Protein C; Chromogenix, Mölndal, Sweden). Total protein S levels were determined with an ELISA, as described previously [18]. Free protein S was measured by precipitating the C4b-binding protein-bound fraction with poly(ethylene glycol) 8000 and measuring the concentration of free protein S in the supernatant [18]. Thrombin–antithrombin complex (TATc), prothrombin fragment 1 + 2 (F_{1+2}) and plasmin-α2–antiplasmin complex (PAP) levels were measured with specific commercially available ELISAs, according to the manufacturers' instructions (Siemens Healthcare Diagnostics and DRG, Marburg, Germany).

Thrombin generation assay

The Calibrated Automated Thrombogram assays the generation of thrombin in clotting plasma by use of a microtiter plate reading fluorometer (Fluoroskan Ascent; ThermoLab Systems, Helsinki, Finland) and Thrombinoscope software (Thrombinoscope BV, Maastricht, the Netherlands). The assay was carried out as described by Hemker *et al*. [19] and the Thrombinoscope manual. Coagulation was triggered by recalcification in the presence of 5 pM recombinant human tissue factor (Innovin; Siemens Healthcare Diagnostics), 4 μM phospholipids, and 417 μM fluorogenic substrate Z-Gly-Gly-Arg-AMC (Bachem, Bubendorf, Switzerland). Fluorescence was monitored, and the parameters (lag time, peak thrombin, area under the curve, and endogenous thrombin potential [ETP]) were calculated with the Thrombinoscope software.

Thromboelastometry

With ROTEM (Tem International, Munich, Germany), three separate assays were carried out, including EXTEM to assess tissue factor-initiated coagulation, INTEM to assess the intrinsic pathway, and FIBTEM to qualitatively assess fibrinogen status. For EXTEM, 20 μL of 0.2 mol L^{-1} $CaCl_2$ (star-tem; Tem International GmbH, Munich, Germany) and 20 μL of recombinant tissue factor (*r* EXTEM; Tem International GmbH) were added to a test vial, and 300 μL of the citrated blood sample was then added. For INTEM, 20 μL of 0.2 mol L^{-1} $CaCl_2$ (star-tem), 20 μL of partial thromboplastin made of rabbit brain (in-tem; Tem International GmbH) and 300 μL of blood were added to the test cuvette. FIBTEM was carried out by adding 20 μL of recombinant human tissue factor (*r* EXTEM), 20 μL of platelet-inactivating cytochalasin D solution, 0.2 mol L^{-1} $CaCl_2$ and 300 μL of the blood sample to the test vial. The electronic pipette program guided all test steps. For INTEM and EXTEM, clotting time (CT), clot formation time (CFT), clot firmness (MCF), α-angle and maximum lysis were recorded. For FIBTEM, CT and MCF were recorded.

Statistics

All variables are expressed as median (interquartile range [IQR]). To compare groups, a two-group *t*-test or a Mann–Whitney test was used. Paired data were compared by use of the Wilcoxon signed rank test. Fisher's exact test was used for comparisons in crosstabs. A *P*-value of < 0.05 was considered to be significant. Statistical analyses were performed with SPSS 20.0 (SPSS, Chicago, IL, USA) and PRISM Version 5.0 (Graphpad Software, San Diego, CA, USA).

Results

Patients

In total, 38 patients were randomized to FFP transfusion, and all samples before and after transfusion were available for analysis. Patients were critically ill, as reflected by a high disease severity score (e.g. APACHE IV and SOFA score) (Table 1). Half of the patients had sepsis, and more than one-third had DIC.

Baseline coagulation tests in critically ill patients with a prolonged INR

Median INR levels at baseline were 1.8 (IQR 1.5–2.2). As expected, median baseline levels of coagulation factors were reduced, with an FII level of 34% (IQR 26–46), an FV level of 48% (IQR 28–76), and an FVII level of 25%

Table 1 Patient characteristics

	FFP transfusion *N* = 38
General characteristics	
Male gender, % (*n*)	68 (26)
Age (years), median (IQR)	64 (54–70)
APACHE IV score, median (IQR)	107 (80–129)
SOFA score, median (IQR)	12 (10–14)
Medical condition, % (*n*)	
Liver disease	16 (6)
Sepsis	47 (18)
DIC	45 (17)
Antiplatelet agents, % (*n*)	
Aspirin	11 (4)
Clopidogrel	3 (1)
Anticoagulation, % (*n*)	
Heparin*	11 (4)
Low molecular weight heparin*	24 (9)
Transfusion	
FFP (units), median (IQR)	3 (2–4)
Outcome	
ICU length of stay (days), median (IQR)	12 (6–19)
28-day mortality, % (*n*)	50 (19)

APACHE, Acute Physiology and Chronic Health Evaluation; DIC, disseminated intravascular coagulation; FFP, fresh frozen plasma; ICU, intensive care unit, IQR, interquartile range; SOFA, Sequential Organ Failure Assessment. *Therapeutic dose.

(IQR 16–38) (Fig. 1). Also, medians of levels of endogenous anticoagulant factors were decreased, including antithrombin level (47% [IQR 35–78]), protein C activity (33% [IQR 21–50]), total protein S level (51% [IQR 36–70]), and free protein S level (53% [IQR 32–75]) (Fig. 2). Markers of activation of coagulation, i.e. TATc and F_{1+2}, were above upper reference values at baseline (10 μg L^{-1} [IQR 5–22] and 370 pmol L^{-1} [IQR 113–608], respectively). Also, the fibrinolytic marker PAP was above the upper reference value at baseline (842 μg L^{-1} [IQR 322–1267]).

For all patients included in one center ($n = 16$), ROTEM was also performed on paired samples. ROTEM variables were within reference ranges, except for prolonged INTEM CFT (Table 2). In addition, EXTEM CFT was at the upper reference range, and both INTEM and EXTEM MCF were at the lower reference range.

Effect of FFP transfusion on coagulation tests, factor levels, and anticoagulants

FFP transfusion reduced the median INR to 1.4 (IQR 1.3–1.6) (Table 2). As expected, FFP transfusion increased the median levels of FII (44% [IQR 38–52]), FV (58% [IQR 44–90]), and FVII (37% [IQR 28–55]). However, the medians of factor levels remained under the lower limit of reference values after transfusion (Fig. 1). Protein C, protein S and antithrombin levels also increased in response to FFP transfusion (Fig. 2). FFP did not increase the levels of activation markers of coagulation, but rather reduced the levels of F_{1+2}. TATc levels were unaffected by FFP transfusion. We also measured parameters of fibrinolysis. FFP did not affect PAP levels, although D-dimer levels were reduced after FFP transfusion (Table 3).

The responses of patients with and without bleeding complications following FFP transfusion did not differ, with no differences being seen in the levels of FII, FV, FVII, antithrombin, protein C, and protein S (data not shown).

Effect of FFP transfusion on thrombin generation

In 27 patients, paired samples were available for thrombin generation tests. At baseline, patients had a prolonged lag time, which is the time from the start of the assay until the first detection of thrombin, and peak values were slightly reduced. Median ETP values were normal before FFP transfusion, and did not change (72% [IQR 51–88] before vs. 71 [IQR 42–89] after, $P = 0.27$). It is of note that, at baseline, approximately half of the patients had peak or ETP values below the reference values at baseline (Fig. 3). FFP transfusion only improved peak values, whereas the other thrombin generation parameters were unaffected by FFP transfusion, with persistent prolonged lag time and time to peak (Fig. 3; Table S2).

Effect of FFP transfusion on thromboelastometry (ROTEM) variables

Transfusion with 12 mL kg^{-1} FFP reduced the median EXTEM CT and improved the median EXTEM MCF, indicating enhanced coagulation. However, in most patients, variables changed only marginally after FFP transfusion (Fig. 4). FIBTEM MCF was unaffected by FFP transfusion, and the same applied for the INTEM variables (Table 2).

Bleeding complications

In eight of 38 patients, minor bleeding complications occurred in the first 24 h after the intervention. Complications consisted of prolonged oozing needing extra compression ($n = 4$) or an extra suture at the insertion site ($n = 1$), or hematoma formation not requiring additional interventions ($n = 3$). None of these events required transfusion of extra FFP or an intervention to stop the bleeding. Coagulation parameters of bleeding and non-bleeding patients are shown in Table 4.

Discussion

In the current study, we have demonstrated that critically ill patients with a coagulopathy as reflected by a prolonged INR have reduced levels of individual coagulation factors, with a concurrent decrease in the levels of naturally occurring anticoagulant factors, associated with delayed thrombin-generating capacity and a tendency towards hypocoagulability as measured by whole blood assays. Transfusion of a fixed dose of FFP improved indi-

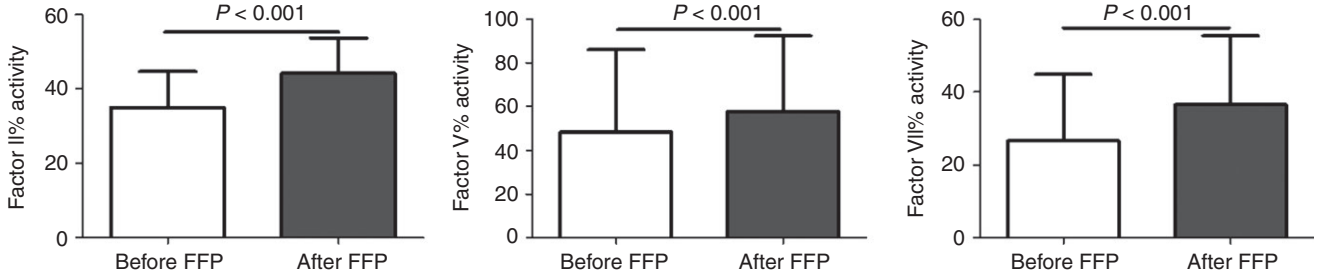

Fig. 1. Levels of individual coagulation factors at baseline and after fresh frozen plasma (FFP) transfusion (12 mL kg^{-1}).

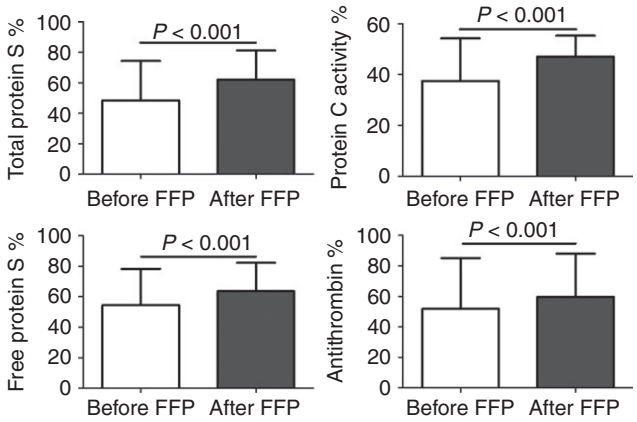

Fig. 2. Levels of anticoagulant proteins before and after fresh frozen plasma (FFP) transfusion (12 mL kg^{-1}).

vidual factor levels, but also increased the levels of natural anticoagulants. Both thrombin generation and thromboelastometry variables improved only marginally in response to FFP transfusion. Therefore, FFP transfusion at a dose of 12 mL kg^{-1} has a marginal effect on hemostatic balance in non-bleeding coagulopathic patients.

Critically ill patients with an increased INR have reduced levels of individual coagulation factors, as found previously [8]. These reduced levels may make them susceptible to bleeding complications. Concurrently, also in line with previous reports [6], we found that the levels of the natural anticoagulants antithrombin, protein C and protein S were also reduced. As the INR fails to reflect altered levels of anticoagulants, the contribution of cellular components to hemostasis, and the amount of fibrinolysis, this test poorly reflects *in vivo* coagulation and actual bleeding risk. In patients with liver disease, it has been shown that INR elevation fails to predict bleeding complications [20]. In these patients, reduced levels of clotting factors, natural anticoagulants [21] and profibrinolytic and antifibrinolytic factors [22] led to the concept of 'rebalanced hemostasis' [23]. Reductions in the levels of coagulation factors and natural anticoagulants, accompanied by relatively normal clot formation, has also been observed in severe sepsis patients [6]. On the basis of our

Table 2 ROTEM variables at baseline and after fresh frozen plasma (FFP) transfusion (12 mL kg^{-1})

	Reference values	Before FFP transfusion $N = 16$	After FFP transfusion $N = 16$	*P*-value
INTEM				
CT (s)	100–240	183 (175–218)	175 (159–197)	0.402
CFT (s)	30–110	133 (51–162)	115 (60–140)	0.327
α-Angle (°)	71–83	74 (63–80)	72 (68–78)	0.634
MCF (mm)	50–72	52 (43–70)	55 (49–69)	0.191
ML (%)		1 (0–4)	0 (0–3)	0.672
EXTEM				
CT (s)	38–79	70 (46–94)	58 (46–82)	0.039
CFT (s)	34–159	156 (60–218)	149 (43–229)	0.140
α-Angle (°)	63–83	67 (55–79)	71 (56–82)	0.102
MCF (mm)	50–72	51 (43–65)	55 (47–75)	0.041
ML (%)		3 (0–5)	1 (0–5)	0.428
FIBTEM				
MCF (mm)	9–25	20 (10–31)	20 (11–31)	0.624

CFT, clot formation time; CT, clotting time; MCF, maximum clot firmness; ML, maximum lysis. Data are expressed as median (interquartile range).

Table 3 Markers of coagulation at baseline and after fresh frozen plasma (FFP) transfusion (12 mL kg^{-1})

	Reference values	Before FFP transfusion $N = 38$	After FFP transfusion $N = 38$	*P*-value
INR	1.0	1.8 (1.5–2.2)	1.4 (1.3–1.6)	< 0.001
APTT (s)	22–30	43 (38–52)	39 (32–46)	< 0.001
Platelet count ($\times 10^9$ L^{-1})	150–400	89 (51–183)	96 (45–158)	0.001
D-dimer (mg L^{-1})	≤ 0.5	7.5 (2.1–11.3)	6.4 (3.3–11.0)	0.009
Fibrinogen (g L^{-1})	1.5–4.0	3.1 (2.1–5.2)	2.8 (2.3–5.4)	0.97
F_{1+2} (pmol L^{-1})	53–271	370 (113–608)	323 (162–480)	0.02
TATc (μg L^{-1})	< 4.6	10 (5–22)	11 (6–22)	0.56
PAP (μg L^{-1})	47–563	842 (322–1267)	833 (411–1151)	0.11

APTT, activated partial thromboplastin time; F_{1+2}, prothrombin fragment 1 + 2; INR, International Normalized Ratio; PAP, plasmin-α2–antiplasmin complex; TATc, thrombin–antithrombin complex. Data are expressed as median (interquartile range). Wilcoxon signed rank test.

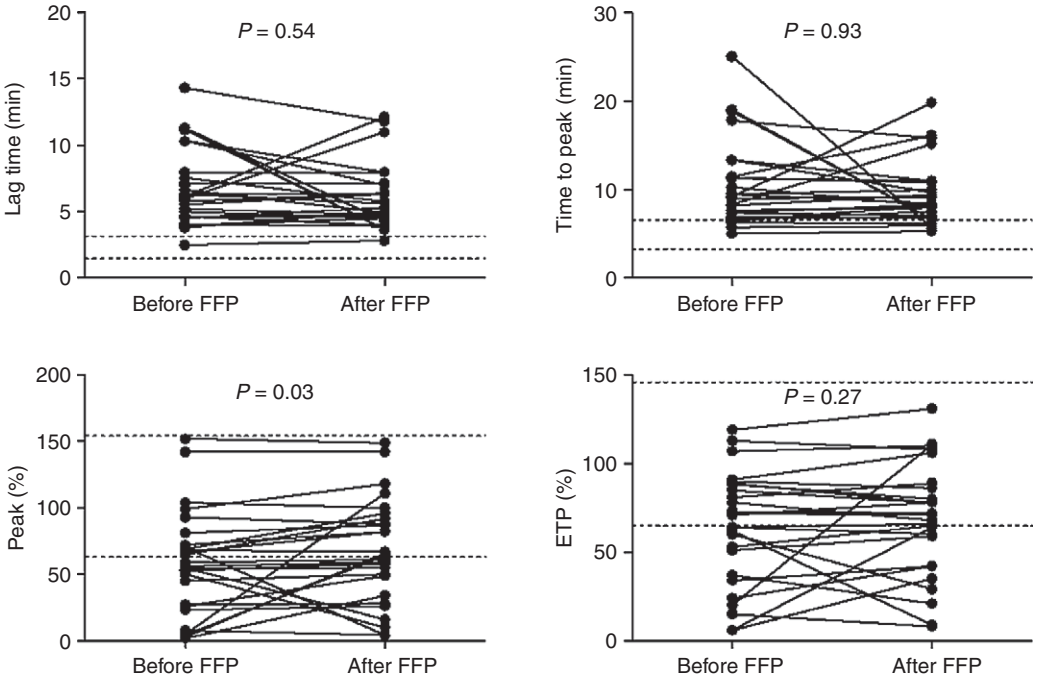

Fig. 3. Thrombin generation test results before and after fresh frozen plasma (FFP) transfusion (12 mL kg^{-1}). Dotted lines indicate reference ranges. ETP, endogenous thrombin potential.

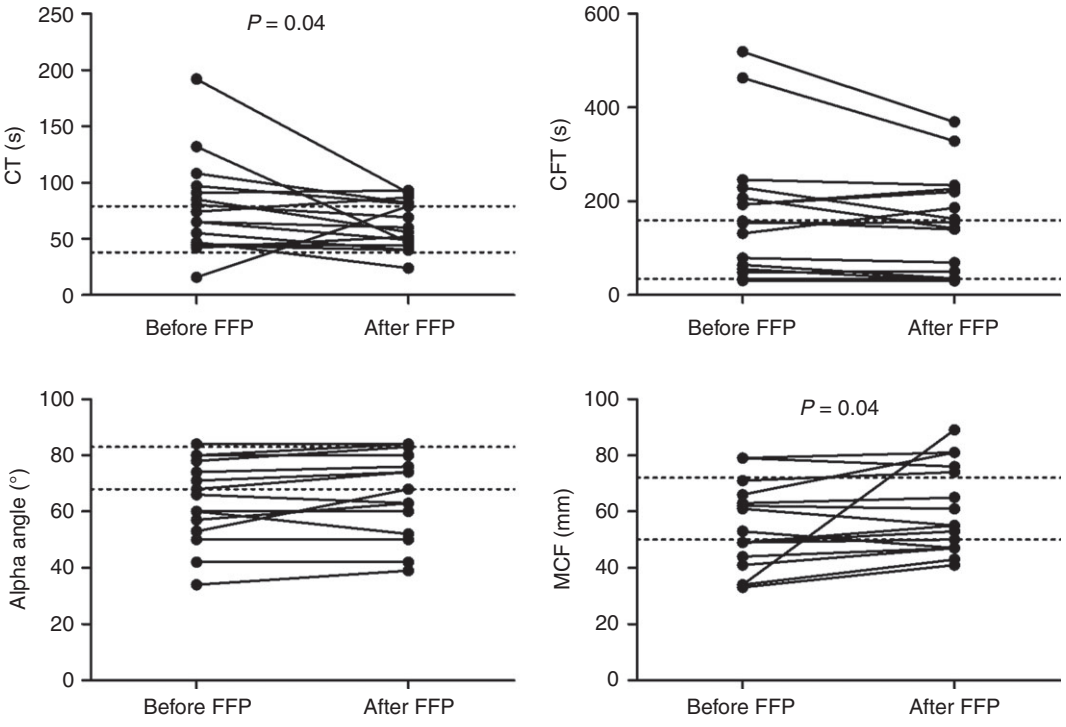

Fig. 4. ROTEM EXTEM variables before and after fresh frozen plasma (FFP) transfusion (12 mL kg^{-1}). Dotted lines indicate reference ranges. CFT, clot formation time; CT, clotting time; MCF, maximum clot firmness.

findings, including near-normal viscoelastic test results, it is conceivable that this concept of rebalanced hemostasis also applies to non-bleeding critically ill patients with a coagulopathy.

The present study aimed to establish the effect of a standardized dose of FFP on individual components of the coagulation system, as well as on global tests of coagulation. First, we demonstrated that FFP transfusion did

Table 4 Coagulation parameters after fresh frozen plasma (FFP) transfusion (12 mL kg^{-1}) in patients with and without minor bleeding complications

	Minor bleeding $N = 8$	No bleeding $N = 30$	*P*-value
INR	1.5 (1.3–1.6)	1.4 (1.3–1.6)	0.75
APTT (s)	37 (30–48)	43 (36–52)	1.00
Hemoglobin (mmol L^{-1})	5.7 (5.4–6.1)	5.8 (5.2–6.4)	0.65
Platelet count ($\times 10^9$ L^{-1})	64 (40–114)	103 (46–167)	0.27
Fibrinogen (g L^{-1})	1.9 (0.9–2.8)	4.0 (2.5–5.5)	0.11
Factor II (%)	40 (30–52)	46 (40–52)	0.23
Factor V (%)	48 (38–56)	67 (50–95)	0.05
Factor VII (%)	44 (30–58)	37 (28–54)	0.56

APTT, activated partial thromboplastin time; INR, International Normalized Ratio. Data are expressed as median (interquartile range).

indeed reduce the INR, in line with an increase in individual factor levels. However, all individual levels remained under the lower limit of the reference values. It is of note that the increase in factor levels was equal in those patients experiencing minor bleeding after the intervention and those who did not. Equally important for the concept of the ability of FFP to mitigate the risk of bleeding is the effect of FFP on the levels of anticoagulant factors. Indeed, FFP resulted in concomitant increases in the levels of the natural anticoagulants antithrombin, protein C, and protein S. In a study on critically ill neonates, prophylactic FFP transfusion resulted in similar findings to ours, with increased coagulation factor levels but also increased anticoagulant levels [24]. A frequently referenced study investigating factor levels after FFP transfusion in critically ill patients also showed decreased levels of all factors and small increases after FFP transfusion [8]. In this study, different doses of FFP were used in small patients groups. We used a fixed dose of 12 mL kg^{-1} FFP. In addition, only individual factor levels were measured, making the net effect of FFP on hemostatic balance unknown.

In this study, the effect of FFP on hemostasis was assessed with thrombin generation and whole blood assays. The results of thrombin generation tests in our patients demonstrated prolonged lag times and times to peak, although the total amount of thrombin generated (expressed by ETP) was preserved. The profile with delayed thrombin generation most likely results from reduced levels of FII and FVII [6,25], and is in line with previous reports [6,26]. The concomitant reduction in antithrombin levels in addition to reduced prothrombin levels is probably responsible for the maintenance of ETP values, as was demonstrated previously [25]. In our study, FFP transfusion did not affect this pattern of delayed thrombin generation and only marginally improved peak values, without an effect on ETP values. F_{1+2} levels were slightly reduced and TATc levels were unaffected by FFP administration, also contradicting enhanced thrombin generation as a result of FFP transfusion. In line with this, in a similar study performed in critically ill neonates, prophylactic FFP transfusion even attenuated thrombin formation, which was thought to be attributable to increased levels of anticoagulants following FFP [24]. Altogether, FFP transfusion has a limited effect on thrombin generation in critically ill patients with a coagulopathy. In whole blood assays, a fixed dose of FFP reduced EXTEM CT and increased EXTEM MCF, whereas INTEM variables remained unaffected, suggestive of a decrease in time to stable clot formation. It is of note, however, that the observed increases were only small, and all values remained within reference ranges. Together, these findings indicate that, despite a reduction in the INR, administration of FFP fails to alter the hemostatic balance to a more procoagulant state. Although the original clinical trial was terminated prematurely [14], the findings of the current substudy suggest that the trial would have been negative if completed.

Our study has several limitations. First, our group size was relatively small. However, the patient characteristics correspond well with those of patients with a coagulopathy in a large prospective cohort study in mixed medical/surgical ICUs [1], supporting the generalizability of our results. It is of note that we found almost no differences in hemostatic test results between patients with and without bleeding following an intervention, but assessment of the ability of hemostatic tests to predict bleeding complications requires a larger sample size. Second, ROTEM testing was performed at only one center, yielding a small sample size. The test precision of ROTEM is a subject of debate, with reported high coefficients of variance [27]. In order to limit variation as much as possible, all tests were carried out with the same device by only two experienced researchers. Third, we did not perform thrombin generation assays with the addition of thrombomodulin or direct activators of protein C. In patients undergoing liver transplantation, thrombin generation was maintained after addition of thrombomodulin, demonstrating a defective endogenous anticoagulant system. Indeed, these patients had reduced levels of antithrombin, protein C, and protein S [21]. It is of note that the levels of antithrombin, protein C and protein S were reduced to similar extents in our patients. Fourth, one could reasonably debate the use of the INR as a test to estimate coagulation status. We chose this test

because the INR is often measured as part of clinical practice to determine coagulation status [1,28,29], and tried to relate effects on the INR to the effects on other hemostatic tests. We feel that the results provide valuable information to the practicing physician. Finally, the dose of FFP was chosen with the aim of reduceing the INR to < 1.5 and not to fully correct the INR [13]. The observed limited increases in coagulation factor levels could have been calculated beforehand, and a higher dose of FFP than 12 mL kg^{-1} would have been required to normalize factor levels. However, transfusion with higher doses of FFP is not current practice [2,15,30,31]; moreover, audits have revealed that a substantial number of patients are transfused with a dose of < 10 mL kg^{-1} [16,32]. Again, we think that the results are relevant to the practicing physician.

Conclusion

Critically ill patients with an increased INR show delayed thrombin generation, but preserved viscoelastic test results. Prophylactic FFP transfusion at a dose of 12 mL kg^{-1} increased the levels of individual coagulation factors, with concomitant increases in the levels of anticoagulants. However, the effects of FFP transfusion on thrombin generation and thromboelastometry were very limited, and failed to induce a more procoagulant state.

Addendum

M. C. A. Müller, E. de Jonge, M. J. Schultz, M. B. Vroom, and N. P. Juffermans designed the study. M. C. A. Müller, M. Straat, M. S. Arbous, J. C. M. Meijers, J. H. Klinkspoor, and N. P. Juffermans performed the study. M. C. A. Müller, J. C. M. Meijers, and N. P. Juffermans analyzed data and wrote the paper. M. Straat, J. H. Klinkspoor, E. de Jonge, M. S. Arbous, M. B. Vroom, and M. J. Schultz critically revised the manuscript.

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Disclosure of Conflict of Interests

The authors state that they have no conflict of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. ISTH score for disseminated intravascular coagulation (Taylor FB *et al.*, *Thromb Haemost* 2001;86:1327–30).

Table S2. Thrombin generation values at baseline and after fresh frozen plasma transfusion (12 mL kg^{-1}).

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