Coagulopathy and plasma transfusion in critically ill patients

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Chapter 6

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

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Submitted for publication
Abstract

Coagulopathy has a high prevalence in critically ill patients. A prolonged INR is a common trigger to transfuse fresh frozen plasma (FFP), even in the absence of bleeding. Thereby, FFP is frequently administered in these patients. However, efficacy of FFP to correct hemostatic disorders in non-bleeding recipients is questioned. We aimed to assess the effect of a fixed dose of FFP transfusion on the hemostatic balance in non-bleeding critically ill patients with a coagulopathy. Markers of coagulation, individual factor levels and levels of natural anticoagulants were measured and thrombin generation assays were performed before and after FFP transfusion (12 ml/kg) to 38 non-bleeding critically ill patients with a prolonged INR (1.5-3.0). At baseline, levels of procoagulant factor II, V and VII as well as levels of anticoagulants protein C, S and antithrombin were below normal values, associated with impaired thrombin generation. FFP transfusion increased both levels of individual coagulation factors as well as levels of anticoagulant proteins. Thrombin generation was not affected by FFP transfusion. In conclusion, in critically ill patients with a coagulopathy, FFP transfusion does not alter hemostatic balance or thrombin generation.
Background

Coagulopathy occurs in up to 30% of critically ill patients [1]. Seventy percent 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 effectiveness of FFP transfusion, 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] and is a common trigger to administer FFP [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, INR only represents a part of the coagulation cascade and is insensitive to the activity or concentration of natural anticoagulants and fibrinolytic proteins. In the critically ill, and particularly in patients with disseminated intravascular coagulation (DIC), levels of natural anticoagulants are reduced and fibrinolysis is attenuated [5,6].

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

The efficacy of FFP to correct coagulopathy has only been evaluated in small and heterogeneous studies [8-10]. Notably, the efficacy of FFP to prevent bleeding in coagulopathic critically ill patients has not been demonstrated [11]. Besides coagulation factors, FFP also contains natural occurring anticoagulants antithrombin, protein C and S. The net effect of FFP transfusion on anticoagulant proteins and the hemostatic balance in the critically ill is not well known [12].

In a predefined sub study of a multicenter randomized controlled trial on the efficacy of prophylactic FFP transfusion to prevent 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 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 entry in 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 NTR 2262 and NCT01143909.

**Setting and patients**

The study was performed between May 2010 and June 2013 in four mixed medical-surgical ICUs in the Netherlands. Patients were eligible when INR was 1.5-3.0 and needed to undergo an intervention. Patients younger than 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 hours for heparin and >12 hours for low molecular weight heparin. Use of low molecular weight heparin in a prophylactic dose was part of standard care in all patients.

**Design**

A predefined post hoc study of a randomized controlled clinical trial on risk and benefit of FFP transfusion in critically ill patients with a coagulopathy [13]. After inclusion, patients were randomized to a single dose of 12 ml/kg FFP or no FFP transfusion before a scheduled intervention. The FFP was quarantine plasma manufactured by Sanquin, the Dutch National Bloodbank. The dose of FFP was based on clinical practice and a previous study with a target of INR reduction to <1.5 [9,14]. Patients were observed until 24 hours after the intervention for bleeding complications.
**Patient data and sample collection**

Patient data were collected from the electronic patient data management system (PDMS) and consisted of medical history, admission diagnosis, use of anticoagulant medication and occurrence of bleeding. Disseminated intravascular coagulation (DIC) was assessed using the ISTH DIC score, which defines overt DIC as a score of ≥5 points [15].

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 (0.109M 3.2%) tubes. Samples were centrifuged within 30 minutes at 2000 x g for 15 mins at 18°C and subsequently 5 mins at 15000 x g also at 18°C. Supernatant was collected and stored at -80°C until measurements were performed.

**Assays**

PT, INR, aPTT 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 CA 7000 and all reagents, Siemens Healthcare Diagnostics, Marburg, Germany) according to manufacturers protocols. After termination of the study the remaining assays were performed collectively in all patients. Levels of factors II, V and VII were determined by a PT based one stage clotting assay (ACL TOP 700, Instrumentation Laboratory, USA), using recombiplastin and factor II, V and VII deficient plasma (Instrumentation Laboratory, USA). Antithrombin was assessed by chromogenic substrate method (Symex CA7000) with reagents and protocols of the manufacturer.

Protein C activity was measured by a kinetic assay (Coamatic Protein C, Chromogenix, Mölndal, Sweden). Total protein S levels were determined by enzyme-linked immunosorbent assay (ELISA) as described previously [16]. Free protein S was measured by precipitating the C4b-binding protein-bound fraction with polyethylene glycol 8000 and measuring the concentration of free protein S in the supernatant [16]. Thrombin-antithrombin (TATc), prothrombin fragment 1 + 2 (F1+2) and plasmin-α2-antiplasmin complex (PAP) levels were measured using specific commercially available ELISAs according to the instruction of the manufacturer (Siemens Healthcare Diagnostics and DRG, Marburg, Germany).
**Thrombin generation assay**

The Calibrated Automated Thrombogram® assays the generation of thrombin in clotting plasma using 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. [17] and the Thrombinoscope® manual. Coagulation was triggered by recalcification in the presence of 5 pM recombinant human tissue factor (Innovin®, Siemens Healthcare Diagnostics, Marburg, Germany), 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 or endogenous thrombin potential) were calculated using the Thrombinoscope software.

**Statistics**

All variables are expressed as mean (standard deviation) or median (interquartile ranges) depending on whether distribution was normal or not. To compare groups two group t-test or Mann-Whitney test was used. Paired data were compared using the Wilcoxon signed rank test. A p value of less than 0.05 was considered significant. Statistical analyses were performed with SPSS 20.0 (SPSS, Inc, Chicago, IL, USA) and Prism Version 5.0 (Graphpad Software, San Diego, 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 a third had DIC. A considerable number of patients received heparin or low molecular weight heparin treatment in a therapeutic dose, which was discontinued for at least an appropriate period prior to baseline measurements. In 8 of 38 patients, minor bleeding complications occurred in the first 24 hours after the intervention. None of these events required transfusion of extra FFP or an intervention to cease bleeding.
Effects of FFP on coagulation in the critically ill

Table 1: Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>FFP transfusion n=38</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>General characteristics</strong></td>
<td></td>
</tr>
<tr>
<td>Gender, male, % (n)</td>
<td>67 (26)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>64 (54-70)</td>
</tr>
<tr>
<td>APACHE IV score</td>
<td>107 (80-129)</td>
</tr>
<tr>
<td>SOFA score</td>
<td>12 (10-14)</td>
</tr>
<tr>
<td><strong>Medical condition</strong></td>
<td></td>
</tr>
<tr>
<td>Liver disease, % (n)</td>
<td>16 (6)</td>
</tr>
<tr>
<td>Sepsis, % (n)</td>
<td>47 (18)</td>
</tr>
<tr>
<td>DIC, % (n)</td>
<td>45 (17)</td>
</tr>
<tr>
<td><strong>Anticoagulation</strong></td>
<td></td>
</tr>
<tr>
<td>Aspirin, % (n)</td>
<td>10 (4)</td>
</tr>
<tr>
<td>Clopidogrel, % (n)</td>
<td>3 (1)</td>
</tr>
<tr>
<td>Heparin, % (n)*</td>
<td>10 (4)</td>
</tr>
<tr>
<td>Low Molecular Weight Heparin, % (n)*</td>
<td>23 (9)</td>
</tr>
<tr>
<td><strong>Transfusion</strong></td>
<td></td>
</tr>
<tr>
<td>FFP (units)</td>
<td>3 (2-4)</td>
</tr>
<tr>
<td><strong>Outcome</strong></td>
<td></td>
</tr>
<tr>
<td>ICU length of stay (days)</td>
<td>12 (6-19)</td>
</tr>
<tr>
<td>28 day mortality, % (n)</td>
<td>51 (19)</td>
</tr>
</tbody>
</table>

Data expressed as median and interquartile ranges.
* therapeutic dose

APACHE = Acute Physiology and Chronic Health Evaluation
SOFA = Sequential Organ Failure Assessment
DIC = Disseminated Intravascular Coagulation
FFP = Fresh Frozen Plasma
ICU = Intensive Care Unit

Baseline coagulation tests in critically ill with a prolonged INR

Median INR levels at baseline were 1.8 [1.5-2.2] in all patients. As expected, median baseline levels of coagulation factors were all reduced, with a factor II of 34% [26-46], factor V of 48% [28-76] and VII level of 25% [16-38] (figure 1). Also, patients had decreased levels of endogenous anticoagulant factors, including antithrombin (47% [35-78]), protein C activity (33% [21-50]), as well as of levels of total protein S (51% [36-70]) and free protein S (53% [32-75]) (figure 2). Markers of activation of coagulation TATc and F1+2 were above upper reference value at baseline (10 ug/L [5-22])
Figure 1: Levels of individual coagulation factors at baseline and after Fresh Frozen Plasma transfusion (12 ml/kg).

Figure 2: Levels of anticoagulant proteins before and after Fresh Frozen Plasma transfusion (12 ml/kg).

and 370 pMol/L [113-608] respectively). Also, the fibrinolytic marker PAP was above upper reference value at baseline (842 ug/L [322-1267]).

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

FFP transfusion reduced median INR to 1.4 [1.3-1.6] (table 2). As expected, FFP transfusion increased median levels of coagulation factors II (44% [38-52]), V (58% [44-
Figure 3: Thrombin generation test results before and after Fresh Frozen Plasma transfusion (12 ml/kg).

Dotted lines indicate reference ranges.

FFP = Fresh Frozen Plasma
ETP = endogenous thrombin potential

90]) and VII (37% [28-55]). However, all factor levels remained under the lower limit of reference values after transfusion (figure 1). Protein C, S and antithrombin levels also increased in response to FFP transfusion (figure 2). FFP did not increase markers of coagulation, but rather reduced levels of F1+2. TATc levels were unaffected by FFP transfusion. We also measured parameters of fibrinolysis. FFP did not affect PAP levels, although D-dimer concentration was reduced after FFP transfusion (table 2). The response of patients with and without bleeding complications following FFP transfusion did not differ, with no differences in levels of factors II, V and VII and anticoagulants antithrombin, protein C and S (data not shown).

**Effect of FFP transfusion on thrombin generation**

In 27 patients, paired samples were available to perform thrombin generation tests. At baseline, patients had prolonged lag time, which is the time from start of
Table 2: Markers of coagulation at baseline and after Fresh Frozen Plasma transfusion (12 ml/kg).

<table>
<thead>
<tr>
<th></th>
<th>Before FFP n=38</th>
<th>After FFP n=38</th>
<th>p value</th>
</tr>
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<tbody>
<tr>
<td>INR</td>
<td>1.8 (1.5-2.2)</td>
<td>1.4 (1.3-1.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>aPTT (sec)</td>
<td>43 (38-52)</td>
<td>39 (32-46)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Platelet count (*10^9/L)</td>
<td>89 (51-183)</td>
<td>96 (45-158)</td>
<td>0.001</td>
</tr>
<tr>
<td>D dimer (mg/L)</td>
<td>7.5 (2.1-11.3)</td>
<td>6.4 (3.3-11.0)</td>
<td>0.009</td>
</tr>
<tr>
<td>Fibrinogen (g/L)</td>
<td>3.1 (2.1-5.2)</td>
<td>2.8 (2.3-5.4)</td>
<td>0.97</td>
</tr>
<tr>
<td>F1+2 (pMol/L)</td>
<td>370 (113-608)</td>
<td>323 (162-480)</td>
<td>0.02</td>
</tr>
<tr>
<td>TATc (ug/L)</td>
<td>10 (5-22)</td>
<td>11 (6-22)</td>
<td>0.56</td>
</tr>
<tr>
<td>PAP (ug/L)</td>
<td>842 (322-1267)</td>
<td>833 (411-1151)</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Data expressed as median (IQR)
Wilcoxon Signed Rank test.
FFP = Fresh Frozen Plasma
INR = International Normalized Ratio
aPTT = Activated Partial Thromboplastin Time
F1+2 = Prothrombin Fragment 1+2
TATc = Thrombin-antithrombin complex
PAP = Plasmin-α2-antiplasmin complex

the assay until detection of the first thrombin (figure 3). Also, peak values and ETP (endogenous thrombin potential) were both reduced, indicating reduced thrombin generation, which is in line with the low individual factor levels in these patients. Transfusion of FFP resulted in slightly increased peak values, however values were still lower compared to the normal range, indicating a persistent hypocoagulable profile. The other thrombin generation parameters were unaffected by FFP transfusion, with persistent prolonged generation lag time and time to peak and reduced ETP (figure 3).

Discussion

In the current study, we demonstrated that critically ill patients with a prolonged INR have reduced levels of individual coagulation factors with a concurrent decrease in levels of natural occurring anticoagulant factors. Transfusion of a fixed dose of FFP improved individual factor levels, but also increased levels of natural anticoagulants. Thrombin generation was impaired in these patients and only improved marginally...
Effects of FFP on coagulation in the critically ill

in response to FFP transfusion. Thereby, FFP transfusion appears not to influence hemostatic balance in non-bleeding patients with an increased INR.

INR is frequently used to assess bleeding risk and elevation is often a trigger to prophylactically administer FFP [18]. Indeed, we found that critically ill patients with an increased INR have reduced levels of individual coagulation factors, as found previously [8]. These reduced levels may render them susceptible for enhanced bleeding. Concurrently, also in line with previous reports [6], we found that levels of natural anticoagulants antithrombin, protein C and S were reduced at the same time, hereby suggesting an unchanged hemostatic balance. As INR does not reflect altered levels of anticoagulants, this test poorly reflects in vivo coagulation and actual bleeding risk. In patients with liver disease, it has been shown that INR elevation indeed fails to predict bleeding complications [19]. In these patients, reduced levels of clotting factors, natural anticoagulants [20] and pro- and antifibrinolytic factors [21] led to the concept of “rebalanced hemostasis” [22]. Our results confirm this concept of rebalanced hemostasis in 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. We demonstrated that FFP transfusion indeed reduced INR value, in line with an increase in individual factor levels. However, all individual levels remained under the lower limit of the reference values. Of note, the increase in factor levels was equal in those patients experiencing bleeding after the intervention compared to those who did not. Equally important to the concept of the ability of FFP to mitigate the risk of bleeding, is the effect of FFP on levels of anticoagulant factors. FFP resulted in a concomitant rise of levels of natural anticoagulants antithrombin, protein C and S. Data on the effect of FFP on the hemostatic balance in non-bleeding critically ill patients are scarce. A frequently referenced study investigating factor levels after FFP transfusion in the critically ill showed decreased levels of all factors and small increments after FFP transfusion [8]. However, in this study, different doses of FFP were used in small patients groups and anticoagulant factor levels were not assessed, rendering the net effect of FFP on hemostatic balance unknown. In a study in critically ill neonates, prophylactic FFP transfusion resulted in similar results to ours, with increased coagulation factor levels but also levels of anticoagulants [23]. In our study, levels of F1+2 were slightly reduced and TATc levels were unaffected by
FFP administration, contradicting enhanced thrombin generation as a result of FFP transfusion and suggesting that hemostatic balance is unaltered by FFP. Of note, in line with the concept of “rebalanced hemostasis”, the paradigm on the use of FFP in liver failure patients has also shifted and liver transplantations are increasingly performed without any transfusion despite increased INR values [24]. Taken together, administration of FFP supplies both pro-coagulant and anti-coagulant coagulation factors, resulting in an unchanged hemostatic balance. Despite INR correction, any change to a more pro-coagulant state following FFP transfusion is highly limited.

Results of thrombin generation tests in our patients varied widely, but predominantly showed a hypocoagulable profile. Reduced levels of individual coagulation factors, in particular factor VII, probably contributed to a delay in initial thrombin generation, resulting in increasing lag time and time to thrombin peak. In addition, low thrombin peak and ETP values indicate reduced overall thrombin generation. These results are in line with those previously reported in sepsis patients [6,25]. FFP transfusion resulted in only marginally increased peak values and did not affect ETP values, indicating a very limited effect of FFP transfusion on thrombin generation in our patients. In line with this, in a study similar to this one performed in critically ill neonates, prophylactic FFP transfusion was demonstrated to even attenuate thrombin formation, which was thought to be due to increased levels of anticoagulants following FFP [23]. We did not perform thrombin generation assays with the addition of thrombomodulin or activated protein C, which is a limitation of our study. In patients undergoing liver transplantation, thrombin generation was preserved after addition of thrombomodulin hereby demonstrating a defective endogenous anticoagulant system. Indeed these patients had reduced levels of antithrombin and protein C and S [20]. Of note, levels of antithrombin, protein C and S were reduced to a similar extent in our patients. Therefore, the observed hypocoagulable profiles in thrombin generation assays do not preclude a preserved hemostatic balance, as stated previously.

Our study has several other limitations. First, our group size was relatively small. However, 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. Second, the dose of FFP was chosen aiming to reduce INR to <1.5 and to not fully correct INR [13]. The observed
limited increment in coagulation factor levels could have been calculated beforehand and higher dose of FFP than 12 ml/kg would have been required to normalize factor levels. However, transfusion with higher doses of FFP is not current practice [2,18,26,27], moreover audits have revealed that a substantial number of patients is transfused with a dose less than 10 ml/kg [14,28]. As transfusion with doses of FFP used in this study reflect current practice, we think that results are relevant to the practicing physician.

**Conclusion**

Critically ill patients with increased INR display a hypocoagulable state with impaired thrombin generation. Prophylactic FFP transfusion resulted in an increase, but not in correction, of individual coagulation factors with a concomitant increase in levels of anticoagulants. In addition effect on thrombin generation was highly limited, hereby the net hemostatic balance remained unchanged after FFP transfusion. These results underline the lack of rationale to administer FFP to non-bleeding ICU patients with a coagulopathy.
References


