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### Coagulopathy and plasma transfusion in critically ill patients

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# Chapter 8

## **Effect of transfusion of fresh frozen plasma on parameters of endothelial condition and inflammatory status in non-bleeding critically ill patients**

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*Submitted for publication*

## Abstract

Much controversy exists on the effect of a fresh frozen plasma (FFP) transfusion on systemic inflammation and endothelial damage. Adverse effects of FFP have been well described, including acute lung injury. However, it is also suggested that a higher amount of FFP decreases mortality in trauma patients requiring a massive transfusion. Furthermore, FFP has an endothelial stabilizing effect in experimental models. In 38 coagulopathic non-bleeding critically ill patients receiving a prophylactic transfusion of FFP (12 ml/kg) prior to an invasive procedure, we measured inflammatory cytokines and markers of endothelial condition before and after transfusion. At baseline, systemic cytokine levels were mildly elevated in critically ill patients. Surprisingly, FFP transfusion resulted in a decrease of TNF- $\alpha$  (from 11.3 to 2.3 pg/ml,  $p=0.01$ ). Other cytokines were not affected. FFP also resulted in a decrease in systemic syndecan-1 levels from 675 to 565 pg/ml ( $p=0.01$ ) and a decrease in median factor VIII levels (from 246 to 246%,  $p<0.01$ ), suggestive of an improved endothelial condition. This was associated with an increase in ADAMTS13 levels (from 24 to 32%,  $p<0.01$ ), with a concomitant decrease in von Willebrandfactor (vWF)(from 474 to 423%,  $p<0.01$ ). In conclusion, a fixed dose of FFP transfusion seems to stabilize endothelial condition, possibly by increasing ADAMTS13 which cleaves vWF.

## **Introduction**

Substantial amounts of Fresh Frozen Plasma (FFP) are utilized in the intensive care unit (ICU)[1,2]. FFP is effective in correcting clotting factor deficiencies [3] and is therefore transfused in patients with active bleeding, but also frequently in patients with abnormal coagulation tests to prevent bleeding [2,4]. In sepsis patients, FFP transfusion rates of up to 57% have been reported [5]. However, there is an association between FFP transfusion and adverse outcome in the critically ill, including transfusion-related acute lung injury (TRALI) [4,6-8], transfusion-related circulatory overload [9,10], multi-organ failure [8,11] and an increased risk of infections [12]. Although not entirely understood, the pathological mechanisms underlying the association between FFP transfusion and lung injury is thought to result from an inflammatory response including a neutrophil influx into the lungs and elevated pulmonary levels of IL-8 and IL-1, as demonstrated in TRALI patients [13,14]. In line with this, FFP increased expression of endothelial adhesion molecules in human pulmonary endothelial cells [15]. Together, these data suggest that endothelial cell activation and disruption may be an early event following lung injury due to transfusion [16].

On the other hand, FFP also seems to have protective effects. In trauma patients requiring a massive transfusion, it is suggested that resuscitation with a higher ratio of FFP to red blood cell units decreases mortality [17,18]. Of interest, this decreased mortality is irrespective of correction of coagulopathy by restoring coagulation factor levels [18,19]. Instead, a beneficial effect of FFP may be related to the restoration of injured endothelium. Patients in hemorrhagic shock have a disrupted endothelial integrity and glycocalyx layer, with decreased syndecan-1 expression [20]. Syndecan-1 is a proteoglycan on the luminal surface of endothelial cells which inhibits leukocyte adhesion and is shed during endothelial damage, resulting in increased levels of syndecan-1 in the systemic compartment [21]. In a hemorrhagic shock model, FFP was found to improve endothelial integrity, associated with increased expression of syndecan-1 on endothelial cells [22].

Vascular integrity is also disrupted in various populations of critically ill patients, as demonstrated by increased levels of syndecan-1 [23,24]. The effect of a transfusion of a fixed dose of FFP on endothelial and cytokine host response in patients is

not known. In a study investigating the risk-benefit ratio of FFP transfusion in non-bleeding critically ill patients with a coagulopathy, we investigated host response to a fixed dose of FFP transfusion.

## Methods

### *Study design*

This was a predefined post-hoc sub-study of a multicenter trial in which non-bleeding critically ill patients with an increased International Normalized Ratio (INR 1.5-3.0) were randomized between May 2010 and June 2013 to omitting or administering a prophylactic transfusion of FFP (12 ml/kg) prior to an invasive procedure. Only patients randomized to receive FFP were included in this sub-study. Patients were enrolled at 3 sites in The Netherlands: two university hospitals (Academic Medical Center, Amsterdam and Leiden University Medical Center, Leiden) and one large teaching hospital (Tergooi Ziekenhuizen, Hilversum). The Institutional Review Board of the Academic Medical Center - University of Amsterdam, Amsterdam, The Netherlands, approved the study protocol. 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 NTR2262 and NCT01143909 [25].

Exclusion criteria were clinically overt bleeding, thrombocytopenia of  $<30 \times 10^9/L$ , treatment with vitamin K antagonists, activated protein C, abciximab, tirofiban, ticlopidine or prothrombin complex concentrates and a history of congenital or acquired coagulation factor deficiency or bleeding diathesis. Patients treated with low molecular weight heparin (LMWH) or heparin in therapeutic dose were eligible if medication was discontinued for an appropriate period. Sepsis was defined by the Bone criteria [26]. Disseminated intravascular coagulation (DIC) was defined by an ISTH score of  $\geq 5$  [27]. The FFP was quarantine plasma manufactured by Sanquin, the Dutch National Bloodbank. As of 2007, women are deferred from donation for preparation of FFP in the Netherlands.

### **Sample collection**

Citrated blood samples were drawn from an indwelling arterial catheter before and directly after FFP transfusion. Samples were collected in sodium citrate (0.109M 3.2%) tubes and were centrifuged twice within 30 minutes: first 15 minutes at 2000  $\times g$  and then 5 mins at 15000  $\times g$ , both at 18°C. Supernatant was collected and stored at -80°C.

### **Assays**

Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) levels were measured by enzyme-linked immunosorbent assay (ELISA), according to instructions of the manufacturer (R&D Systems Inc., Minneapolis, MN, USA). Serum levels of IL-1 $\beta$ , IL-1RA, IL-8, IL-10, Macrophage Inflammatory Proteins (MIP)-1A, Monocyte Chemotactic Protein (MCP)-1 and soluble CD40 ligand were determined by Luminex, according to instructions of the manufacturer (Merck Millipore Chemicals BV; Amsterdam; the Netherlands). When less than 50 beads were measured by the Luminex assay, samples were excluded from further analysis. Von Willebrand Factor (VWF) antigen (VWF:Ag) levels were determined with an in-house enzyme-linked immunosorbent assay (ELISA) assay using commercially available polyclonal antibodies against VWF (DAKO, Glostrup, Denmark). ADAMTS13 antigen levels were measured using a commercially available ELISA according to the manufacturer's instructions (Sekisui Diagnostics, Stamford, CT) and ADAMTS13 activity was assessed using the FRETTS-VWF73 assay (Peptanova, Sandhausen, Germany) based on the method described by Kokame *et al.* [28].

### **Statistical analysis**

Variables are expressed as medians and interquartile ranges or means and standard deviations. For comparisons, a paired t-test was used, or in case of not normally distributed data the Wilcoxon signed rank test. Statistical uncertainties are expressed in 95% confidence limits, with a significance level of 0.05. For the analyses, we used SPSS 20 and Graphpad Prism 5.

## Results

### ***Patients***

From 38 patients receiving FFP, paired samples from 33 patients were available for analysis before and after FFP transfusion. Patients were ill, as reflected by a high disease severity score and half of the patients had sepsis (table 1). Patients received a mean dosage of 11.2 (2.8) ml/kg FFP.

### ***Inflammatory cytokine and chemokine levels before and after transfusion of 12 ml/kg FFP***

At baseline, levels of cytokines were mildly elevated in this cohort. After FFP transfusion, median TNF- $\alpha$  decreased ( $p=0.01$ , table 2). Levels of all other cytokines were not affected by FFP transfusion. Chemokine levels IL-8 and MCP1 were elevated at baseline but also not influenced by FFP transfusion. Levels of soluble CD40L, which has been implicated as a mediator in TRALI [29], were also not significantly altered by FFP transfusion.

### ***Parameters of endothelial condition before and after transfusion of 12 ml/kg FFP***

After FFP transfusion, levels of ADAMTS13 increased ( $p<0.01$ , figure 1). This increase was accompanied by a decrease in VWF ( $p<0.01$ ) and in levels of syndecan-1. Factor VIII levels were slightly decreased following FFP transfusion ( $p=0.01$ ).

## Discussion

Our patients had mildly elevated levels of inflammatory cytokines at baseline, which corresponds to levels measured before in critically ill patients [30]. We observed no aggravation of this inflammatory response after FFP transfusion. Rather, there was a decrease in TNF- $\alpha$  level. This is not in line with a study in which FFP elicited an inflammatory response in endothelial cells [31], nor with an *in vitro* model of transfusion, in which whole blood incubated with FFP induced TNF production [32]. Of the cytokines we measured, only TNF- $\alpha$  changed after FFP transfusion. As TNF- $\alpha$  is known to be the quickest responder among all cytokines, we may have timed our measurement too early after FFP transfusion to note an effect of FFP on other

**Table 1:** Patient characteristics

	<b>FFP transfusion n=33</b>
<b>General characteristics</b>	
Gender, male, % (n)	64 (21)
Age (years)	61 (50-70)
APACHE IV score	96 (79-128)
SOFA score	11 (10-14)
<b>Medical history</b>	
Pulmonary disease, % (n)	8 (3)
Liver disease, % (n)	16 (6)
Cardiac failure, % (n)	16 (6)
<b>Medical condition 24 hours before transfusion</b>	
Mechanical ventilation, % (n)	82 (27)
Sepsis, % (n)	45 (15)
Disseminated intravascular coagulation, % (n)	49 (16)

Data expressed as median and interquartile ranges

**Table 2:** Inflammatory cytokines in critically ill patients with a coagulopathy before and after a transfusion of fresh frozen plasma (12 ml/kg)

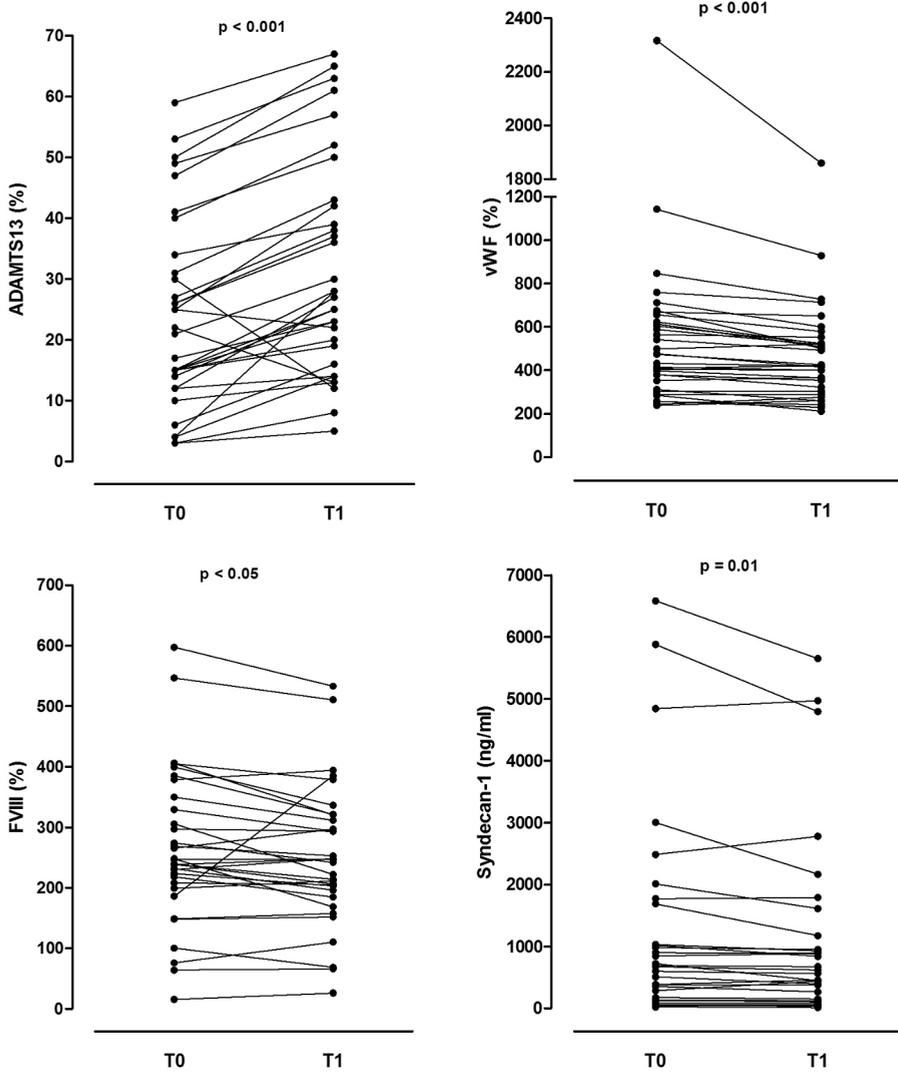
	<b>Before FFP</b>	<b>After FFP</b>	<b>p value</b>
<b>pro-inflammatory parameters (pg/ml)</b>			
TNF- $\alpha$	11.3 (2.3 – 52.3)	2.3 (2.3 – 41.0)	0.01
IL1- $\beta$	15.0 (11.7 – 18.8)	14.4 (13.1 – 23.3)	0.97
IL-8	178 (124 – 418)	187 (113 – 412)	0.23
MCP1	1255 (503 – 3376)	1101 (434 – 5802)	0.89
MIP1A	19.6 (15.7 – 33.6)	19.1 (13.3 – 34.4)	0.12
sCD40L	409 (257 – 614)	324(216 – 537)	0.08
<b>anti-inflammatory parameters (pg/ml)</b>			
IL-1RA	69.3 (52.1 – 110.6)	73.5 (47.8 – 104.9)	0.11
IL-10	36.1 (15.5 – 100.1)	31.5 (14.8 – 279.6)	0.62

Data expressed as median (IQR)

FFP = Fresh Frozen Plasma

cytokine levels. However, lung injury following transfusion is thought to be an early event. Also, we choose this early time point to minimize confounding by other factors. Taken together, FFP does not appear to elicit an early inflammatory response.

**Figure 1:** Effect of fresh frozen plasma transfusion (12 ml/kg) on markers of endothelial condition: ADAMTS13, Von Willebrandfactor, Factor VIII and Syndecan-1.



vWF = von Willebrandfactor  
FVIII = Factor VIII

Of interest, recent *in vitro* studies support an endothelial stabilizing role of FFP, as FFP reduced vascular endothelial cell permeability [22,33] and decreased expression of endothelial adhesion markers [34] and endothelial white blood cell binding [22,34,35]. Effects of FFP were investigated in a rat hemorrhagic shock model, characterized by systemic shedding of syndecan-1, decreased syndecan-1 expression on pulmonary cells and increased pulmonary vascular permeability. Syndecan-1 is a proteoglycan on the luminal surface of endothelial cells which inhibits leukocyte adhesion during inflammation and is shed in case of endothelial damage [21]. Resuscitation with FFP was found to abrogate these effects, whereas resuscitation with crystalloids did not [22], associated with preservation of the endothelial glycocalyx [36] and improvement of lung injury [37].

In trauma patients with hemorrhagic shock, syndecan-1 levels are also increased [20]. Studies of the effect of FFP on endothelial condition in patients are however lacking. Of note, recent evidence in trauma patients requiring a massive transfusion suggests that more and earlier administration of FFP decreases mortality [17,18,38,39]. This effect was not associated with improved coagulation ability, as the reduction in mortality in their study was irrespective of the admission INR [18] and coagulopathy does not seem to improve with higher amounts of FFP [19]. Given that FFP restores coagulation factors but also anti-coagulant proteins and that the net effect on hemostasis is unclear, FFP may exert protection via other mechanisms. This study found that FFP decreased levels of syndecan-1, associated with decreased levels of factor VIII, the latter which may also reflect improved endothelial condition. These results support earlier experimental work indicating that FFP preserves endothelial integrity.

The mechanism underlying this beneficial effect of FFP has not yet been described. We found that FFP transfusion was associated with an increase in ADAMTS13 and a decrease in vWF. Thereby, ADAMTS13 may have increased the ability to cleave large vWF multimers present on the activated endothelium. As large vWF multimers damage the endothelium, this effect may have preserved endothelial condition. This is also the rationale behind the treatment of thrombotic thrombocytopenic purpura by therapeutic plasma exchange.

A protective effect of FFP on the endothelium is in apparent contrast with studies that have linked FFP to the occurrence of TRALI [4,6-8]. In an effort to reconcile

these findings, we suggest that FFP associated with TRALI occurs as a result of an antibody-mediated pathogenesis. Indeed, efforts to reduce antibody positive blood products by male only policies, are associated with a significant reduction in TRALI [40]. In patients in whom transfusion is associated with lung injury in the absence of antibodies, other products such as red blood cells and platelets may be more important in inducing lung injury. Although dissecting these effects in multiple transfused patients is a challenge, future research should focus on differential effects of different blood products.

This study is limited by a small and heterogeneous patient population. Thereby, some of the effects may be caused by chance or by regression to the mean. Findings need to be confirmed in a larger sample. Also, as mentioned, the timing of measurement may have been too early following FFP transfusion. We cannot exclude that FFP induces a pro-inflammatory host response later in time.

In conclusion, this study is the first to describe the effect of a fixed dose of FFP transfusion in detail in critically ill patients. Results suggest that FFP stabilizes endothelial condition.

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