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FXIII Deficiency Occurs Frequently in Major Trauma and Influences Transfusion Requirements While Levels of Von Willebrand Factor are well above Thresholds

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Abstract

Background: Coagulation Factor XIII (FXIII) and von Willebrand Factor (VWF) are essential for initial and final clot formation, however, no data on FXIII have been reported so far, and only a few studies addressed VWF in major blunt trauma.

Methods: Patients were part of a cohort study including 334 adult trauma patients (DIA-TRE-TIC Study) of whom 274 had severe poly-trauma and measurements of FXIII (n=274) and VWF (n=239) at admission, 4, 6 and 24h thereafter. Study endpoints were FXIII and VWF levels, their association with transfusion (RBC) and clinical outcome.

Results: At admission, half the study patients showed FXIII below the reference value of 70%, and 27% had even FXIII <60%. These patients received significantly more RBC/ 24 hours [6 (1, 12) U vs 0 (0, 3) U, p <0.0001] and showed a worse clinical outcome. Logistic regression analysis adjusted for ISS revealed FXIII <60% as independently influencing RBC transfusion. In contrast, the majority of study patients exhibited elevated VWF levels without a detectable association with transfusion requirements or clinical outcome. The immediate available parameters haemoglobin and clot firmness showed a sensitivity of 72 and 74% and a specificity of 83% and 85%, respectively, to predict FXIII plasma levels ≥60% at admission.

Conclusion: In major trauma VWF levels are commonly increased while nearly 30% of trauma patients show FXIII levels <60% at admission and this independently increased transfusion requirements. Further studies should clarify as to whether substituting FXIII may limit blood loss, transfusion requirements and ultimately improve clinical outcome.

Keywords: Coagulation Factors; Factor XIII; Von Willebrand Factor; Trauma; Blood Transfusion

Introduction

Trauma-induced coagulopathy is commonly diagnosed from the results of prothrombin time and activated partial thromboplastin time and numerous studies have reported that abnormal results correlate with increased transfusion requirements and poor outcome [1-3]. However, besides sufficient thrombin generation the formation of stable clots is a precondition for cessation of bleeding [4]. The quality of clot formation can be assessed with viscoelastic tests like TEG®/ROTEM®. The referring parameters Maximum Amplitude (MA) or Maximum Clot Firmness (MCF) and functional fibrin polymerisation (FibTEM) have been shown to correlate with transfusion requirements and mortality, which is excessively increased in case of fulminante hyperfibrinolysis [5-8]. The strength of the formed clot depends on fibrinogen concentration and polymerisation, numbers and quality of platelets, and on Factor XIII (FXIII). Thrombin activated FXIIIa enables cross-linking of fibrin monomers by introducing covalent binding sites. FXIIIa increases the resistance against fibrinolytic attack by binding α-antiplasmin and Thrombin-Activatable-Fibrinolysis-Inhibitor (TAFI) into fibrin. Former studies in neurosurgical and cardiac patients have shown that FXIII levels <60% are associated

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with increased bleeding [9-13]. The effect of FXIII on clot firmness can be demonstrated by TEG®/ROTEM®, whereas FXIII is not detected by global plasmatic coagulation tests [14-20].

Another rarely measured coagulation factor is Von Willebrand Factor (VWF), which is crucial for initial platelet aggregation and FVIII liberalisation, thereby augmenting thrombin formation. It is an indicator of endothelial activation and injury. Bleeding as a consequence of congenital or acquired VWF-deficiency varies and can be considerable [21]. To our knowledge only a few studies assessed VWF in patients with isolated head injury [22,23] and one study focusing on the significance of VWF in septic and non-septic patients with acute lung injury also enrolled 50 trauma patients [24], while data on FXIII levels in trauma patients are lacking.

We hypothesize that FXIII is critically reduced in polytrauma trauma patients and low FXIII levels are associated with increased bleeding and transfusion requirements. We further hypothesize that in contrast, trauma-induced systemic endothelial activation/injury results in elevated plasma levels of VWF in the majority of patients. The primary objective of the present analysis was to evaluate FXIII and VWF levels. Secondary objectives were to explore whether FXIII and VWF levels are associated with red blood cell transfusions and clinical outcome.

Patients and Methods

Patients were part of the The Diagnosis and Treatment of Trauma-Induced Coagulopathy (DIA-TRE-TIC) [7] study, which was conducted as a single-center, prospective, cohort study including adult trauma patients admitted to the Level I Trauma Center at Innsbruck Medical University Hospital to determine the characteristics of coagulation abnormalities in severe blunt polytrauma (Injury Severity Score, ISS ≥15) and in those patients exhibiting isolated head injury. Exclusion criteria were age below 18 years, penetrating injuries, pregnancy, admittance to the study hospital >12 hours after trauma, known pre-existing coagulopathy and isolated burn injury. The study protocol was approved by the Ethics Committee of Innsbruck Medical University. The need for written informed consent was waived because study-related blood sampling was judged a minimal-risk intervention and all patients were treated according clinical routine. We here present the analysis on measurements of FXIII and VWF in patients with polytrauma. Patients with isolated brain injury were excluded from this analysis (Table 1).

Male gender n (%)	218 (79.6)
Age (years)	42 (27, 54)
ISS (pts)	35 (25, 50)
15-29	104
30-50	116
51-75	54
Pattern of injury n (%)	
Head	176 (64.2)
Chest	210 (76.6)
Abdomen	148 (54.0)
Extremities	160 (58.4)
GCS (pts)	13 (6, 15)
SBP (mmHg)	120 (100, 140)
Heart rate (bpm)	90 (80, 110)
Shock n (%)	50 (18.2)
Prehospital crystalloids (mL)	1000 (500, 1500)
Prehospital colloids (mL)	500 (0, 1000)
Time until admission (min)	75 (60, 120)
Surgery within 4 hours n (%)	145 (52.9)
24h mortality n (%)	21 (7.7)
ISS: Injury Severity Score; GCS: Glasgow Coma Scale; SBP: Systolic Blood Pressure. Data are given as median values with interquartile ranges or numbers (%).	

Table 1: Characteristics of the Study Cohort.

Blood Sampling and Analysis

Blood for analysis of FXIII, VWF antigen (VWF:Ag), and VWF Ristocetin activity (VWF:RiCo) plasma levels, standard coagulation parameters and ROTEM® assays was drawn simultaneously into citrated tubes immediately after Emergency Department Admission (ED), 4, 6, 24h after hospital admission and then sent to the central laboratory for immediate work-up and plasmastorage at -80°C. Laboratory personnel were unaware of patient details (Table 2).

pH value	7.33 (7.28, 7.38)
Base excess (mmol/L)	-3.5 (-6.1, -1.6)
Haemoglobin (g/dL)	11.3 (9.4, 12.8)
PT (%)	75 (58, 89)
INR	1.3 (1.1, 1.4)
aPTT (sec)	32 (29, 39)
Antithrombin (%)	66 (53, 79)
Fibrinogen (mg/dL)	203 (148, 246)
Platelets (G/L)	169 (137, 202)
FXIII (%)	75 (57, 93)
VWF:Ag (%)	209 (163, 280)
VWF:RiCo (%)	202 (157, 267)
EXTEM CT (sec)	64 (55, 75)
EXTEM MCF (mm)	51 (45, 56)
FIBTEM MCF (mm)	9 (6, 13)
Hyperfibrinolysis (n)	20 (8.1)

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PT: Prothrombin Time; INR: International Normalized Ratio; aPTT: Activated Partial Thromboplastin Time; FXIII: coagulation Factor XIII; VWF: Ag-VWF Antigen, VWF:RiCo, VWF Ristocetin activity, EXTEM: Extrinsically Activated Thrombelastometric Assay, CT: Coagulation Time, MCF: Maximum Clot Firmness, FIBTEM MCF, Fibrinpolymerisation. Data are given as median and interquartile range.

Table 2: Laboratory Values at Emergency Department Admission.

FXIII, VWF :Ag, VWF:RiCo and standard coagulation parameters were determined using the following assays (Siemens Healthcare AG, Erlangen, Germany): FXIII (Berichrom®; reference range, 70%-144%), VWF antigen (von Willebrand Antigen®; reference range, 69%-169%), VWF:ristocetin (BC von Willebrand Reagent®; reference range, 50%-150%), prothrombin time (Thromborel S®; reference range, 70%-110%), activated partial thromboplastin time (Pathromtin SL®; reference range, 26-37 seconds), fibrinogen concentration (Multifibren®; reference range, 180-350 mg/dL), and D-Dimer (DD, chromogenic latex immunoassay DD, STA Roche Diagnostics, Mannheim, Germany, reference range 0-190µg/L; Innovance D-Dimer® Siemens Healthcare AG, reference range 0-500 µg/L). D-Dimer ratio was calculated by dividing the measured value by the upper normal value of the used assay.

Viscoelastic measurements were performed using rotational thrombelastometry (ROTEM®, Tem International GmbH (formerly Pentapharm), Munich, Germany) and the extrinsically activated assays (EXTEM®, FIBTEM®). The ROTEM parameters coagulation time, CT (reference range <80sec), clot formation time, CFT (reference range<150sec), maximum clot firmness, MCF (reference range >50mm), lysis index after 60min, LI60 (reference range <15%) and fibrin polymerization, FIBTEM MCF (reference range (>8mm) were registered.

Data Collection

Patient demographics, type of injury, Injury Severity Score (ISS), Glasgow Coma Scale (GCS), details of pre-hospital treatment and time elapsed between trauma and hospital admission was documented at emergency department admission. The following data were recorded at admission, 4, 6, and 24hours later: type and number of transfused blood components, dosage of coagulation factors administered and amount of fluids infused. All study patients were followed up until hospital discharge. The need for intensive care unit admission, days on mechanical ventilation, occurrence of multiple organ dysfunction syndrome or sepsis, intensive care unit and hospital length of stay, as well as 24h and hospital mortality were recorded.

The numbers of ventilator-free days were calculated as follows: 28 days minus days on mechanical ventilation, patients without need for mechanical ventilation were allocated as 28, those who did not survived as 0. Hyperfibrinolysis was diagnosed in cases showing a reduction of clot firmness in the extrinsically activated ROTEM assay >15% within 60min. Clinical significant FXIII deficiency was defined as FXIII levels below 60% according to previously published data [9-13].

Shock was defined as the presence of a systolic blood pressure <95 mmHg at ED admission despite ongoing fluid resuscitation. Multiple organ dysfunction syndrome was defined as the simultaneous failure of two or more organs. Organ failures were defined as presence of >2 points in single organ subcategories of the Sequential Organ Failure Assessment Score. Sepsis was defined according to the criteria suggested by the American College of Chest Physicians and the Society of Critical Care Medicine.

Transfusion of Blood Components and Administration of Coagulation Factor Concentrates

At our institution blood components are leukocyte-depleted and quarantine FFP are available within 30-45 min. Haemostatic therapy is guided by results of plasmatic coagulation test, ROTEM measurements and clinical decision. Coagulation Factor Concentrates (CF) are used first for immediate treatment. Fibrinogen concentrate (Haemocomplettan P 1g®, CSL Behring, Marburg, Germany) is used for correction of low fibrinogen concentration and/or poor fibrin polymerization (fibrinogen concentration <150-200 mg dL-1 and/or FIBTEM MCF <7mm) at dosages of 25-50 mg kg-1 body weight. Prothrombin complex concentrate (Beriplex P/N 500 IU® CSL Behring, Marburg, Germany) containing Factors II, VII, IX and X is used at dosages of 20-30IU kg^{-1} body weight in cases showing delayed initial thrombin formation (PT <50% or INR >1.5 and/or EXTEM CT >90sec). FXIII concentrate (Fibrogammin® CSL Behring, Marburg, Germany) is administered at 20-30 IU kg^{-1} body weight in bleeding patients showing FXIII levels below 60% and/or insufficient fibrin polymerization following administration of fibrinogen concentrate.

FFP are transfused according to the clinical experience of the anesthesiologist in charge and coagulation test results (INR>1.5, aPTT >50sec and/or EXTEM CT >90sec). Apheresis platelet concentrates are used in bleeding patients showing platelet counts <50-100 G L-1 and/or poor clot firmness (EXTEM MCF <45 mm). Haemoglobin levels <8-9 g dL-1 are the usual transfusion trigger in actively bleeding trauma patients. At the time, the study was conducted antifibrinolytics were not used prophylactically.

Statistical Analysis

The primary study endpoint was to evaluate plasma levels of FXIII and VWF in blunt trauma patients. Secondary study endpoints were to 1) compare clinical and laboratory variables, transfusion requirements and outcome between trauma patients displaying VWF levels within reference ranges and those showing elevated levels, as well as between patients with FXIII below and above 60% at ED admission, 2) evaluate the influence of FXIII < 60% at admission on cumulative Red Cell Concentrate (RBC)

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requirements during the first 24 hours, and 3) identify whether immediate available routine laboratory variables can indicate FXIII plasma levels ≥60%.

The SPSS software package (Version 18.0.; SPSS Inc; Chicago, IL, United States) was used for statistical analysis. Kolmogorov-Smirnov tests were applied to test for normality distribution of study variables. Because several variables departed significantly from normal at an alpha level of .05, non-parametric tests were used throughout the analyses. The Kruskal-Wallis-Test and Mann-Whitney-U-Test were used to analyze metric data; for categorical data, the chi-square test and Fisher's-exact-test were used, as applicable. In order to assess the association between coagulation tests and the admission independently of injury severity, a multiple logistic regression model was calculated that included the need for RBC transfusion as the dependent variable, different coagulation tests as the independent variable and ISS (reflecting injury severity) as a covariate.

Receiver operating characteristic curves were used to identify the predictive value and cut-off levels of surrogate routine laboratory parameters to indicate FXIII plasma levels ≥60%. Cut-off values were determined as those with the highest sum of sensitivity and specificity. A p value <0.05 was considered to designate statistical significance. Data are presented as median values with interquartile ranges, if not otherwise indicated.

Results

Among the 334 patients enrolled in the DIA-TRE-TIC study 274 exhibited multiple injuries (polytrauma) and had complete data on FXIII levels. In 239 of these patients measurements of VWF were also available (missing blood samples in 35 patients). Characteristics of the study cohort and laboratory measurements at admission are given in Tables 1, 2.

At admission, nearly half of the study population exhibited FXIII levels below the normal value, while VWF antigen and VWF ristocetin plasma levels were elevated or within the normal range in the vast majority (99.2%) of study patients, except two (0.8%) presenting with decreased plasma levels (Figure 1). FXIII, VWF antigen, and VWF ristocetin plasma levels significantly changed during the observation period as shown in Figure 2a-c.

Fig. 1

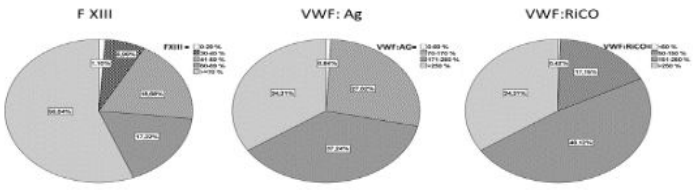

Figure 1: Shows the prevalence of the concentration of coagulation Factor XIII (FXIII, reference range 70-144%), von Willebrand factor antigen (VWF:Ag, referencerange 69-169%) and von Willebrand factor Ristocetin activity (VWF:RiCo, reference range 50-150%) in polytrauma patients at admission to the emergency department.

Fig. 2

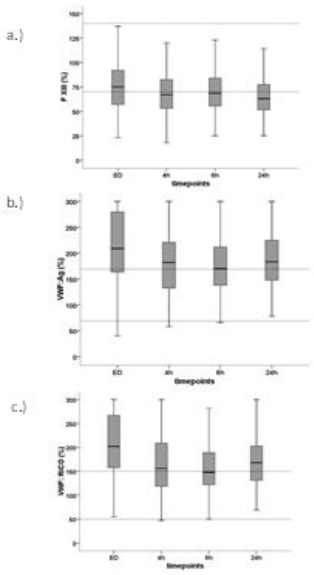

Figure 2: (a-c) shows the time course of coagulation Factor XIII (FXIII), von Willebrand factor antigen (VWF:Ag) and von Willebrand factor ristocetin activity (VWF:RiCo) in blunt trauma patients at admission to the Emergency Department (ED), as well as 4, 6, and 24 hours later. Levels of FXIII, VWF:Ag and VWF:RCo changed significantly during the observation period (p<0.001). Reference ranges are indicated as dashed lines.

For further analysis patients were grouped in those showing FXIII below 60% [FXIII 47 (40, 54)%, n=73] as compared to those exhibiting FXIII >60% [FXIII 82 (70-98)%, n=201]. Study patients with FXIII below 60% at admission had higher ISS, received more fluids during the prehospital period, were more often in shock (Table 3) and showed more abnormal laboratory test results than those with FXIII >60% (Table 4). Median levels of FXIII at admission decreased with increasing ISS (Figure 3), however patients exhibiting very low levels of FXIII were observed in all ISS groups.

	FXIII <60% (n=73)	FXIII ≥60% (n=201)	p value
Age (years)	43 (26, 54)	42 (27, 54)	0.813
Male sex n (%)	56 (76.7)	161 (80.5)	0.493
Pattern of injury n (%)			
Head/Neck	42 (57.5)	133 (66.5)	0.156
Chest	55 (75.3)	154 (77.0)	0.723
Abdomen/Pelvis	53 (72.6)	95 (47.5)	<0.001
Extremities	51 (69.9)	108 (54)	0.021
Time trauma-ED admission (min)	70 (56, 98)	78 (60, 136)	0.27
Prehospital crystalloids (mL)	1000 (1000, 2000)	1000 (500, 1500)	0.001

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Prehospital colloids (mL)	1000 (500, 1000)	500 (0, 500)	<0.001
Injury Severity Score (pts)	43 (34, 59)	34 (24, 41)	<0.001
Glasgow Coma Scale (pts)	11 (5, 15)	13 (7, 15)	0.998
SBP (mm Hg)	103 (90, 121)	120 (110, 140)	<0.001
Heart rate (bpm)	95 (80, 115)	90 (80, 105)	0.008
Shock n (%)	23 (31.5)	27 (13, 5)	<0.001
Temperature (°C)	35.0 (33.2, 36.2)	35 (34.2, 36.1)	0.677
ED: Emergency Department; SPB: Systolic Blood Pressure; Data are given as median values with interquartile ranges, if not otherwise indicated.			

Table 3: Characteristics of patients with Factor XIII levels < 60 and ≥ 60% at emergency department admission.

	FXIII <60% (n=73)	FXIII ≥60% (n=201)	p value
FXIII (%)	47 (40, 54)	82 (70, 98)	<0.001
PT(%)	53 (40, 66)	82 (68, 93)	<0.001
INR	1.5 (1.4, 1.8)	1.2 (1.1, 1.3)	<0.001
aPTT (sec)	43 (37, 64)	30 (27, 34)	<0.001
Antithrombin (%)	48 (39, 56)	72 (62, 82)	<0.001
Fibrinogen (mg dL-1)	122 (100, 156)	217 (186, 262)	<0.001
DD ratio	16.1 (7.5, 32.3)	10.0 (4.7, 21.3)	0.02
EXTEM CT (s)	75 (65, 113)	60 (54, 69)	<0.001
EXTEM CFT (s)	209 (170, 321)	126 (100, 168)	<0.001
EXTEM MCF (mm)	44 (39, 48)	54 (49, 58)	<0.001
FIBTEM MCF (mm)	5 (1, 8)	10 (7, 14)	<0.001
Hyperfibrinolysis n (%)	11 (15.1)	9 (4.5)	0.003
Platelets (G L-1)	132 (110, 168)	182 (154, 214)	<0.001
Haemoglobin (g dL-1)	8.4 (6.7, 10.2)	12.0 (10.4, 13.4)	<0.001
vWF:Ag (%)	181 (137, 255)	223 (173, 290)	0.007
vWF:RiCO (%)	188 (131, 253)	207 (163, 282)	0.091
Arterial pH	7.30 (7.25, 7.35)	7.34 (7.29, 7.39)	<0.001
Base deficit (mmol L-1)	-5.1 (-8.0, -3.2)	-2.9 (-5.2, -1.4)	<0.001
FXIII: Factor XIII; INR: International Normalized Ratio; aPTT: Activated Partial Thromboplastin Time; vWF:Ag- von Willebrand Factor Antigen; vWF:RiCo- von Willebrand Ristocetin Activity; DD ratio: D-Dimer ratio; EXTEM: Extrinsically Activated Thrombelastometric Assay; CT, coagulation time; MCF: Maximum Clot Firmness; FIBTEM MCF: Fibrinpolymerisation. Data are given as median values (interquartile range).			

Table 4: Laboratory variables in patients with Factor XIII levels < 60% and ≥ 60% at emergency department admission.

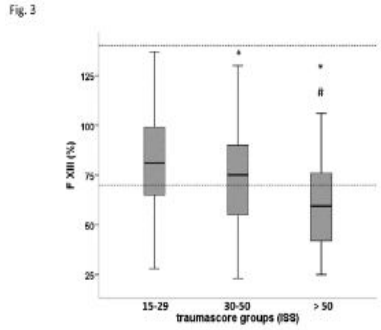

Figure 3: shows concentration of coagulation factor XIII (F XIII) at admission to emergency department according to ISS grouped as ISS 15-29, 30-50 and >50. (reference ranges are indicated as dashed line). The Kruskal-Wallis test and the Mann- Whitney U Test was used for analysing differences between ISS groups. *p<0.05 as compared to ISS group 15-29, # p<0.05 as compared to ISS group 30-50.

Trauma patients presenting with FXIII <60% required transfusion of more blood components and coagulation factor concentrates during the first 24 hours, developed more often multiple organ dysfunction syndrome, had fewer ventilator-free days and longer ICU stay, than did study patients without FXIII deficiency (Table 5). In logistic regression models, FXIII at admission was a strong predictor of the cumulative 24 h RBC transfusion, as were prothrombin time, haemoglobin, presence of hyperfibrinolysis and poor fibrin polymerisation (Table 6). Among the immediately available routine laboratory parameters haemoglobin, maximum clot firmness of the EXTEM® and fibrin polymerisation (FIBTEM®) were predictors of FXIII deficiency at emergency department admission. Although not immediately available PT values showed an acceptable sensitivity and specifity to detect FXIII deficiency (Table 7).

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	FXIII <60% (n=73)	FXIII ≥60% (n=201)	p value
RBC (U)	6 (1, 12)	0 (0, 3)	<0.0001
PC (aphaeresis U)	0 (0, 2)	0 (0, 0)	<0.0001
FFP (U)	5 (0, 10)	0 (0, 0)	<0.0001
Fibrinogen concentrate (g)	4 (2, 7)	0 (0, 3)	<0.0001
PCC (IU)	600 (0, 2000)	0 (0, 0)	<0.0001
MOF n (%)	22 (30.1)	28 (14.0)	0.001
Sepsis n (%)	13 (17.8)	33 (16.5)	0.573
Length of ICU stay (days)	13 (5, 28)	9 (3, 17)	0.009
Ventilator-free days (days)	13 (0, 23)	24 (12, 26)	<0.001
Length of hospital stay (days)	19 (9, 41)	18 (9, 31)	0.52
Hospital mortality n (%)	17 (23.3)	18 (9.0)	0.002
GOS (pts)	4 (2, 5)	5 (4, 5)	<0.001

FXIII: Factor XIII plasma level; RBC, red blood cells; PC: Platelet Concentrate; FFP: Fresh Frozen Plasma; PCC: Prothrombin Complex Concentrate (containing factors II, FVII, IX and X); MOF: Multiple Organ Failure; GOS: Glasgow Outcome Scale; RC: Red Cell Concentrate; PC: Platelet Concentrate; FFP: Fresh Frozen Plasma;
Data are presented as median values with interquartile ranges, if not otherwise indicated.

Table 5: Differences in 24h transfusion and coagulation factor requirements and outcome between trauma patients with FXIII below or above 60% at emergency room admission.

	Regression coefficient	p value	OR (odds ratio)	95 % CI	
FXIII (%)	-0.035	<0.001	0.966	0.953	0.978
FXIII <60% (categorical)	1.366	<0.001	3.921	2.084	7.379
PT (%)	0.014	<0.001	0.96	0.945	0.975
aPTT (sec)	0.009	0.272	1.009	0.993	1.024
Fibrinogen (mg/dL)	-0.005	0.003	0.995	0.991	0.998
Platelets (G/L)	-0.007	0.006	0.993	0.988	0.998
EXTEM CT (sec)	-0.001	0.342	0.999	0.996	1.001
EXTEM CFT (sec)	0	0.291	1	0.999	1
EXTEM MCF (mm)	-0.19	0.25	0.981	0.95	1.013
Hyperfibrinolysis (categorical)	1.401	0.012	4.061	1.353	12.19
FIBTEM MCF (mm)	-0.082	0.004	0.921	0.871	0.974
Haemoglobin (g/dL)	-0.47	<0.001	0.954	0.941	0.967

FXIII: coagulation Factor XIII; PT: Prothrombin Time, aPTT: Activated Partial Prothrombin Time; EXTEM: Extrinsically Activated ROTEM assay; CT: Coagulation Time; CFT: Clot Formation Time; MCF: Maximum Clot Firmness; FIBTEM MCF: fibrin polymerization

Table 6: Summary of single logistic regression models (including ISS as a covariate) evaluating the influence of coagulation parameters at admission on 24h red blood cell requirements in patients with severe blunt trauma.

	AUC ROC	CI 95%	p value	Cut-off value	Sensitivity (%)	Specificity (%)
EXTEM CT (sec)	0.774	0.711-0.835	<0.001	69	67	74
EXTEM CFT (sec)	0.859	0.816-0.903	<0.001	161	81	73
EXTEM MCF (mm)	0.862	0.817-0.908	<0.001	50	74	85
FIBTEM MCF (mm)	0.849	0.796-0.901	<0.001	6	93	57
PT (%)	0.873	0.827-0.919	<0.001	61	88	74
Fibrinogen (mg/dL)	0.886	0.838-0.934	<0.001	154	89	75
Platelets (G/L)	0.785	0.723-0.847	<0.001	148	80	69
Haemoglobin (g/dL)	0.839	0.783-0.895	<0.001	10.7	72	83
Base deficit (mmol/L)	0.665	0.583-0,747	<0.001	-3.3	55	75

CI: Confidence Interval; EXTEM: Extrinsically Activated Thrombelastometry; CT: Coagulation Time; CFT: Clot Formation Time; MCF: Maximum Clot Firmness; FIBTEM MCF: Fibrin Polymerization.

Table 7: Results of the Receiver Operator Characteristic Curve Analysis (AUC ROC) for identification of the predictive value and cut-off levels of surrogate laboratory parameters to indicate Factor XIII plasma levels ≥60% at admission.

For evaluating VWF significance in polytrauma, patients were grouped in those showing VWF antigen in the normal range [137 (118%-157) %, n=66] vs those showing values above normal [246 (204, >300)%, n=171] at emergency department admission. Those with normal VWF levels at admission had a lower ISS

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[34 (23, 41) vs. 36 (25, 50) points, p=0.085], had received larger amounts of colloids during the prehospital period [500 (0, 1000) vs. 500 (0, 500) mL, p<0.001], showed longer activated partial thromboplastin time [35 (31, 45) vs. 32 (28, 37) s, p<0.001], lower haemoglobin [10.1 (7.4, 12.1) vs. 11.8 (9.8, 13.3) g/dL, p<0.001] and lower FXIII plasma levels [66 (48, 86) vs. 77 (62, 95)%, p=0.002] than did study patients exhibiting elevated VWF antigen. They also showed significantly lower values of PT, AT, fibrinogen, platelets, clot strength and fibrin polymerisation as compared to those patients admitting with elevated VWF antigen (data not shown). The VWF quotient (VWF ristocetin activity/VWF antigen), indicating binding capacity, was similar in both VWF groups (normal VWF 0.99 (0.82, 1.18) vs VWF above normal 0.98 (0.85, 1.04), as was the incidence of hyperfibriolysis (normal VWF six out of 68 patients, 8.8% vs VWF above normal 12 out of 171 patients, 7.0%).

Requirements for blood components [RBC 0 (0, 8) vs. 0 (0,5) U, p=0.90] and need for coagulation factor concentrates [fibrinogen concentrate (1(0, 4) vs. 2 (0, 5) g, p=0.81] were not influenced by levels of VWF or VWF ristocetin activity. Patients with normal or elevated VWF antigen at admission had a similar incidence of sepsis and MOF and showed comparable numbers of ventilator-free days, length of ICU and hospital stay. No difference in levels of VWF antigen at emergency department admission or later time points was detected between surviving and non-surviving study patients (data not shown).

Discussion

We here report for the first time FXIII and VWF levels in a large cohort of patients with major blunt trauma. Nearly half of the study patients exhibited FXIII levels below the reference range of 70%. Twenty-seven percent of the study population even presented with FXIII plasma levels <60%, a value, which has been found as associated with decreased fibrin polymerisation and increased bleeding in surgical patients [9-13]. FXIII level at admission was a strong independent predictor of 24h RBC requirements and univariate analysis of patients with FXIII below or above 60% showed significant differences in clinical outcome.

In contrast, the majority of blunt trauma patients exhibited VWF plasma levels above normal. While VWF plasma levels were higher in patients with more severe injury, abnormal low VWF levels were observed in only two (0.8%) of trauma patients. This prevalence is comparable to the incidence of mild VW disease Type 1 observed in the general population. No association was found between normal or elevated VWF levels and transfusion requirements or clinical outcome. The haemostatic significance of FXIII was first described in 1960 by Duckert [25]. At that time the transglutaminase was mentioned as a „fibrin stabilising factor "As it increases the rigidity of a newly formed fibrin clot by cross linking fibrin γ-chains into dimers and fibrin α-chains into high-molecular-weight polymers once it was activated by thrombin and Ca2+. The fibrin cross-linking makes the fibrin network more resistant against shear stress and directly inhibits plasmin-induced degradation. FXIII also inhibits the fibrinolytic system by covalent cross-linking of α2-antiplasmin (α2-PI), the major plasmin inhibitor, to the fibrin clot [26]. Thrombin Activatable Fibrinolysis Inhibitor (TAFI) and the Plasminogen Activator Inhibitor 2 (PAI-2) have also been shown to be substrates of FXIII [27]. A recently published in vitro study clearly shows that exogeneous FXIII attenuated t-PA induced fibrinolysis nearly as effective as tranexamic acid [28]. The clot strengthening effects of FXIII as well as increased resistance against fibrinolytic attack may be important especially in trauma patients, who frequently show increased fibrinolytic activity and impaired clot strength resulting from low fibrinogen concentrations, impaired fibrin polymerisation and low platelet counts [7,29]. Thus, in these patients the effects of FXIII deficiency cannot be compensated by these other factors governing clot strength. Confirming this assumption, Gerlach and co-workers reported the highest incidence of re-bleeding and the need for revision craniotomy in patients exhibiting combined deficiency of fibrinogen, platelets and FXIII, although single factor deficiency was only moderate [10]. It has also been shown that weaker clots after colloid administration dissolve faster at constant fibrinolytic activity [30]. In contrast, all these pathologies are not present in the rare patients with congenital FXIII deficiency, a fact which might explain why FXIII levels below 10% have been reported in the literature to be sufficient to prevent from spontaneous bleeding [31]. In a recent study using flow conditions for investigating FXIII- mediated cross-linking and resistance to fibrinolysis FXIII levels of 8-9% were clearly not sufficient to achieve normal thrombus resistance to clot lysis [32]. Furthermore, it has been reported that patients with FXIII levels between 5% and 40% can also suffer significant bleeding [33]. Lastly, when interpreting test results one should consider that the sensitivity of laboratory tests varies especially at low concentrations leading to overestimation and the frequently used clot solubility test detects severe deficiency only [34]. All these aspects explain the existing difficulties with establishing general applicable critical FXIII levels. In the present study we used the Berichrom® assay which shows good reproducibility and low variations for normal and pathological controls. Using this assay in more than 1000 samples acquired FXIII deficiency was relatively common in postoperative adult and paediatric patients and in patients on the ICU [34]. FXIII-deficiency is not detected by classical global coagulation test (e.g. PT, INR, aPTT), which are commonly used to guide coagulation management in trauma patients, and may be thus overlooked. FXIII-mediated decreased clot strength and increased fibrinolysis can be assessed using viscoelastic methods, although exact quantification of FXIII is not possible at this time [13,14,16,17,19,20,30]. In the present study we also observed a significantly reduced clot firmness and fibrin polymerisation in patients with FXIII levels below 60% which cannot be

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explained simply by the also lower fibrinogen concentrations and lower platelet counts of these patients. Moreover, hyperfibrinolysis was more frequently detected by ROTEM if FXIII levels were below 60% than in patients with FXIII levels above 60%.

Since FXIII analysis is time-consuming and not available at many institutions, we analyzed the usefulness of surrogate routine laboratory parameters which are commonly available to predict FXIII levels >60%. Our results suggest that clot strength and haemoglobin at cut-off values of 50 mm and 11 g/dL, respectively, may be used as surrogate parameters with a tolerable sensitivity and specificity to exclude FXIII deficiency. For the first look it seems likely that low FXIII at admission and increased need for red cell transfusion primarily reflect severity of injury. However, multivariate analysis including ISS as a covariate revealed that FXIII <60% was a strong factor associated with need for red cell transfusion during the first 24h. Because prolonged bleeding and increased transfusion requirements have been shown to impact patient outcome, future studies investigating the benefit of monitoring and optimizing FXIII plasma levels appear justified.

Von Willebrand factor (vWf) is a multimeric glycoprotein which is synthesized and stored by endothelial cells. Its release occurs in response to several stimuli (e.g. histamine, estrogens, thrombin, fibrin) what makes it a good indicator of endothelial activation. VWF mediates platelet adhesion via the glycoprotein Ib platelet receptor and serves as carrier for factor VIII, needed for sufficient thrombin generation. Yokota and colleagues reported a significant correlation between Fibrinogen Degradation Product (FDP) as a marker of cerebral tissue injury and elevated VWF levels as a marker of the accompanying cerebral endothelial activation in severe head injury [23]. The plasma levels of VWF were significantly higher in focal brain injury than in diffuse axonal injury reflecting the more pronounced mechanical injury of focal brain damage. Oliveira and co-workers showed a significant correlation between elevated VWF levels and tomographic signs of intracranial pathologies and consecutive mortality in severe traumatic brain injury indicating that high VWF levels are a marker of unfavourable outcome [22].

Another study in patients with acute lung injury/acute respiratory distress syndrome found that baseline VWF levels in 54 trauma patients were lower than in 505 non-trauma patients. For the total study population the risk for death increased in patients with baseline VWF levels above 450% of control [24]. In the present study survivors showed slightly lower VWF levels than did non-survivors, but this difference did not reach statistical significance. When comparing patients within and above VWF reference ranges we were not able to establish an association between VWF levels and patient outcome. This discrepancy might be related to the fact that the upper detection limit of the VWF assay used in our study was 300% which refers to about 170% above control, while the assays used in the above-cited studies were relevantly higher. Another possibility is that in patients with isolated organ injury VWF might be released mainly at local sites and systemically measured elevated levels may specifically reflect severity of the single organ failure and thus patient outcome. In contrast, in multiple trauma patients VWF levels are more likely to originate from systemic endothelial injury/activation. Accordingly, VWF plasma levels were higher in trauma patients with severe injury, but were not associated with a specific trauma pattern in this study cohort. Lastly, the here reported lower levels of VWF in major trauma patients may be influenced by the significant blood loss and fluid resuscitation in major trauma patients which counterbalances the increase in VWF plasma levels. Nevertheless, the VWF levels observed in this large collective were clearly above normal. This finding might explain in part why thrombin generation is initially increased in trauma patients, as well as their susceptibility for hyperfibrinolysis, because VWF liberation is accompanied by FVIII but also t-PA release. In contrast to FXIII which was frequently below normal decreased VWF was detected rarely. Thus a general monitoring of VWF seems not necessary in trauma patients.

Limitations of the present study need to be addressed. Firstly, this was a prospective cohort study and its results may have been influenced by a certain selection bias. However, the study design was appropriate to address the main study endpoint namely VWF and FXIII plasma levels in blunt trauma patients. Given the observational design of this study and the lack of data on VWF and FXIII plasma levels in blunt trauma patients, no power analysis could be calculated. We assumed that inclusion of approximately 250 trauma patients would be sufficient to estimate the prevalence of FXIII levels below normal and VWF levels above normal. The observed prevalence (95% confidence interval) of FXIII <70%, VWF:Ag ≥ 170% and VWF:RiCo > 150% was 44% (95%CI 38-50), 72% (95% CI 65-77) and 78% (95% CI 72-82%), respectively. Thus, the sample size of 274 and 239 patients, respectively detected the investigated prevalence of FXIII and VWF in polytrauma with an accuracy of ±6%.

Secondly, dichotomizing the study population based on VWF within or above reference ranges as well as presence of FXIII below or above 60% could be criticized as arbitrary and clinically irrelevant. Considering that specific VWF plasma levels have not been associated with outcome in blunt trauma patients yet, we decided to focus our analysis on patients showing levels within or above the current reference range. The decision to define FXIII deficiency as FXIII plasma levels <60% in this study population was governed by previous published data of surgical patients clearly indicating a relevant increase in blood loss at FXIII levels below 60% [9-13]. Our results on 24h RBC transfusion requirements confirm these previous data in a blunt trauma population. Lastly, our observational study indicates an association of FXIII with bleeding-related transfusion requirements and consequently clinical outcome, while a firm causal relationship still needs to be established.

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In conclusion, the vast majority of patients exhibited normal or increased VWF plasma levels, but VWF plasma levels did not differ between survivors and non-survivors. In contrast, decreased FXIII plasma levels were observed frequently already at hospital admission and were associated with 24h red blood cell transfusion requirements and clinical outcome. Clot firmness within normal ranges and hemoglobin levels above 10 g/dL can exclude FXIII deficiency in the emergency department with an acceptable sensitivity and specificity. Whether laboratory monitoring of FXIII and substituting of FXIII may reduce blood loss and thus improves outcome of trauma patients needs to be addressed in future clinical trials.

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