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Abstract

***Background**:* Factor XIII (F XIII) is an essential parameter for final clot stability. The purpose of this study was to determine the impact of the addition of F XIII on clot stability as assessed by Rotation Thromboelastometry (ROTEM®).

Methods: In 90 intensive care patients ROTEM® measurements were performed after in vitro addition of F XIII 0.32 IU, 0.63 IU, 1.25 IU and compared to diluent controls (DC; aqua injectabile) resulting in approximate F XIII concentrations of 150, 300 and 600%. Baseline measurements without any additions were also performed. The following ROTEM® parameters were measured in FIBTEM and EXTEM tests: clotting time (CT), clot formation time (CFT), maximum clot firmness (MCF), maximum lysis (ML), maximum clot elasticity (MCE) and α-angle (αA). Additionally, laboratory values for F XIII, fibrinogen (FBG), platelets and hematocrit were contemporaneously determined.

Results: In the perioperative patient population mean FBG concentration was elevated at 5.2 g/L and mean F XIII concentration was low at 62%. The addition of F XIII led to a FBG concentration dependant increase in MCF both in FIBTEM and EXTEM. Mean increases in MCF (F XIII vs. DC) of approximately 7 mm and 6 mm were observed in FIBTEM and EXTEM, respectively. F XIII addition also led to decreased CFT, increased αA, and reduced ML in FIBTEM and EXTEM.

Conclusions: In vitro supplementation of F XIII to supraphysiologic levels increases maximum clot firmness, accelerates clot formation and increases clot stability in EXTEM and FIBTEM as assayed by ROTEM® in perioperative patients with high fibrinogen and low F XIII levels.

Words: 249

Introduction

Blood coagulation factor XIII (F XIII) is a protransglutaminase that is present in plasma, platelets, monocytes and monocyte-derived macrophages. It becomes activated by the combined action of thrombin and Ca^{2+} in the final phase of the clotting cascade. Plasma F XIII circulates in association with its substrate precursor, fibrinogen, which has an important regulatory impact in the activation of plasma F XIII [1, 2]. Relevant physiologic functions of F XIII include cross-linking of fibrin monomers amongst themselves to generate a stable fibrin strand, cross-linking fibrinogen with α2 antiplasmin, a potent plasmin inhibitor, to protect the fibrin clot from fibrinolysis, and cross-linking the fibrin clot with subendothelial collagen and fibronectin resulting in clot localization at a site of injury [3, 4]. While Fibrinogen is an acute phase protein whose concentration increases in relation to the extent of the acute phase reaction, F XIII is not up-regulated under the same circumstances. We thus hypothesized that a relative F XIII deficiency might occur in the presence of an acute phase as manifested in our patient population.

After severe trauma and in major surgery severe bleeding leads to considerable decrease of all coagulation factors. Secondary to its loss as well as its consumption, hemodilution by volume replacement therapy additionally reduces the plasma coagulation factor fraction. Like in burn patients it is assumed that F XIII concentration may be low following trauma and in major surgery and may be responsible for otherwise inexplicable intra- and postoperative bleeding [5-7]. F XIII is not detected by routine laboratory screening such as prothrombin time (PT) and partial thromboplastin time (PTT). F XIII assays are often only available in specialized laboratories. Furthermore, determination of F XIII using plasma-based testing is time consuming due to the obligatory prior sample centrifugation.

Point of care monitoring of blood coagulation at the patients bed side is becoming increasingly relevant and provides real-time results. Detecting F XIII deficiency with point of care devices would be desirable. Most recently, Weber and colleagues [8] reported the detection of unknown F XIII deficiency by Thromboelastometry in one case. Should ROTEM® indeed have the ability to reliably detect the functional consequences of changes in F XIII concentrations on clot formation, it might become a point of care device for guiding the treatment with F XIII in real time.

The goal of this study was to investigate in-vitro supplementation of F XIII with 0.32 IU F XIII, resulting in F XIII concentration of approximately 150 %, 0.63 IU FXIII resulting in F XIII concentration of 300 % and 1.25 IU resulting in F XIII concentration of 600 % to see whether clot characteristics as assayed by ROTEM® in the EXTEM and FIBTEM tests, both of which are fibrinogen and F XIII dependent, are to be improved in patients with laboratory proven F XIII deficiency. No comparison of ROTEM® tests after in vivo treatment with F XIII was made in the current study, and therefore no direct extrapolation to any clinical situation should be made.

Materials and Methods

This study was performed after obtaining authorization by the local ethic committee (Kantonale Ethikkommission, Kanton Zürich, Switzerland, Study number StV 27-2007, amendment 3). As patients were in a critical and life threatening situation not allowing direct written informed consent, consent was obtained by signed consent form by exception [9]. Inclusion criteria were: Patients needing ROTEM® measurements postoperatively because of a presumed coagulation problem and F XIII, platelets and fibrinogen being determined by the institutional laboratory. Exclusion criteria were: known coagulopathic disorders and thrombocytopenia ≤ 100 000/µl.

Ninety critical care patients were enrolled consecutively after major surgery in pre-specified order in this prospective single-center trial and each 30 patients were allocated to one of three ROTEM® groups, respectively. Enrollment occurred within six hours after major surgery. The ROTEM groups were defined by the F XIII added to the ROTEM® tests (0.32 IU in 5 µl, 0.63 in 10 µl or 1.25 IU in 20 µl). In the intensive care unit (ICU) two samples of citrated and one sample of EDTA whole blood were collected in each patient, contemporaneously. Platelet count was determined in the EDTA sample and fibrinogen and F XIII concentrations were determined in one of the two citrated blood samples of each patient by the institutional laboratory. The second citrated whole blood sample was used for EXTEM and FIBTEM measurements i) after addition of 0.32, 0.63 and 1.25 IU of F XIII in a volume of 5, 10 and 20µl, ii) after addition of 5, 10 and 20 µl of aqua injectabile (diluent controls, DC) and iii) without addition of any further reagent (baseline measurements).

ROTEM® tests were performed according to the manufacturer's instructions at 37° C and ran for 62 minutes using two ROTEM® devices simultaneously. To optimize comparability, F XIII-supplemented samples and diluent control samples were run on the same machine, while baseline samples were measured in parallel on the second machine.

Parameters of Rotation Thromboelastometry: ROTEM® assesses the kinetics and quality of clot formation and clot lysis in real-time. The clotting time (CT) is defined as the period of time from the start of the analysis until the start of clot formation, normally until the 2 mm amplitude is reached. The clot formation time (CFT) is defined as the period until the 20 mm amplitude is reached. The α-angle (αA) is defined as the angle between the centre line and a tangent to the curve through the 2 mm amplitude point, which is the end of the CT. The maximum amplitude of the curve is defined as the maximum clot firmness (MCF). The maximum lysis (ML) represents the maximum fibrinolysis detected during the measurement. The maximum clot elasticity (MCE) is a calculated parameter from MCF values, and is intended to allow a better interpretation of MCF in case of high amplitudes in MCF. Further details on that subject haven been recently been published by Theusinger and colleagues. [10] For the purpose of this paper we define ΔMCF as being the difference in MCF of a FXIII supplemented sample and the related diluent control. The same definition was used for ΔMCE.

The following parameters were collected: Clotting time (CT), Clot formation time (CFT), α-angle (αA), Maximum clot firmness (MCF), Maximum clot elasticity (MCE) and Maximum lysis (ML) for EXTEM. In FIBTEM only CT, MCF, MCE and ML were analyzed.

Test procedure

The two ROTEM® devices being used in this study were set up by a representative of the manufacturer; both devices had been calibrated and tested before the study started. All tests were performed according to the instructions of the manufacturer. For each measurement a new pin was positioned on the axis of the measurement channel and a new cup was put into

the special cup holder of the device. The automated pipette programs was used for baseline measures, with 20 µl re-calcification reagent (200 mmol/l calcium chloride solution) and 20 µl of the respective activation reagent (FIBTEM: cytochalasin D, EXTEM: thromboplastin from rabbit brain) were added into the pre-warmed cup. Then 300 µl of citrated whole blood was added to the cup and, after a semi-automated mixing step, the cup holder was placed to the measuring position of the ROTEM® device. The measurement started automatically when blood was added to the cup and stopped after 62 min. (ex-TEM lot 41194401, fib-TEM lot 41147601, star-TEM lot 41166101)

For FXIII supplemented tests as for diluent controls, the appropriate volumes of F XIII (Fibrogammin P® 250 IU in 4 ml, lot 23164211 J, CSL Behring, Zürich, Switzerland) and diluent (aqua injectabile, lot 71511011, CSL Behring GmbH, Marburg, Germany) were added to the cup prior to initialization of the pipetting program.

The laboratory analyses were performed in quality controlled ISO 17025 accredited university laboratories. F XIII was determined on Behring Coagulation System (Dade Bering, Düdingen, Switzerland) using the Berichrom® F XIII test (Dade Bering, Düdingen, Switzerland; normal range 70-140% which equals 0.7-1.4 IU/ml). This test measures the function of F XIII. Fibrinogen was also analyzed by the Behring Coagulation System (Dade Bering, Düdingen, Switzerland) using the Multifibrin® U test (Dade Bering, Düdingen, Switzerland; normal range 1.5 – 4.0 g/l). Platelet count was measured with Advia® 2120 (Siemens Healthcare Diagnostics GmbH**,** Eschborn, Germany; normal range 143 – 400 $x10^9$/l).

Statistical Analyses

Data were transferred from the ROTEM® devices into Microsoft Excel (Microsoft Office 2007, Microsoft Corporation Redmond, WA, US) and analyzed using SPSS® (version 16, SPSS Inc. Chicago, Illinois, USA). Continuous variables are summarized as mean ± SD. Within each of the three F XIII supplementation groups 0.32, 0.63 and 1.25 IU, values of CT, CFT, ML, α-angle, MCE and MCF of EXTEM, and CT, ML, MCE and MCF of FIBTEM were compared between different samples (baseline, DC, and F XIII). ANOVA for repeated measures with Bonferroni post hoc test was used to analyze these differences. Because of expected high fibrinogen concentrations and hence high MCF values particularly in the FIBTEM, ΔMCF values for EXTEM and FIBTEM were calculated by subtracting the MCF of the diluent control sample from those of the F XIII added sample. Thereafter, the resulting ΔMCF values were correlated with the fibrinogen concentration, F XIII concentration and the fibrinogen to F XIII ratio (FBG:F XIII ratio) using linear regression and reporting r-square values. P-values of 0.05 or less are considered significant.

Results

1. Patient characteristics

Ninety critically ill patients after cardiac, major vascular and major non cardiac surgery were included. Age, sex, BMI, fibrinogen and F XIII concentrations, platelet count and hematocrit were not significantly different in the three groups (table 1).

2. Effects of FXIII supplementation on FIBTEM

As compared to DC, F XIII addition led to significantly increased mean MCF and significantly decreased ML in FIBTEM (table 2). The effect on mean MCF was not dependent on F XIII dose (table 2) or basal F XIII concentration (figure 3). The ML of the F XIII group was reduced significantly vs. diluent controls also in a non dose dependent manner.

3. Effects of FXIII supplementation on EXTEM

As shown in table 3, the addition of 0.32, 0.63 and 1.25 IU of F XIII led to a significant increase of MCF as compared to diluent control. The effect on mean MCF in the three groups (0.32, 0.63 and 1.25 IU) was not dose-dependent. We also observed a significant non dose dependent reduction of mean ML for all three groups.

4. Effects on MCE of F XIII supplementation on EXTEM and FIBTEM

As MCE is calculated via the MCF values [MCE = (100*MCF)/(100-MCF)] descriptive statistic was made and is shown in table 2 and 3. Highly significant ($p<0.01$) were the changes of F XIII compared to DC. MCE increased after addition of F XIII and decrease in the DC group. Those results are similar to the MCF changes.

5. Correlation of ΔMCF and ΔMCE with determining factors of MCF

MCF of FIBTEM is determined by the FBG and F XIII concentration. We thus plotted ΔMCF vs. FBG concentration (figure 1), ΔMCF vs. F XIII concentration (figure 3) and ΔMCF vs. FBG:F XIII ratio (figure 2) for the three groups (0.32, 0.63 and 1.25 IU). Table 4 illustrates the mean ΔMCF for FIBTEM and EXTEM measurements. For ΔMCE which is calculated via the MCF values, only the correlations of ΔMCE in the 0.32 IU group versus the platelets for EXTEM and FIBTEM, were significant (r^2 respectively for EXTEM 0.22, $p<0.01$, and FIBTEM 0.19, $p<0.05$). All other correlations were non-significant.

ΔMCF increased with increasing FBG concentration. The correlation was positive in all three groups with values of $r^2=0.36$ ($p<0.01$), $r^2=0.04$ ($p=0.19$), $r^2=0.55$ ($p<0.01$) in the F XIII 0.32, 0.63 and 1.25 IU groups, respectively. At low FBG concentration there were no or only small ΔMCF irrespective of the dose of F XIII added. At high FBG concentration ΔMCF increased proportionately. Accordingly, the slope of the regression is highest for 1.25 IU and lower for the other two groups (figure 1).

ΔMCF did hardly increase with increasing basal F XIII concentration (figure 3). For both the 0.32 and the 0.63 IU groups the regression curves are nearly horizontal with $r^2=0.01$($p=0.33$ and $p=0.49$). In the 1.25 IU group r^2 was 0.05 ($p=0.67$). The correction of the pre-existing absolute F XIII deficiency (mean F XIII concentration was 63% for 0.32 IU group, 66% for 0.63 IU group and 56% for the 1.25 IU group; table 1) led to mean increases in MCF of 4.9, 8.0 and 8.3 mm respectively (table 4). We also plotted ΔMCF against a parameter known not to be associated with MCF in FIBTEM, namely platelet count and found nearly horizontal regression lines (data not shown).

Comparing the ΔMCF of FIBTEM with the ratio of fibrinogen concentration to F XIII (FBG:F XIII ratio) showed that the lower the FBG:F XIII ratio, the higher is the effect of

added F XIII on FIBTEM (figure 2). These findings were significant ($p<0.01$) in the 0.32 IU ($r^2=0.22$) and the 1.25 IU ($r^2=0.31$) group.

Discussion

Factor XIII (F XIII) is essential for clot stability as assayed by ROTEM® tests. Utilizing an in-vitro supplementation model, we investigated the impact of F XIII substitution on EXTEM and FIBTEM measurements indicative of clot firmness and stability in 90 critically ill patients with high FBG and low F XIII concentrations. The main findings of the current study are that in vitro supplementation of F XIII to supraphysiologic levels increases maximum clot firmness, accelerate clot formation and increases clot stability in EXTEM and FIBTEM postoperative patients. The positive effect of F XIII supplementation on MCF was dependent on basal FBG concentration, with higher ΔMCF reached at high FBG concentration. The effect was independent of basal F XIII concentration or basal platelet count. The FBG concentration dependent effect on MCF suggests a relative F XIII deficiency in states of high FBG and concomitantly low F XIII concentrations.

Due to sample preparation and test procedures, current plasma-based tests for F XIII function or antigen are time consuming and cumbersome [11] [12]. Furthermore, these tests are frequently not readily available or not available on a 24 hour 7 day a week basis. Finally, these tests have test-related limitations. Point of care testing using viscoelastic methods can be utilized on site in emergency facilities and operating theaters permitting constant and immediate availability of analyses around the clock. Ours findings suggest that, in vitro monitoring of acquired or inherited F XIII deficiency may be possible using ROTEM®. However, since no ROTEM ® test before and after in vivo treatment with F XIII were performed, no direct extrapolation to any clinical situation should be made.

F XIII has an impact on thromboelastography and thromboelastometry, which has been previously reported by several investigators [13-15]. In an in-vitro and plasma-based study using thromboelastography, Nielsen and colleagues [14] have shown that increasing

concentrations of F XIII improved clot strength as evidenced by an increased maximum amplitude (MA) and alpha angle, and a decreased coagulation time (r). The same investigators reported in a subsequent study that exposure of normal plasma to anti-F XIII antibodies resulted in a significant decrease in clot strength (63%). In addition, they found that F XIII-induced cloth strength varied between 44 and 50% in hypercoagulable and hypocoagulable plasma, respectively. In the current investigation the MCF of EXTEM and FIBTEM of ROTEM® significantly increased by addition of F XIII resulting in final concentrations near (150% in the 0.32 IU group) or well above the physiologic range (300 and 600% in the 0.63 and 1.25 IU group). Additionally the clot formation time of the EXTEM was considerably shortened. CFT of EXTEM mainly depends on the activity of platelets and those of F XIII, whereas in FIBTEM the platelets are inhibited. More than two thirds and approximately half of all critically ill patients included in this study suffered F XIII plasma concentrations < 70% and < 60%, respectively.

Recently Weber and colleagues [8] described the detection of F XIII deficiency by ROTEM® in a patient undergoing a Whipple's operation. They observed significant maximum lysis in both EXTEM and APTEM (in vitro addition of aprotinin) and a reduction in the maximum lysis after addition of F XIII in the in EXTEM measure. This patient had an acquired F XIII deficiency. In another case report, Dargaud and co-workers reported [16] a patient with known mutation of factor V Leiden and severe F XIII deficiency. They observed reduced clot strength (MCF) and impaired clot stability (increased maximum lysis) in the baseline measurement of ROTEM®. After addition of F XIII an increase in the MCF and a decrease of maximum lysis was observed. They concluded that ROTEM® was able to detect viscoelastic changes of fibrin clot in whole blood samples with acquired low F XIII activity. MCF thus may be a valuable surrogate marker in patients treated with F XIII. These findings [8, 16] were confirmed in our investigation. In the same manner, the addition of F XIII increased the

MCF in EXTEM and reduced significantly the ML in EXTEM and FIBTEM. EXTEM and FIBTEM seem to be sensitive tools to detect the protective and stabilizing effect of F XIII on clot strength and the degradation caused by fibrinolysis. These findings are supported by the observations, that a reduction of maximum lysis of EXTEM has been reported in patients with very low levels of F XIII as well as by the fact that an aprotinin resistant lysis can be corrected by F XIII and is an indicator for a F XIII deficiency [14, 17].

The impact of F XIII on clot firmness, as presented in the current study, is in keeping with the recently published investigation of Korte and colleagues [18]. They were able to show in gastrointestinal cancer patients that substitution of F XIII led to a smaller reduction of MCF in EXTEM and a reduction of blood loss, compared a control group of patients with no F XIII supplementation. Possible reasons discussed were that either high doses of F XIII given reduce the consumption of fibrinogen due to increased clot firmness or that F XIII protects fibrinogen from plasmin degradation.

Our current investigation has limitations. One regards F XIII supplementation to supraphysiologic levels ranging from 150 (in the 0.32 IU group) to 600% (1.25 IU group). We hypothesize that the lack of a F XIII dependent dose response effect is due to this fact. With the dose of 0.32 IU the resultant F XIII concentration in the sample was already above the physiologic level. It was thus only under the extreme FBG values, where at higher doses of FXIII an additional effect on MCF could be observed. Again we interpret these findings to be indicative of a relative F XIII deficit. Other limitations include supplementation with various volumes (5, 10 and 20 µl). Compared to the total sample volume the relation is small and we did perform baseline tests without any supplementation that permit to evaluate the relative contribution of sample dilution. The final limitation is that there were no ROTEM ® tests

performed before and after in vivo treatment with F XIII and thus no direct extrapolation to any clinical situation should be made.

Fibrinogen is an acute phase reactant and thus frequently elevated in the periopertive context. In contrast, low F XIII concentrations are prevalent in critically ill patients [18]. This constellation of high FBG and low F XIII concentrations or a high FBG:F XIII ratio thus appears to be of high prevalence. In all groups, the fibrinogen concentration was elevated and F XIII concentration was reduced (table 2). Interestingly, the effect of adding F XIII on ΔMCF-FIBTEM did not depend on the F XIII concentration (figure 3). However, the effect of adding F XIII was greater at higher fibrinogen concentrations in the 0.32 and 1.25 IU groups (figure 1). This may represent a relative F XIII deficiency at high fibrinogen levels. We therefore introduced the ratio between fibrinogen and F XIII concentrations (FBG:F XIII ratio). The higher the FBG:F XIII ratio the greater was the effect of adding F XIII on ΔMCF-FIBTEM in the 0.32 and 1.25 IU groups (figure 2). In our in vitro model we observed improved clot qualities after F XIII supplementation to supraphysiologic final concentrations. Future clinical studies will have to show whether or not the concept of relative F XIII deficiency that we describe will prove clinically useful. Placebo-controlled trials comparing F XIII substitution according to the ratio of FBG to F XIII concentrations vs. F XIII concentration alone will clarify whether the current in vitro observations, will result in a clinically relevant benefit to the perioperative patient.

Conclusion: The results of the current investigation suggest that in vitro supplementation of high doses F XIII improves clot firmness and stability in EXTEM and FIBTEM as assessed by ROTEM® in the case of absolute or relative F XIII deficiency. Further investigations need to demonstrate whether supplementation of F XIII to physiological concentrations has a similar impact on clot firmness.

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Figure legends

Figure 1: Regression analysis of ΔMCF-FIBTEM vs. basal FBG for different doses of F XIII supplementation (0.32, 0.63, 1.25 IU).

Annotation: ΔMCF-FIBTEM, difference between MCF-FIBTEM with added F XIII and MCF-FIBTEM diluent control (DC). (0,32 IU r^2=0.36, p<0.01; 0,63 IU r^2=0.04, p=0.19; 1.25 IU r^2=0.55, p<0.01)

Figure 2: Regression analysis of different doses of F XIII supplementation (0.32, 0.63, 1.25 IU) between ΔMCF-FIBTEM and ratio FBG:F XIII concentrations.

Annotation: ΔMCF-FIBTEM, difference between MCF-FIBTEM with added F XIII and MCF-FIBTEM diluent control (DC); ratio FBG:F XIII (%), concentration of fibrinogen divided by F XIII concentration, expressed in a percentage decimal count. (0,32 IU r^2=0.22, p<0.01; 0,63 IU r^2=0.03, p=0.34; 1.25 IU r^2=0.31, p<0.01)

Figure 3: Regression analysis of ΔMCF-FIBTEM vs. basal F XIII for different doses of F XIII supplementation (0.32, 0.63, 1.25 IU).

Annotation: ΔMCF-FIBTEM, difference between MCF-FIBTEM with added F XIII and MCF-FIBTEM diluent control (DC). (0,32 IU r^2=0.01, p=0.33; 0,63 IU r^2=0.01, p=0.49; 1.25 IU r^2=0.05, p=0.67)

Tables

Table 1 Patient characteristics

Parameter	0.32 IU group	0.63 IU group	1.25 IU group
Age	62.6±15.5	60.8±16.3	61.4±15.0
Sex (m/f)	26/4	27/3	23/7
BMI (kg/m^2)	25.5±4.1	26.2±3.1	26.1±3.2
Fibrinogen (g/L)	5.1±1.9	5.6±2.0	5.0±2.5
F XIII (%)	63±23	66±24	56±15
Platelet count (G/L)	198±95	199±99	174±89
Hematocrit (%)	27.0±2.7	26.2±2.3	26.7±3.1

Table 2: FIBTEM data

Sample	Group	MCF (mm)	CT (s)	ML (%)	MCE
Baseline	0.32 IU	23.9±7.6	64.3±11.3	8.1±4.9	31.2±13.8
	0.63 IU	28.7±10.1	56.3±11.0	3.3±4.2	43.0±19.6
	1.25 IU	22.4±13.1	86.1±30.4	2.2±2.8	32.8±27.0
FXIII	0.32 IU	28.0±9.9 **	62.6±9.5*	0.1±0.6**	40.0±20.2**
	0.63 IU	36.0±12.3 **	53.9±9.9*	0.4±1.0**	60.2±37.5**
	1.25 IU	29.5±17.1 **	77.9±29.0**	0.1±0.4**	49.4±48.5**
DC	0.32 IU	23.1±8.2	69.6±14.1	2.5±5.2	32.6±13.1
	0.63 IU	28.2±10.7	60.6±10.1	2.9±4.2	42.3±21.7
	1.25 IU	21.3±13.3	99.4±34.3	1.9±2.9	31.5±27.6

Significances were addressed DC vs. F XIII: * denotes $p < 0.05$; ** denotes $p < 0.001$

Table 3 EXTEM data

Sample	Group	MCF (mm)	CT (s)	CFT (s)	αA (°)	ML (%)	MCE
Baseline	0.32	63.2±7.3	65.3±11.6	85.7±43.6	73.5±7.4	8.1±4.9	180.7±54.0
	0.63	66.0±8.9	57.6±9.2	74.4±37.0	75.5±6.6	5.9±3.8	211.9±74.0
	1.25	61.3±9.3	96.6±40.9	95.6±36.0	71.5±6.8	5.9±3.8	175.5±75.5
FXIII	0.32	67.3±8.5 **	63.0±11.4	78.5±42.3 *	75.0±7.0 **	2.9±1.8**	221.7±72.9**
	0.63	68.9±8.4 **	54.9±6.4**	66.8±40.5 **	77.3±7.0 **	3.0±1.7**	242.6±84.6**
	1.25	64.4±9.9 **	83.7±29.6 *	90.9±43.9**	72.8±7.9**	2.5±1.4**	206.8±99.3**
DC	0.32	61.1±7.9	67.8±9.4	88.4±50.8	73.3±7.8	9.8±3.6	180.7±50.9
	0.63	64.1±9.8	61.8±9.5	81.1±43.0	74.4±7.3	7.5±7.0	196.2±72.4
	1.25	58.7±9.5	100.8±37.1	107.7±44.2	69.5±7.7	8.6±5.7	156.9±70.3

Significances were addressed DC vs. F XIII: * denotes $p < 0.05$; ** denotes $p < 0.001$.

Table 4: ΔMCF data for F XIII vs. DC

Group	FIBTEM ΔMCF (mm)	EXTEM ΔMCF (mm)
0.32 IU	4.9±2.6 **	6.0±3.6 **
0.63 IU	8.0±6.5 **	5.0±4.2 **
1.25 IU	8.3±6.0 **	5.6±4.2 **

Significances were addressed DC vs. F XIII: ** denotes $p < 0.001$.

Figures

Figure 1: Regression analysis of the different doses of F XIII supplementation (0.32, 0.63, 1.25 IU) between ΔMCF-FIBTEM and fibrinogen concentrations.

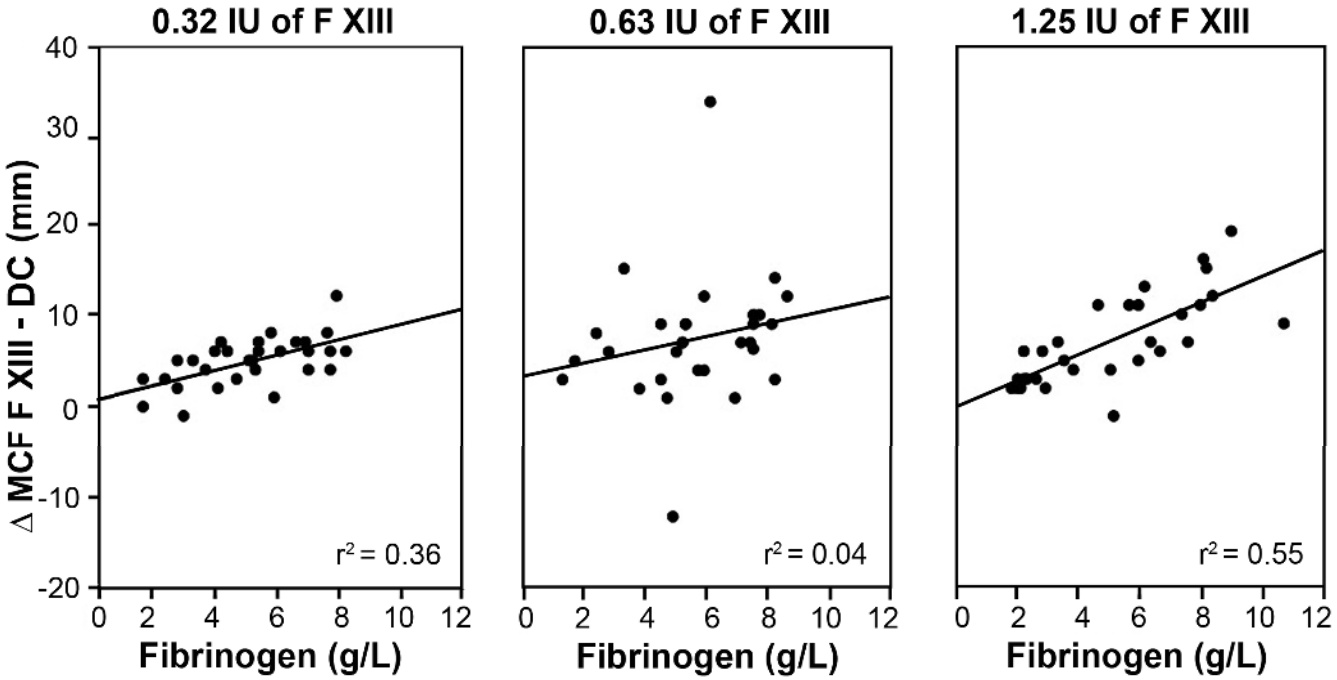

Figure 2: Regression analysis of the different doses of F XIII supplementation (0.32, 0.63, 1.25 IU) between ΔMCF-FIBTEM and ratio FBG: F XIII concentrations.

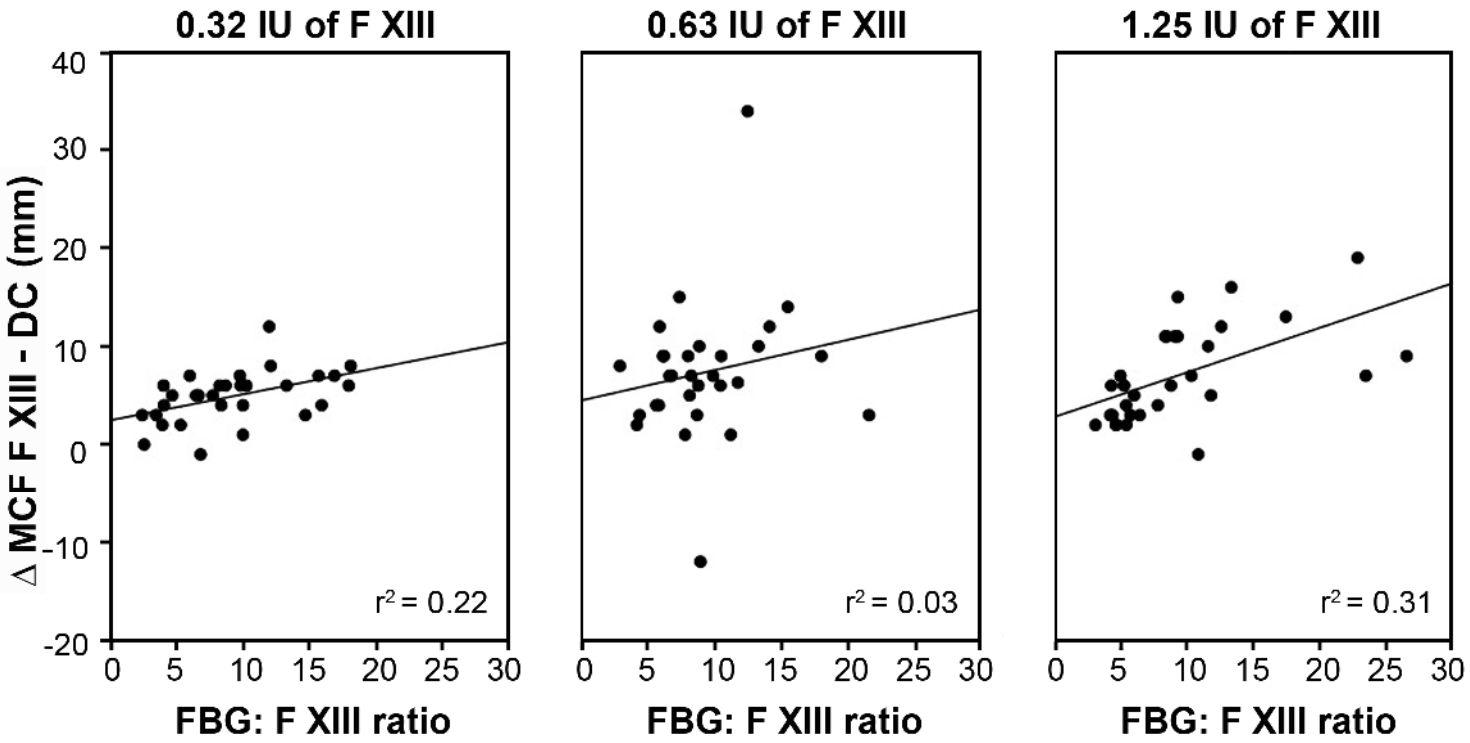

Figure 3: Regression analysis of the different doses of F XIII supplementation (0.32, 0.63, 1.25 IU) between ΔMCF-FIBTEM and F XIII concentrations.