# *In vitro* factor XIII supplementation increases clot firmness in Rotation Thromboelastometry (ROTEM®)

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## Summary

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 factor (F)XIII on clot stability as assessed by Rotation Thromboelastometry (ROTEM®). 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 FXIII, fibrinogen (FBG), platelets and haematocrit were contemporaneously determined. In the perioperative patient population mean FBG concentration was elevated at 5.2 g/l and mean FXIII concentration was low at 62%. The addition of FXIII led to a FBG concentration-dependent increase in MCF both in FIBTEM and EXTEM. Mean increases in MCF (FXIII 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. *In vitro* supplementation of FXIII 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 FXIII levels.

## Keywords

Thromboelastometry, blood coagulation, haemostasis, thromboelastography, factor XIII

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## Introduction

Blood coagulation factor XIII (FXIII) 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 FXIII circulates in association with its substrate precursor, fibrinogen (FBG), which has an important regulatory impact in the activation of plasma FXIII (1, 2). Relevant physiologic functions of FXIII include cross-linking of fibrin monomers amongst themselves to generate a stable fibrin strand, cross-linking FBG 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 localisation at a site of injury (3, 4). While FBG is an acute phase protein whose concentration increases in relation to the extent of the acute phase reaction, FXIII is not up-regulated under the same circumstances. We thus hypothesised that a relative FXIII 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, haemodilution by volume replacement therapy additionally reduces the plasma coagulation factor fraction. Like in burn patients it is assumed that FXIII concentration may be low following trauma and in major surgery and may be responsible for otherwise inexplicable intra- and postoperative bleeding (5–7). FXIII is not detected by routine laboratory screening such as prothrombin time (PT) and partial thromboplastin time (PTT). FXIII assays are often only available in specialised laboratories. Furthermore, determination of FXIII using plasma-based testing is time consuming due to the obligatory prior sample centrifugation.

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

The goal of this study was to investigate *in vitro* supplementation of FXIII with 0.32 IU FXIII, resulting in FXIII concentration of approximately 150%, 0.63 IU FXIII resulting in FXIII concentration of 300% and 1.25 IU resulting in FXIII concentration of 600% to see whether clot characteristics as assayed by ROTEM® in the EXTEM and FIBTEM tests, both of which are FBG and FXIII-dependent, are to be improved in patients with laboratory proven FXIII deficiency. No comparison of ROTEM® tests after *in vivo* treatment with FXIII 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 authorisation 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 FXIII, platelets and FBG 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. Enrolment occurred within 6 hours after major surgery. The ROTEM® groups were defined by the FXIII 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 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 FBG and FXIII 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 FXIII in a volume of 5, 10 and 20 µl, 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 (min) using two ROTEM® devices simultaneously. To optimise comparability, FXIII-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 ROTEM®

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 et al. (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 analysed.

### Test procedure

The two ROTEM® devices 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 mM 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 FXIII (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 initialisation of the pipetting program.

The laboratory analyses were performed in quality controlled ISO 17025 accredited university laboratories. FXIII was determined on Behring Coagulation System (Dade Bering, Düdingen, Switzerland) using the Berichrom® FXIII test (Dade Bering, Düdingen, Switzerland; normal range 70–140% which equals 0.7–1.4 IU/ml). This test measures the function of FXIII. FBG was also analysed 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 x$10^9$/l).

### Statistical analyses

Data were transferred from the ROTEM® devices into Microsoft Excel (Microsoft Office 2007, Microsoft Corporation Redmond, WA, USA) and analysed using SPSS® (version 16, SPSS Inc. Chicago, IL, USA). Continuous variables are summarised as mean ± SD. Within each of the three FXIII supplementation groups 0.32, 0.63 and 1.25 IU, values of CT, CFT, ML, αA, MCE and MCF of EXTEM, and CT, ML, MCE and MCF of FIBTEM were compared between different samples (baseline, DC, and FXIII). ANOVA for repeated measures with Bonferroni post-hoc test was used to analyse these differences. Because of expected high FBG 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 FXIII added sample. Thereafter, the resulting ΔMCF values were correlated with the FBG concentration, FXIII concentration and the FBG to FXIII ratio (FBG:FXIII ratio) using linear regression and reporting r-square values. P-values of 0.05 or less are considered significant.

**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 (male/female) | 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 |
| Haematocrit (%) | 27.0 ± 2.7 | 26.2 ± 2.3 | 26.7 ± 3.1 |

## Results

### Patient characteristics

Ninety critically ill patients after cardiac, major vascular and major non-cardiac surgery were included. Age, sex, body mass index (BMI), FBG and FXIII concentrations, platelet count and haematocrit were not significantly different in the three groups (▶Table 1).

### Effects of FXIII supplementation on FIBTEM

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

### Effects of FXIII supplementation on EXTEM

As shown in ▶Table 3, the addition of 0.32, 0.63 and 1.25 IU of FXIII led to a significant increase of MCF as compared to diluent

**Table 2: FIBTEM data.** Significances were addressed DC vs. FXIII: * denotes p < 0.05; ** denotes p < 0.001.

| 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. FXIII: * denotes p < 0.05; ** denotes p < 0.001. | | | | | |

| 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. FXIII: * denotes p < 0.05; ** denotes p < 0.001. | | | | | | | |

**Table 3: EXTEM data.** Significances were addressed DC vs. FXIII: * denotes p < 0.05; ** denotes p < 0.001.

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.

### Effects on MCE of FXIII 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 Tables 2 and 3. Highly significant (p<0.01) were the changes of FXIII compared to DC. MCE increased after addition of FXIII and decrease in the DC group. Those results are similar to the MCF changes.

### Correlation of ΔMCF and ΔMCE with determining factors of MCF

MCF of FIBTEM is determined by the FBG and FXIII concentration. We thus plotted ΔMCF vs. FBG concentration (▶Fig. 1), ΔMCF vs. FXIII concentration (Fig. 3) and ΔMCF vs. FBG:FXIII ratio (▶Fig. 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 vs. the platelets for EXTEM and FIBTEM, were significant ($r^2$ for EXTEM 0.22, p<0.01, and FIBTEM 0.19, p<0.05, respectively). 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 FXIII 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 FXIII 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 (Fig. 1).

ΔMCF did hardly increase with increasing basal FXIII concentration (▶Fig. 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 FXIII deficiency (mean FXIII 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 FBG concentration to FXIII (FBG:FXIII ratio) showed that the lower the FBG:FXIII ratio, the higher is the effect of added FXIII on FIBTEM (Fig. 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.

**Table 4: ΔMCF data for FXIII vs. DC.** Significances were addressed DC vs. FXIII: ** denotes p < 0.001.

| 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. FXIII: ** denotes p < 0.001. | | |

## Discussion

Factor XIII (FXIII) is essential for clot stability as assayed by ROTEM® tests. Utilising an *in vitro* supplementation model, we investigated the impact of FXIII substitution on EXTEM and FIBTEM measurements indicative of clot firmness and stability in 90 critically ill patients with high FBG and low FXIII concentrations. The main findings of the current study are that *in vitro* supplemen-

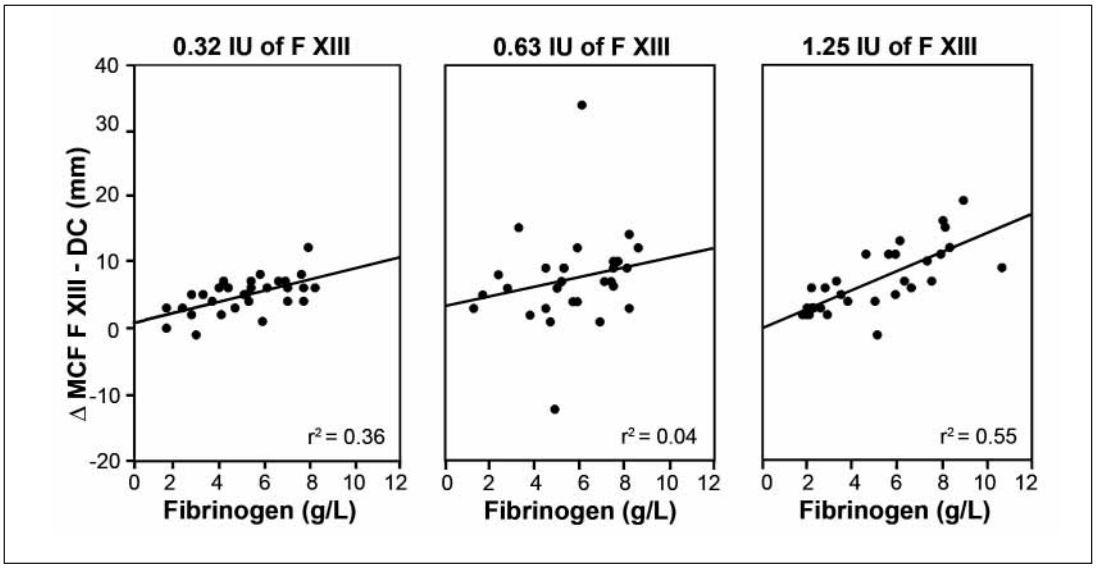


**Figure 1: Regression analysis of ΔMCF-FIBTEM vs. basal FBG for different doses of FXIII supplementation (0.32, 0.63, 1.25 IU).** ΔMCF-FIBTEM, difference between MCF-FIBTEM with added FXIII and MCF-FIBTEM diluent control (DC). (0.32 IU $r^2$=0.36, p<0.01; 0,63 IU $r^2$=0.04, p=0.19; 1.25 IU $r^2$=0.55, p<0.01)

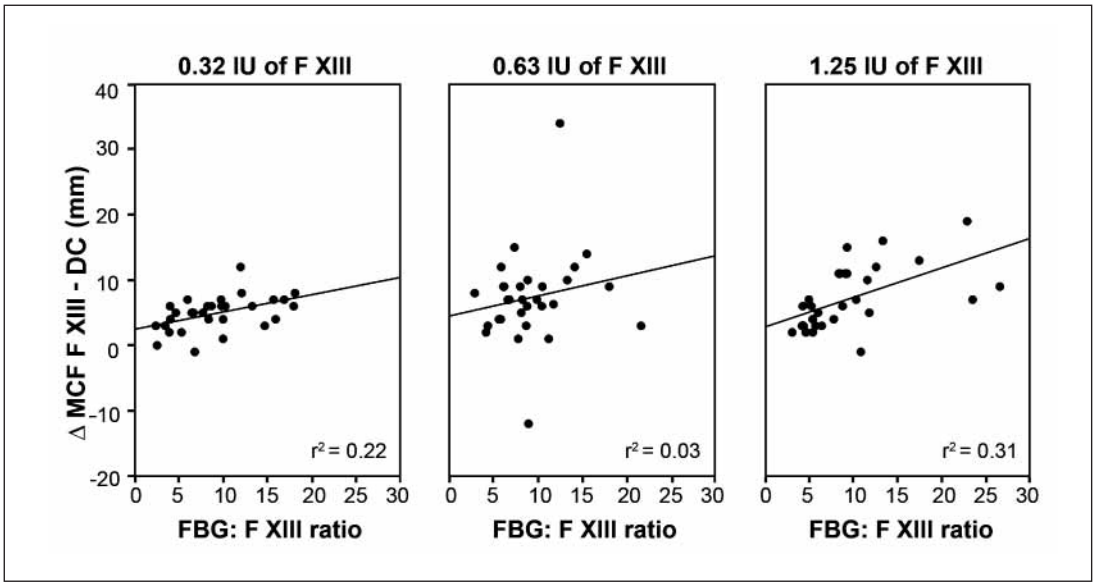


**Figure 2: Regression analysis of different doses of FXIII supplementation (0.32, 0.63, 1.25 IU) between ΔMCF-FIBTEM and ratio FBG:FXIII concentrations.** ΔMCF-FIBTEM, difference between MCF-FIBTEM with added FXIII and MCF-FIBTEM diluent control (DC); ratio FBG:FXIII (%), concentration of fibrinogen divided by FXIII 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)

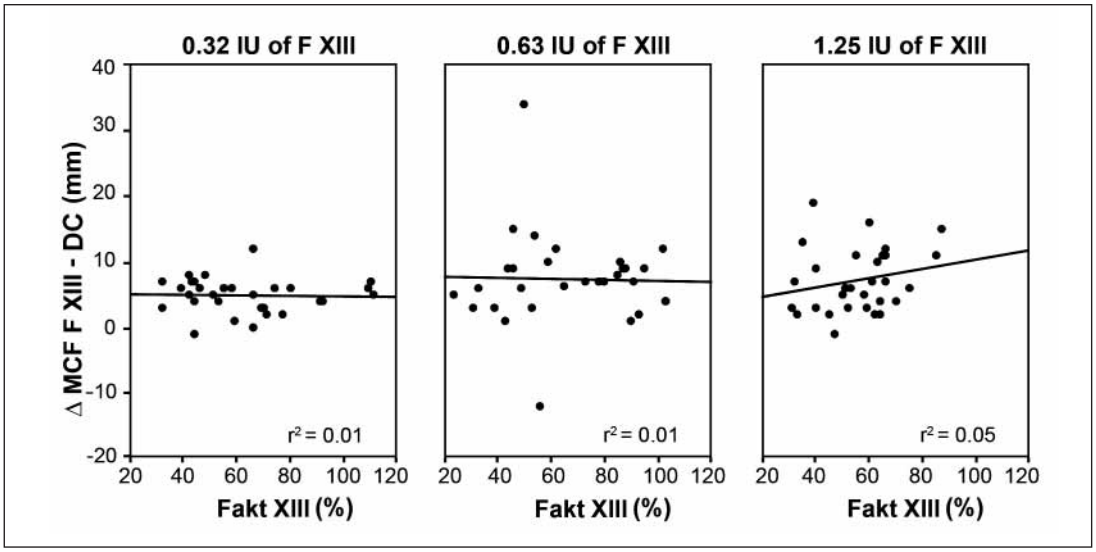


**Figure 3: Regression analysis of ΔMCF-FIBTEM vs. basal FXIII for different doses of FXIII supplementation (0.32, 0.63, 1.25 IU).** ΔMCF-FIBTEM, difference between MCF-FIBTEM with added FXIII and MCF-FIBTEM diluent control (DC). (0.32 IU $r^2$=0.01, p=0.33; 0,63 IU $r^2$=0.01, p=0.49; 1.25 IU $r^2$=0.05, p=0.67).

tation of FXIII to supraphysiologic levels increases maximum clot firmness, accelerate clot formation and increases clot stability in EXTEM and FIBTEM postoperative patients. The positive effect of FXIII supplementation on MCF was dependent on basal FBG concentration, with higher ΔMCF reached at high FBG concentration. The effect was independent of basal FXIII concentration or basal platelet count. The FBG concentration dependent effect on MCF suggests a relative FXIII deficiency in states of high FBG and concomitantly low FXIII concentrations.

Due to sample preparation and test procedures, current plasma-based tests for FXIII function or antigen are time consuming and cumbersome (11, 12). Furthermore, these tests are frequently not readily available or not available 24 hours 7 days a week. Finally, these tests have test-related limitations. POC testing using viscoelastic methods can be utilised 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 FXIII deficiency may be possible using ROTEM®. However, since no ROTEM® test before and after *in vivo* treatment with FXIII were performed, no direct extrapolation to any clinical situation should be made.

FXIII 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 et al. (14) have shown that increasing concentrations of FXIII improved clot strength as evidenced by an increased maximum amplitude (MA) and αA, and a decreased coagulation time (r). The same investigators reported in a subsequent study that exposure of normal plasma to anti-FXIII antibodies resulted in a significant decrease in clot strength (63%). In addition, they found that FXIII-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 FXIII 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 FXIII, whereas in FIBTEM the platelets are inhibited. More than two thirds and approximately half of all critically ill patients included in this study suffered FXIII plasma concentrations < 70% and < 60%, respectively.

Recently Weber et al. (8) described the detection of FXIII 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 FXIII in the in EXTEM measure. This patient had an acquired FXIII deficiency. In another case report, Dargaud et al. (16) reported a patient with known mutation of factor V Leiden and severe FXIII deficiency. They observed reduced clot strength (MCF) and impaired clot stability (increased maximum lysis) in the baseline measurement of ROTEM®. After addition of FXIII 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 FXIII activity. MCF thus may be a valuable surrogate marker in patients treated with FXIII. These findings (8, 16) were confirmed in our investigation. In the same manner, the addition of FXIII 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 FXIII 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 FXIII as well as by the fact that an aprotinin resistant lysis can be corrected by FXIII and is an indicator for a FXIII deficiency (14, 17).

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

Our current investigation has limitations. One regards FXIII supplementation to supraphysiologic levels ranging from 150%

### What is known about this topic?

- Relevant physiologic functions of factor (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 localisation at a site of injury.
- ROTEM® assesses the kinetics and quality of clot formation and clot lysis in real-time.
- There is only one case report on the topic of ROTEM® and FXIII.

### What does this paper add?

- *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 FXIII levels.
- Point-of-care testing using viscoelastic methods can be utilised on site in emergency facilities and operating theatres permitting constant and immediate availability of analyses around the clock.
- Our findings suggest that *in vitro* monitoring of acquired and inherited FXIII deficiency may be possible using ROTEM®. Such a ROTEM®-based test may prove useful in guiding FXIII substitution of patients in the acute periopertaive setting.
- The constellation of high fibrinogen (FBG) and low FXIII concentrations or a high FBG:FXIII ratio thus appears to be of high prevalence in intensive care patients. In our *in vitro* model we observed improved clot qualities after FXIII supplementation to supraphysiologic final concentrations.

(in the 0.32 IU group) to 600% (in the 1.25 IU group). We hypothesise that the lack of a FXIII-dependent dose-response effect is a result of this. With the dose of 0.32 IU the resultant FXIII 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 FXIII 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 FXIII and thus no direct extrapolation to any clinical situation should be made.

FBG is an acute phase reactant and thus frequently elevated in the periopertive context. In contrast, low FXIII concentrations are prevalent in critically ill patients (18). This constellation of high FBG and low FXIII concentrations or a high FBG:FXIII ratio thus appears to be of high prevalence. In all groups, the FBG concentration was elevated and FXIII concentration was reduced (Table 2). Interestingly, the effect of adding FXIII on ΔMCF-FIBTEM did not depend on the FXIII concentration (Fig. 3). However, the effect of adding FXIII was greater at higher FBG concentrations in the 0.32 and 1.25 IU groups (Fig. 1). This may represent a relative FXIII deficiency at high FBG levels. We therefore introduced the ratio between FBG and FXIII concentrations (FBG:FXIII ratio). The higher the FBG:FXIII ratio the greater was the effect of adding FXIII on ΔMCF-FIBTEM in the 0.32 and 1.25 IU groups (Fig. 2). In our *in vitro* model we observed improved clot qualities after FXIII supplementation to supraphysiologic final concentrations. Future clinical studies will have to show whether or not the concept of relative FXIII deficiency that we describe will prove clinically useful. Placebo-controlled trials comparing FXIII substitution according to the ratio of FBG to FXIII concentrations vs. FXIII 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 FXIII improves clot firmness and stability in EXTEM and FIBTEM as assessed by ROTEM® in the case of absolute or relative FXIII deficiency. Further investigations need to demonstrate whether supplementation of FXIII to physiological concentrations has a similar impact on clot firmness.

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