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In seeking diagnostic tool for laboratory monitoring of FXII-targeting agents, could assessment of rotational thromboelastometry (ROTEM) in patients with factor XII deficiency be useful?

Paulina Stelmach[1], Weronika Nowak[2], Marta Robak[3], Emilia Krzemińska[1], Marzena Tybura-Sawicka[3], Krzysztof Chojnowski[3], Jacek Treliński[3*]

[1]Department of Hematology, Copernicus Memorial Hospital in Lodz, Łódź, Poland
[2]Department of Hematooncology, Copernicus Memorial Hospital in Lodz, Łódź, Poland
[3]Department of Hemostasis Disorders, Medical University of Lodz, Łódź, Poland

Abstract

Introduction: Targeting factor XII (FXII) is a new concept for safe thrombosis prophylaxis. Global hemostasis tests offer promise in terms of the laboratory monitoring of FXII inhibition. The present study examines selected parameters of rotational thromboelastometry (ROTEM) in patients with FXII deficiency.

The objective of this study was to assess the impact of FXII deficiency on selected parameters of ROTEM, which can be significant in the laboratory monitoring of FXII inhibition.

Material and methods: The study included 20 patients with FXII deficiency ≤40% and 21 volunteers free of it. Clotting time (CT), clot formation time (CFT), alpha angle (α), maximum clot firmness (MCF), and maximum lysis (ML) were recorded in ROTEM.

Results: For the INTEM test, CT and CFT readings were markedly higher in FXII deficient patients than in controls. No marked differences in relation to MCF and ML were found.

Conclusion: The results of ROTEM show that FXII deficiency has a great impact on the initiation and amplification of coagulation. This was confirmed by a number of marked correlations between FXII activity and certain ROTEM parameters. ROTEM tests merit further investigation as treatment control strategies in the context of FXII inhibition.

Key words: factor XII deficiency, ROTEM, FXII as a target for thrombosis prevention, laboratory monitoring of FXII inhibition

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Introduction

Medical device-induced thrombosis poses a significant medical challenge. Annually, several million people worldwide undergo the implementation of medical devices such as a cardiopulmonary bypass, renal hemodialysis, extracorporeal membrane oxygenation (ECMO), left ventricular assist devices, and intravenous catheters. The standard prevention of thrombosis relies on using high doses of heparin, but such treatment is associated with an increased risk of bleeding complication. The optimal treatment should prevent thrombosis without such risk.

*Address for correspondence: Jacek Treliński, Department of Hemostasis Disorders, Medical University of Lodz, Ciołkowskiego 2, 93–510 Łódź, Poland, phone +48 42 689 50 58, e-mail: jacek.trelinski@umed.lodz.pl

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Table I. Characteristics of study population

Parameter	FXII deficient patients (n = 20)	Controls (n = 21)	*p*
Median age in years (range)	49 (21–73)	46 (23–67)	NS
Sex (F/M)	13/7	19/2	
PLT [× 10^9/L]	254 (142–369)	229 (151–376)	NS
PT [s]	11.8 (10.1–14.8)	11.5 (10.2–13.1)	NS
APTT [s]	83 (37.8–500)*	25,4 (22.4–28.2)	**<0.001**
Fibrinogen [mg/dL]	288 (224–475)	266 (206–376)	ns
FXII activity [%]	18.5 (0–40)**	105 (54–142)	**<0.001**

*Patients with APTT value >500 s (n = 5) were reported as 500 s; **patients with FXII activity <1% (n = 8) were reported as 0; FXII — factor XII; NS — non significant; F — female; M — male; PLT — platelets; PT — prothrombin time; APTT — activated partial thromboplastin time

In recent years, there has been growing interest in the preventive role of inhibition of factor XII, with a very low risk of bleeding [1–3].

The role of factor XII (FXII) was underestimated for decades, since even severe deficiency did not cause bleeding complication. However, recent studies have shown that this factor plays a crucial role in initiating plasma contact system [4–6]. Exposure of blood to negatively charged artificial or biological surfaces induces conformational changes in the catalytic domain of FXII, thereby triggering a series of proteolytic reactions eventually resulting in thrombin generation, fibrin deposition and activation of proinflammatory kallikrein–kinin system [1]. Although FXIIa triggers fibrin formation through activation of the intrinsic coagulation pathway, it appears to have no critical significance in fibrin clot formation during normal hemostasis due to vessel injury. Inhibition of factor XII, by blocking device surface-induced blood coagulation without bleeding risk, seems to be the optimal treatment target [7–10]. A number of potential therapeutic FXII-targeting agents are in preclinical and clinical trials for the treatment of thrombotic and inflammatory condition [3].

Even though the suspected bleeding risk related to such anticoagulant therapy is minimal, it will require laboratory assessment of hemostasis. Commonly used coagulation tests [such as activated partial thromboplastin time (APTT), and prothrombin time (PT)] are not suitable for monitoring hemostasis after FXII activity knockdown, providing information only on the initiation of clot formation. These tests are not adequate to rate the balance of hemostasis; for example, they remain within normal ranges in patients with antithrombin, protein C or protein S deficiency, where higher amounts of thrombin are generated compared to healthy subjects [11]. In fact, since 95% of the generated thrombin is not estimated in this test, nor is the amount of thrombomodulin sufficient for protein C activation, i.e. the initiation of anticoagulant activity, APTT is only suitable for the evaluation of the drivers of the initial phase of intrinsic and common pathway coagulation [12, 13].

Given this background, a more comprehensive and sensitive test is needed. Global hemostasis tests e.g. rotational thromboelastometry (ROTEM) appears to be a better choice. The objective of this study was to assess the impact of FXII deficiency on selected parameters of ROTEM, which can be significant in the laboratory monitoring of FXII inhibition.

Material and methods

Twenty patients diagnosed with FXII deficiency ≤40% were enrolled in the study (Table I). The study protocol was approved by the local ethics committee. All participants gave informed consent. The exclusion criteria were as follows: known liver disorder (plasma alanine transaminase concentration >2 upper limit range), renal failure (creatinine concentration ≥2 mg/dL), thrombocytopenia (platelet count <100 × 10^9/l), and taking any drug that strongly influences platelet function or coagulation for 10 days prior to study entry. The control group consisted of 21 subjects without FXII deficiency, of comparable age and with no history of any bleeding or thrombosis.

PT, APTT, concentration of fibrinogen, and FXII activity were measured using an ACL Top 500 system (Werfen, Le Pré-Saint-Gervais, France).

Activated rotation whole blood thromboelastometry was conducted using a computerized ROTEM device (Rotation Thromboelastometry, Pentapharm GmbH, Munich, Germany, software version 1.5.3). Four ROTEM tests, i.e. INTEM, EXTEM, FIBTEM and APTEM, were performed according to the manufacturer's instructions. The coagulation time (CT), clot formation time (CFT), α-angle, maximum clot firmness (MCF), maximum lysis (ML), and clot lysis index at 30, 45 and 60 minutes (LI 30, LI 45, LI 60 respectively) were among the investigated parameters.

The ROTEM output (TEMogram) reflects the coagulation process by indicating clot initiation (CT), followed by its amplification (CFT and a angle) and then the propagation phase (MCF). The next parameters of the TEMogram (e.g. LI 30 and ML) describe clot stabilization and lysis,

Table II. ROTEM data. Data expressed as median, range

ROTEM test		CT [s]	*p*	CFT [s]	*p*	Alpha (°)	*p*	MCF [mm]	*p*
INTEM	FXII DEF	216.5 (44–1,204)	**0.04**	101.5 (37–212)	**0.003**	72 (53–82)	NS	63 (49–76)	NS
	Controls	173 (148–339)		62 (48–167)		78 (59–82)		62 (54–70)	
EXTEM	FXII DEF	51 (28–1,057)	**0.03**	127 (76–186)	**0.002**	69.5 (56–81)	NS	63.5 (53–73)	NS
	Controls	59 (45–80)		94 (49–134)		71 (65–81)		63 (54–71)	
FIBTEM	FXII DEF	59 (45–734)	**<0.001**	108 (90–553)	NS	70.5 (37–78)	NS	13.5 (4–63)	NS
	Controls	52 (44–65)		493.5 (73–2,082)		66 (46–80)		13 (9–30)	
APTEM	FXII DEF	57.5 (46–76)	NS	103.5 (55–179)	NS	70 (28–81)	NS	62 (9–75)	NS
	Controls	57.5 (46–69)		100 (61–130)		73.5 (65–78)		63.5 (56–70)	

CT — coagulation time; CFT — clot formation time, α-angle, MCF — maximum clot firmness in patients with FXII deficiency and in control group; FXII — factor XII; NS — non significant

these being parts of the subsequent fibrinolysis process. The INTEM test, based on activators such as ellagic acid and phospholipids, gives information comparable to APTT, while EXTEM (tissue factor activation) provides information similar to that of PT. In general, CT values are influenced by the activity of coagulation factors, while CFT results depend on the activity of coagulation factors, platelet count and function, fibrinogen concentration, fibrin polymerization and hematocrit level. MCF is additionally influenced by thrombin concentration and the activity of FXIII [14, 15].

ROTEM technology is used among others in cardiac surgery, trauma, obstetrics and liver transplantation, but it is also tested experimentally in hemophilia to assess the individual response to factor replacement therapy [16]. Full details of the ROTEM laboratory technique have been provided in previous publications [14, 17–21].

Statistical analysis

The Mann-Whitney U-test was used to assess the significance of differences between studied groups. Correlations between variables were assessed by the Spearman rank correlation coefficient (r). In all measurements, $p < 0.05$ was considered statistically significant. Analyses were performed using STATISTICA v. 13.1 software (StatSoft, Tulsa, OK, USA).

Results

The screening coagulation tests, platelet count and activity of FXII data are shown in Table I. In the FXII deficient patient group, the median FXII activity was 18.5%, ranging from FXII activity below 1% (eight patients) to 40%. APTT values were significantly higher in the FXII-deficient group than in controls (83 s vs. 25.4 s, $p \leq 0.001$). In five patients, APTT values exceeded 500 s but these were recorded as 500 s.

ROTEM

The ROTEM values are displayed in Tables II–III.

Parameters concerning initiation and speed at which solid clot forms (clotting time, clot formation time, α-angle)

CT readings were found to be markedly higher in FXII deficient patients than in the control group according to the INTEM and FIBTEM tests. An opposite significant relationship was identified in relation to CT readings by the EXTEM test. CFT values were markedly higher in FXII deficient patients than in controls according to the INTEM and EXTEM tests. No significant differences concerning alpha angle values were observed.

However, the INTEM test found the median alpha angle to be significantly smaller in the subgroup of patients with FXII <1% (n = 8) than in controls (66 vs. 78, $p < 0.001$). There were also marked differences in CFT readings (126.5 vs. 101.5, $p = 0.02$) and alpha angle readings (66 vs. 72, $p = 0.01$) between the FXII <1% subgroup (n = 8) and the whole FXII-deficient group (n = 20).

Marked negative correlations were found between FXII activity and INTEM CT (r = –0.46), INTEM CFT (r = –0.62), while a positive correlation was observed between FXII activity and INTEM alpha angle (r = 0.75).

Table III. ROTEM data. Data expressed as median, range

ROTEM test		ML (%)	*p*	LI 30 [%]	*p*	LI 45 [%]	*p*	LI 60 [%]	*p*
INTEM	FXII DEF	18 (2–25)	NS	100 (98–100)	**<0.001**	97 (93–100)	<0.001	93 (88–98)	**0.002**
	Controls	18 (14–23)		98 (94–100)		93 (88–98)		89 (84–96)	
EXTEM	FXII DEF	20 (14–33)	NS	100 (98–100)	**0.009**	97 (92–100)	0.002	93 (88–98)	**0.004**
	Controls	22 (15–29)		99 (96–100)		94 (89–98)		90 (84–95)	
FIBTEM	FXII DEF	0 (0–23)	NS	100 (94–100)	NS	100 (95–100)	NS	100 (90–100)	NS
	Controls	1 (0–11)		100 (96–100)		100 (92–100)		100 (90–100)	
APTEM	FXII DEF	19.5 (0–26)	NS	100 (98–100)	NS	97 (92–100)	**0.04**	93.5 (88–100)	**0.02**
	Controls	21 (15–29		100 (97–100)		95.5 (91–98)		91.5 (85–95)	

ML — maximum lysis; LI 30 — clot lysis index at 30 min.; LI 45 — clot lysis index at 45 min.; LI 60 — clot lysis index at 60 min. in patients with FXII deficiency and in control group; FXII — factor XII; NS — non significant

Parameters concerning clot firmness (maximum clot firmness)

No marked differences were found between the studied groups regarding MCF readings. Also, there were no significant correlations between FXII activity and MCF values demonstrated in any ROTEM test.

Parameters concerning clot lysis (ML, LI 30, LI 45, LI 60)

ML readings did not differ markedly between the analyzed groups for any studied ROTEM test; however, the INTEM and EXTEM tests found the LI 30, LI 45, LI 60 results to be markedly higher in FXII-deficient patients than controls (Table III). Marked negative correlations were observed between FXII activity and INTEM LI 30 ($r = -0.49$) and EXTEM LI 60 ($r = -0.48$).

Discussion

As expected, the FXII deficient group demonstrated significantly higher APTT values than healthy volunteers. Patients with FXII deficiency (n = 20) demonstrated elongated CT and CFT according to the INTEM test, and no marked differences in α-angle and MCF readings compared to healthy volunteers. However, the median value of the INTEM α-angle was significantly lower among patients with FXII <1% (n = 8) than controls. These results are similar to those observed by Govers-Riemslag et al. [22] in four patients with FXII <5%; they noted that the addition of purified FXII to blood samples resulted in the normalization of all ROTEM parameters.

These findings, taken together with marked negative correlations between FXII activity and INTEM CT, INTEM CFT and a positive correlation between FXII activity and INTEM α-angle, demonstrate that FXII plays a pivotal role in the initiation and amplification of coagulation processes, but not in the propagation phase.

There were no differences seen between FXII patients and controls in relation to ML in the present study. Similarly, the LI 30, LI 45, LI 60 results were markedly higher in FXII-deficient patients than in controls, according to the INTEM and EXTEM tests, which indicates more stable clots. Additionally, we observed marked negative correlations between FXII activity and INTEM LI 30 and EXTEM LI 60. In their ROTEM analysis, Govers-Riemslag et al. [22] found fibrinolysis to be extremely delayed in one patient, with less than 90% of thrombus being lysed within two hours of measurement. This finding may underscore the role of FXII in fibrinolysis initiation. Previous studies demonstrated that activated factor XII (FXIIa) binds to fibrin, leading to higher fibrin density and greater stiffness of fibrin clots [23]. FXIIa has been also found to directly convert plasminogen into plasmin, leading to fibrinolysis acceleration [24]. In FXII-deficient patients, diminished clot lysis / impaired fibrinolysis has been noted [25, 26].

The limitations of the present study are its relatively small number of patients/controls and the fact that it compares ROTEM tests between different subjects. Although the ROTEM tests are thought to describe the hemostatic potential of each person, they still lack standardization, and the results are highly individual, which is still poorly understood [27]. Due to the high heterogeneity of the

individual results, ROTEM may be more accurate as a laboratory diagnostic tool when each subject is his/her own control. Moreover, ROTEM is not normally used to assess fibrinolysis, although, if properly modified, it could be also useful for this purpose [28, 29].

Conclusions

In conclusion, the results of ROTEM show that FXII deficiency has a great impact on the initiation and amplification of coagulation, but not in the propagation phase. This relationship was further confirmed by a number of marked correlations between FXII activity and certain ROTEM parameters. FXII seems to be only a weak activator of plasminogen [30], however it cannot be excluded that such influence disturbs the balance of hemostasis. This aspect should be taken into consideration, especially now that first clinical trials with anti-XII agent have already started. ROTEM tests merit further investigation as treatment control strategies in the context of FXII inhibition, especially when each subject is his/her own control.

Authors' contributions

JT, PS — concept authorship. JT, KC — content supervision. JT, MR, PS — development of assumptions and methods. EK, MT-S, PS — conducting research. JT, PS, WN — analysis of results and formulation of conclusions.

Conflict of interests

None.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; uniform requirements for manuscripts submitted to biomedical journals.

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