ORIGINAL PAPERS

Adv Clin Exp Med 2016, **25**, 6, 1255–1263
DOI: 10.17219/acem/65781

ISSN 1899–5276

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Assessment of Selected ROTEM Parameters, Kinetics of Fibrinogen Polymerization and Plasmin Amidolytic Activity in Patients with Congenital Fibrinogen Defects

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article

Abstract

Background. Congenital fibrinogen disorders (CFD) are rare fibrinogen deficiencies which may be quantitative or functional. The clinical course of hypofibrinogenemia (hypoFI) or dysfibrinogenemia (dysFI) is unpredictable and cannot be determined by the application of standard hemostasis tests.
Objectives. The main aim of this study was to assess ROTEM parameters in CFD patients.
Material and Methods. Nine patients with CFD were studied. The fibrinogen concentration was measured functionally and antigenically. EXTEM, INTEM, FIBTEM and APTEM tests were used to measure selected ROTEM parameters, including maximum clot firmness (MCF). Fibrin plasma polymerization, clot lysis and plasmin amidolytic activity were determined by spectrophotometric methods.
Results. Incorporating the antigenic, ELISA method, to the diagnostic workup allowed the initial diagnosis to be switched from hypoFI to dysFI in 3/7 patients. MCF readings (the most important parameter describing fibrin polymerization capacity) were significantly lower in patients than in controls according to all ROTEM tests. Cases with hypoFI demonstrated markedly lower readings of MCF according to all ROTEM tests than cases with dysFI. All patients demonstrated disturbances of fibrin polymerization process assessed by turbidimetry. In contrast, no marked differences were identified between studied groups in reference to plasmin amidolytic activity.
Conclusions. Our data suggests that ROTEM and fibrin plasma polymerization according to the turbidimetric method have a high sensitivity towards detection of different CFD. Although ROTEM MCF assessment may help discriminate patients with hypo- or dysfibrinogenemia, this finding has to be confirmed on larger groups of patients (**Adv Clin Exp Med 2016, 25, 6, 1255–1263**).

Key words: ROTEM, congenital fibrinogen disorders, fibrin plasma polymerization by turbidimetric method.

Congenital fibrinogen disorders (CFD) are very rare fibrinogen deficiencies, which may be either quantitative (afibrinogenemia and hypofibrinogenemia) or functional (dysfibrinogenemia and hypodysfibrinogenemia) [1–3]. In contrast to afibrinogenemia, a high proportion of hypofibrinogenemia or dysfibrinogenemia patients are asymptomatic at the time of diagnosis, which is often made as a result of different abnormalities in routine tests of hemostasis [2, 4, 5]. However, in rare cases, the diagnosis of congenital dysfibrinogenemia (dysFI) can be challenging and its confirmation requires specialized tests, including genetic analysis [5–7]. The natural history of patients with dysFI is al-

* The present study was supported by Medical University of Łódź grant (number 503/1-093-01/503-01) and University of Łódź grant (number 506/1136).

so very unpredictable since the risk of bleeding, thrombotic events or pregnancy-related complications cannot be precisely determined for the majority using standard hemostasis tests [8].

The last few years have seen a growing interest in the use of thromboelastography, a global hemostasis test which measures the dynamics of the entire clotting and fibrinolysis process, for the assessment of bleeding and thrombotic risk in various clinical settings [9–13]. Recently, it has been shown that rotation thromboelastometry (ROTEM) may be helpful in indicating the prothrombotic state, which is present at diagnosis in patients with multiple myeloma and essential thrombocythemia [14, 15]. Until now, few attempts have been made to characterize the usefulness of thromboelastography in patients with dysFI or hypodysfibrinogenemia [16–18]. The aim of the present study is to compare the spectrum of ROTEM parameters in a cohort of patients with CFD and to determine whether ROTEM can be used to discriminate patients with dysFI and hypoFI. The study also compares the results of fibrin plasma polymerization and clot lysis tests, as well as plasmin amidolytic activity, in different types of CFD.

Material and Methods

The study was performed in 9 CFD patients, 5 women and 4 men, with a median age of 40 years (range 21–85) as given in Table 1. Informed consent was obtained from all subjects. The diagnosis of afibrinogenemia (patient number 4 – Table 1), hypoFI (patients number 1, 2, 6, 7 – Table 1) and dysFI (patients number 3, 5, 8, 9 – Table 1) was made based on the assessment of thrombin time (TT), functional (von Clauss method) and antigenic (ELISA method) fibrinogen concentration following the exclusion of acquired causes of fibrinogen defects. The majority of patients (7/9) revealed no bleeding or thrombotic complications in the medical history, with the exception of 1 female subject with afibrinogenemia (number 4 – several bleeding events since early childhood) and one male subject with hypoFI (number 7 – deep vein thrombosis at the age of 45). The patients enrolled into the study did not receive any drugs strongly influencing hemostasis for at least 14 days prior to taking a blood sample. All patients had platelet counts within normal range. The control group consisted of 15 healthy volunteers, 8 women and 7 men with median age of 38 years (20–73). Fibrin plasma polymerization, clot lysis tests, and plasmin amidolytic activity were assessed at the Department of General Biochemistry, University of Łódź. Reference plasma (in 12 repetitions) was used as a control for the tests.

Methods

General

The following tests were performed in all patients and controls: basic hemostasis screening, fibrinogen concentration analysis by von Clauss and

Table 1. Characteristics of study participants

Patients	Age (years)/ sex	Fibrinogen concentration von Clauss method (g/L)	Fibrinogen concentration ELISA method (g/L)	PT (s)	APTT (s)	TT (s)
1	24/m	0.81	1.04	11.4	31.3	33.2
2	64/f	1.21	1.31	14.0	30.5	28.2
3	40/m	1.86	2.96	9.2	46.8	178.5
4	21/f	< 0.03	0.04	> 120.1	> 180.1	> 240.1
5	40/f	1.47	3.33	12.1	28.2	39.7
6	85/m	1.34	1.48	10.6	36.0	24.1
7	47/m	1.13	1.38	10.4	27.9	27.9
8	24/f	1.27	2.35	10.6	44.7	23.2
9	31/f	1.07	2.32	13.9	34.3	29.5
Reference values		2–4	2–4	7.0–10.5	26.0–40.0	16.0–21.0

APTT – activated partial thromboplastin time; f – female; m – male; PT – prothrombin time; TT – thrombin time.

ELISA, ROTEM analysis, fibrin plasma polymerization, clot lysis and plasmin amidolytic activity assays.

ELISA for the Quantitative Determination of Fibrinogen Concentration in Plasma

Abcam's Fibrinogen Human *in vitro* competitive ELISA kit (Cambridge, USA) is designed for the quantitative measurement of fibrinogen levels in plasma. A fibrinogen specific antibody was pre-coated onto 96-well plates and blocked. Standards and test samples were added to the wells, followed by biotinylated fibrinogen, before the wells were washed with wash buffer. Streptavidin-peroxidase complex was added and unbound conjugates were washed away with wash buffer. TMB was then used to visualize streptavidin-peroxidase enzymatic reaction. TMB was catalyzed by streptavidin-peroxidase to produce a blue color product that changed to yellow after the addition of acidic stop solution. The density of yellow coloration was inversely proportional to the amount of fibrinogen captured in the plate.

ROTEM

Citrated samples of blood were collected under standardized conditions and ROTEM (Pentapharm GmbH, Munich, Germany, software v. 1.5.3.) measurements were processed within a maximum of 2 h. Four routine ROTEM tests (EXTEM, INTEM, FIBTEM and APTEM) were conducted to assess coagulation time (CT), clot formation time (CFT), α – angle, maximum clot firmness (MCF) and maximum lysis (ML) according to the manufacturer's instructions. The details of ROTEM methodology have been provided in our previous publications [19–21].

Determination of Fibrin Plasma Polymerization and Clot Lysis by the Turbidimetric Method

The kinetics of fibrin plasma polymerization and clot lysis were evaluated by turbidimetry according to Kostka et al. [22]. To each microtiter well we added 100 µL of citrated plasma and then 200 µL of activation mixture (0.75 U/mL thrombin, 225 ng/mL rt-PA, 7.5 mM $CaCl_2$ in TBS, pH 7.4), which had been preheated to 37ºC. Immediately after the addition of enzymes, the absorbance changes were monitored every 12 s for 50 min (λ = 360 nm) at 37°C in a SPECTROstar Nano (BMG LABTECH) microplate reader. The following parameters were determined from the fibrin polymerization and clot lysis curve: lag time – time required for the formation and growth of protofibrils from fibrin monomer after the removal of fibrinopeptides; the maximal velocity of the polymerization process (V_{max}) – reflecting the velocity of lateral protofibril association; maximal absorbance (A_{max}) – indicating the fiber thickness and the degree of crosslinking; velocity of clot lysis (V_{Lys}) – the susceptibility of the clot to lysis.

Plasmin Amidolytic Activity Assay by the Spectrophotometric Method [23–25]

Plasma plasmin was assayed by chromogenic substrate (Chromogenix S-2251) after streptokinase activation. Assays were performed at 37ºC in 96-well polystyrene flat-bottom plates. Briefly, 20 µL of test plasma was diluted with 220 µL of buffer (50 mM Tris/HCl, pH 8.2) and preincubated at 37°C for 10 min with 10 µL of streptokinase (10 000 U/mL). Following this, 30 µL of chromogenic substrate (3 mM) was added to each reaction well. The rate of liberation of p-nitroaniline from the substrate was determined by kinetic method at 415 nm in a SPECTROstar Nano (BMG LABTECH) microplate reader.

Statistical Analysis

Data is presented by means ± SD, medians and ranges. Normally distributed data was analyzed by Student's unpaired t-test, and nonparametric data by the Mann-Whitney *U* test. All p-values less than 0.05 were considered as indicating statistical significance in all tests. All analyses were performed using STATISTICA v. 8.0 (Tulsa, USA).

Results

The concentration of fibrinogen, measured using ELISA and the von Clauss method, together with the results of the hemostasis screening tests are shown in Table 1. All patients demonstrated reduced values of functional fibrinogen, while five of the nine patients showed lower than normal values of antigenic fibrinogen. Patients with dysFI demonstrated higher median values of functional and antigenic fibrinogen, APTT (activated partial thromboplastin time) and TT than patients with hypoFI (Table 2).

ROTEM

Parameters Reflecting Initiation and Speed at which a Solid Clot Forms (CT, CFT, α – Angle)

Median CT and CFT readings were found to be markedly higher, while α – angle values were found to be markedly lower in the cohort of pa-

Table 2. Results of fibrinogen concentration and screening hemostasis tests in patients with hypo and dysfibrinogenemia

	Patients with hypofibrinogenemia (4)	Patients with dysfibrinogenemia (4)	Reference values
Fibrinogen concentration (g/l): von Clauss method	mean ± SD 1.13 ± 0.23 median 1.17 range 0.81–1.47	mean ± SD 1.87 ± 0.8 median 1.59 range 1.27–305	2–4
Fibrinogen concentration (g/l): ELISA method	mean ± SD 1.3 ± 0.19 median 1.345 range 1.04–1.48	mean ± SD 2.74 ± 0.49 median 2.66 range 2.32–3.33	2–4
PT (s)	mean ± SD 11.6 ± 1.7 median 11.0 range 10.4–14.0	mean ± SD 11.45 ± 2.02 median 11.35 range 9.2–13.9	7.0–10.5
APTT (s)	mean ± SD 31.4 ± 3.4 median 30.9 range 27.9–36.0	mean ± SD 38.5 ± 8.78 median 39.5 range 28.2–46.8	26.0–40.0
TT (s)	mean ± SD 28.4 ± 3.7 median 28.05 range 27.9–33.2	mean ± SD 67.73 ± 74.1 median 34.6 range 23.2–178.5	16.0–21.0

PT – prothrombin time; APTT – activated partial thromboplastin time; TT – thrombin time; SD – standard deviation.

tients with CFD than in controls according to EXTEM, INTEM, FIBTEM and APTEM tests (Table 3). Patients with hypoFI showed markedly higher readings of CFT according to EXTEM and lower α – angle values according to EXTEM and APTEM than patients with dysFI (Table 4).

Parameters Reflecting Clot Firmness (MCF)

MCF readings were significantly lower in the samples of patients than in controls according to EXTEM, INTEM, FIBTEM or APTEM tests ($p < 0.001$ in all ROTEM tests) (Table 3). Cases with hypoFI demonstrated markedly lower readings of MCF according to all ROTEM tests than cases with dysFI (Table 4). The most significant differences concerned the MCF EXTEM test ($p < 0.001$).

Parameters Reflecting Clot Lysis

None of the ROTEM tests found any significant differences with regard to ML values between the cohort of patients and healthy volunteers, nor between the patients with hypoFI and those with dysFI (Table 4).

Parameters of Fibrin Plasma Polymerization, Clot Lysis and Plasmin Amidolytic Activity

All patients demonstrated different disturbances of fibrin polymerization process, while patients numbered 3 and 4 showed no fibrin polymerization at all. The values for maximal velocity of fibrin polymerization (V_{max}), maximal absorbance (A_{max}) and velocity of clot lysis (V_{Lys}) were found to be significantly lower in the group of CFD patients than in reference plasma (Table 5). In contrast, no marked differences were identified between studied groups in reference to Lag time and plasmin amidolytic activity (Table 5). Patients with hypoFI showed higher median readings of Lag time, V_{max}, A_{max}, V_{Lys} and plasmin amidolytic activity than patients with dysFI (Table 6). Figure 1 presents the process of fibrin plasma polymerization and clot lysis graphically in all studied patients and in the reference plasma.

Discussion

Nine patients with previously diagnosed CFD were enrolled in the study. The initial diagnosis was made based on the assessment of TT and functional fibrinogen concentration (von Clauss method) following the exclusion of acquired causes of fibrinogen defects. Seven patients were initially diagnosed with hypoFI, one with afibrinogenemia and one with dysFI. Incorporation of the antigenic, ELISA, method to the diagnostic workup allowed us to switch the initial diagnosis from hypoFI to dysFI in 3/7 patients. In these 3 cases, the ratio between functional activity to antigen amounted to 0.44 and 0.54 and 0.46 respectively. Previous studies including Krammer et al. [26] indicate that the ratio lower than 0.7 allows the vast majority of the dysFI patients to be to identified [6–8, 27].

Table 3. Thromboelstometry data

Test	CT			CFT			α – angle			MCF			ML		
	patients (n = 8)	controls (n = 15)	p	patients (n = 8)	controls (n = 15)	p	patients (n = 8)	controls (n = 15)	p	patients (n = 8)	controls (n = 15)	p	patients (n = 8)	controls (n = 15)	p
EXTEM	69.6 ± 13.2	59.1 ± 7.8	**< 0.001**	133.1 ± 43.2	79.5 ± 19.6	**< 0.001**	63.3 ± 6.8	74 ± 3.9	**< 0.001**	52.2 ± 7.3	63.1 ± 4.5	**< 0.001**	19 ± 3.9	21.9 ± 3.9	0.13
INTEM	193 ± 14.9	172.7 ± 20.6	**0.02**	117.9 ± 31.7	63.9 ± 13.6	**< 0.001**	66.8 ± 5.7	77.2 ± 2.5	**< 0.001**	49.9 ± 8.3	61 ± 3.9	**< 0.001**	16.9 ± 3.6	18.7 ± 2.8	0.16
FIBTEM	89.8 ± 19.9	52.6 ± 8.1	**< 0.001**	–	–	–	60 ± 7.8	69.9 ± 8.7	**< 0.001**	9.1 ± 5.7	15.7 ± 3.8	**< 0.001**	16.6 ± 14	4.4 ± 5.1	–
APTEM	81.0 ± 22.69	55.9 ± 8.1	**0.002**	133.5 ± 55.4	83.5 ± 21.2	**< 0.001**	62.2 ± 8.3	73.4 ± 4.1	**< 0.001**	51.3 ± 7.9	62.2 ± 4.4	**< 0.001**	19.3 ± 3.6	20.61 ± 3.5	0.53

p-values lower than 0.05 are marked in bold.
CT – coagulation time; CFT – clot formation time; α – angle; MCF – maximum clot firmness; ML – maximum lysis in the cohort of patients with hypo and dysfibrinogenemia and in control group

Table 4. Comparison between selected parameters of thromboelstometry

Test	CT			CFT			α – angle			MCF			ML		
	patients HypoFI (n = 4)	patients DysFI (n = 4)	p	patients HypoFI (n = 4)	patients DysFI (n = 4)	p	patients HypoFI (n = 4)	patients DysFI (n = 4)	p	patients HypoFI (n = 4)	patients DysFI (n = 4)	P	patients HypoFI (n = 4)	patients DysFI (n = 4)	p
EXTEM	63 ± 9.9	76 ± 11.5	0.9	159.5 ± 25.3	83.3 ± 11.2	**< 0.001**	60.8 ± 4.9	77.2 ± 4.4	**0.01**	49 ± 3.8	71.9 ± 4.7	**< 0.001**	19.5 ± 4.1	18 ± 6	0.45
INTEM	198.5 ± 24.5	187 ± 13.3	0.7	128.5 ± 34.6	95 ± 22.3	0.41	67.5 ± 4.8	73.6 ± 4.3	0.16	46.5 ± 4.4	68.7 ± 5.3	**0.01**	18 ± 2.9	16 ± 4.2	0.32
FIBTEM	88.5 ± 32.3	86 ± 9.1	0.8	–	–	–	–	69 ± 8.2	–	7.5 ± 2.1	13.9 ± 4.11	**0.03**	24.3 ± 16.8	13 ± 11.4	0.32
APTEM	72.5 ± 28.4	83.5 ± 21.7	0.6	161.5 ± 47.6	89.5 ± 29.5	0.52	62.0 ± 6.1	77.7 ± 3.3	**0.03**	47.8 ± 5.4	65.6 ± 4.8	**0.02**	19.5 ± 2.4	17.7 ± 3.2	0.28

p-values lower than 0.05 are marked in bold letters.
CT – coagulation time; CFT – clot formation time; α – angle; MCF – maximum clot firmness; ML – maximum lysis in the cohort of patients with hypo- and dysfibrinogenemia

Table 5. Results of selected parameters of fibrin plasma polymerization, clot lysis and plasmin amidolytic activity in the cohort of patients (mean ± SD, median from three tests in each patient) and in reference plasma (mean ± SD, median values from 12 tests). P-values reflect differences between medians

Patients	Lag time (s)	V_{max} [ΔmA/min]	A_{max}	V_{Lys} [ΔmA/min]	Plasmin amidolytic activity [ΔmA/min]
1	432 ± 32	20.0 ± 8.9	0.039 ± 0.005	6.7 ± 5.8	152 ± 14
2	617 ± 27	11.3 ± 3.8	0.018 ± 0.004	5.0 ± 1.0	178 ± 14
3	0	0	0	0	137 ± 9
4	0	0	0	0	120 ± 11
5	410 ± 16	38.3 ± 2.9	0.122 ± 0.009	14.0 ± 1.0	129 ± 5
6	195 ± 8	85.3 ± 4.2	0.166 ± 0.017	21.0 ± 1.7	120 ± 37
7	177 ± 2	81.3 ± 5.0	0.132 ± 0.002	12.3 ± 1.2	138 ± 11
8	150 ± 4	78.3 ± 3.2	0.151 ± 0.004	21.7 ± 1.5	123 ± 17
9	0	7.33 ± 1.5	0.016 ± 0.004	3.0 ± 0.0	155 ± 17
Patients (9) median, range	177.0; 0–617	20.0; 0–85.3	0.04; 0–0.17	6.7; 0–21.7	137; 120–178
Reference values (12) median; range	178; 169–190	240; 201.0–273.0	0.42; 0.35–0.45	46.0; 37.0–49.0	119; 98–135
p	ns.	0.00014	0.00014	0.00014	ns.

p-values lower than 0.05 are marked in bold. Lag time – time required for the formation and growth of protofibrils; V_{max} – maximal velocity of fibrin polymerization process; A_{max} – maximal absorbance; V_{Lys} – velocity of clot lysis; 0 – lack of polymerization; ns – non significant; SD – standard deviation; ΔmA/min – delta mili absorbance/minute.

Table 6. Comparison between selected parameters of fibrin plasma polymerization, clot lysis and plasmin amidolytic activity in the cohort of patients with hypo and dysfibrinogenemia

Lag time (s)		Maximal velocity of polymerization process (V_{max}) [ΔmA/min]		Maximal absorbance (A_{max})		Velocity of clot lysis (V_{Lys}) [ΔmA/min]		Plasmin amidolytic activity [ΔA/min]	
patients hypoFI (n = 4) median range	patients dysFI (n = 4) median range	patients hypoFI (n = 4) median range	patients dysFI (n = 4) median range	patients hypoFI (n = 4) median range	patients dysFI (n = 4) median range	patients hypoFI (n = 4) median range	patients dysFI (n = 4) median range	patients hypoFI (n = 4) median range	patients dysFI (n = 4) median range
313 177–617	75 0–410	50.6 11.3–85.3	22.8 0–78.3	0.086 0.018–0.166	0.069 0–0.15	9.9 5–21	8.5 0–21.7	0.145 0.120–0.178	0.133 0.123–0.155

dysFI – dysfibrinogenemia; hypoFI – hypofibrinogenemia; SD – standard deviation; ΔmA/min – delta mili absorbance/min.

One of the main purposes of this study was to assess the selected parameters of ROTEM in a cohort of patients with CFD and compare these values with those of healthy volunteers. Median CT and CFT readings were markedly higher, and MCF readings were significantly lower, in the CFD group than in controls according to all routine ROTEM tests used. Among them, FIBTEM MCF is of particular value to measure the contribution of fibrinogen to the clot firmness, since cytochalasin D is first used to inactivate sample platelets [28]. In particular, lower MCF readings reflected impaired fibrin polymerization capacity and corresponded well with the results of fibrin plasma polymerization, as assessed by turbidimetry.

To date, ROTEM or TEG (thromboelastography) are commonly used to determine the appropriate use of blood and blood products (in case of impaired clotting) or antifibrinolytics (in case of excessive clot lysis) in trauma resuscitation, cardi-

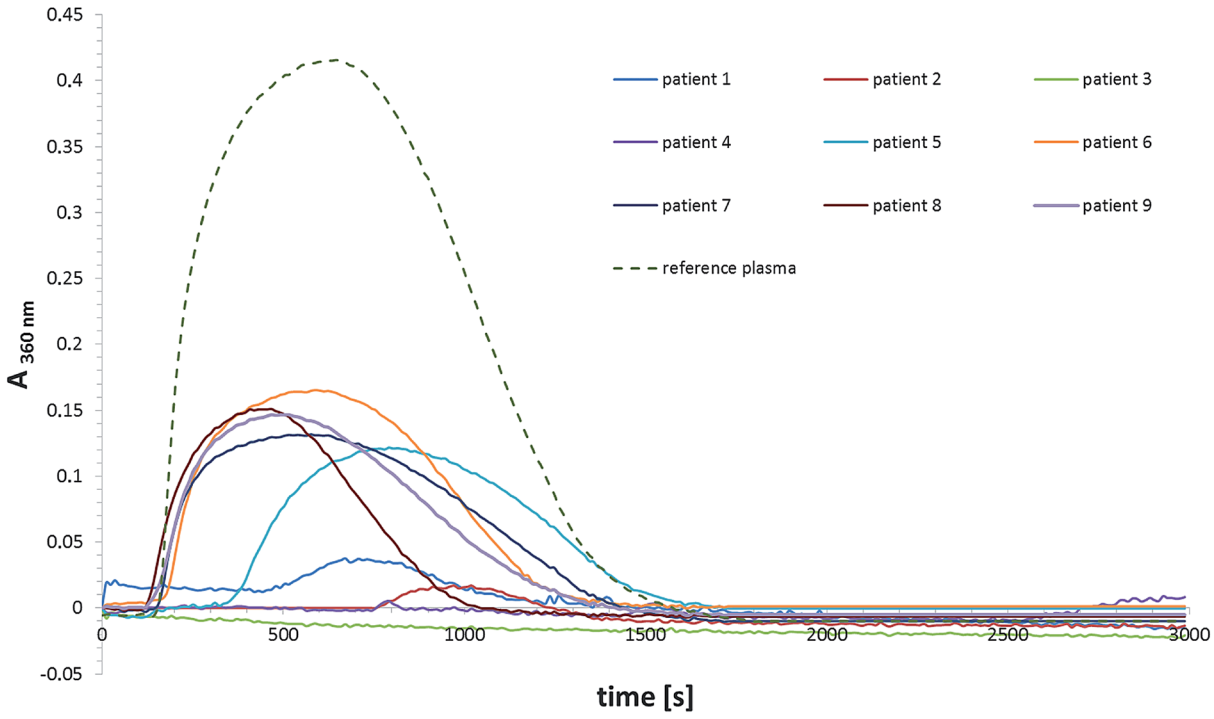

Fig. 1. Graph depicting fibrin plasma polymerization and clot lysis in all studied patients and in reference plasma

ac surgery or liver transplantation [9, 29–31]. This paper is one of the first to assess the value of the ROTEM device in the context of CFD.

In a previous study, Kalina et al. [32] demonstrated that the assessment of MCF by FIBTEM and EXTEM provided a consistent and more predictable response to fibrinogen administered *in vitro* to patients with afibrinogenemia, hypoFI or dysFI, than the assessment of fibrinogen concentration by the von Clauss or ELISA method. In particular, while patients with dysFI demonstrated a good correlation between added fibrinogen and higher MCF readings, the response to fibrinogen spiking was found to be blunted by interactions with abnormal fibrinogen structure when using the von Clauss method. The authors conclude that ROTEM may be useful for monitoring the effects of fibrinogen therapy in bleeding patients with CFD. Our results suggest that patients with dysFI have much higher (similar to controls) median values of MCF than patients with hypoFI, which may possibly reflect differences in the natural history of these disorders.

One of the most important questions is whether the results of ROTEM can help to identify the dysFI patients at a highest risk of thrombotic complications. We speculate that patients who, on diagnosis, have higher than normal MCF values (especially FIBTEM MCF) can be at a higher risk of developing thrombosis, as compared to patients with MCF values within ranges of healthy volunteers. However, an experimental study by Galanakis et al. [18] found that two out of three clots obtained from patients with (dys)fibrinogen variants and positive thrombotic history demonstrated diminished maximal signal amplitudes measuring 31% and 25% of normal control values (MA; equivalent of MCF in ROTEM) when examined by TEG 5000 thromboelastography [29, 30]. Only one patient with dysFI and a history of thrombosis had a normal, undiminished MA value. Among the other 16 CFD patients with a history of hemorrhage, pregnancy complications or who were asymptomatic, the MA values ranged between 0–38% of normal.

In 2015, Zhou J et al. [17] recorded TEG and functional Fg TEG (that corresponds to FIBTEM test in ROTEM) in whole blood samples from 30 patients with congenital dysFI. Significant differences were demonstrated in TEG results between specific fibrinogen mutations. For example, the MA and MA-CFF values measured by functional fibrinogen TEG in patients with mutations at Arg35, Pro37 or Arg38 on Aα chain were almost within normal ranges, while those in patients with γArg301 site mutations were markedly lower than those of healthy volunteers. The authors conclude that the presence of normal results of TEG in dysFI patients may suggest the presence of these three listed above Aα chain mutations. Unfortunately no dependence between TEG results and clinical course of the disease was found, since no TEG parameter could help to identify the patients with the highest risk of bleeding.

In another paper, Zhou J et al. [16] found that women with (hypo)dysfibrinogenemia with values of MA ≤ 54.2 mm and MA-CFF ≤ 12.1 mm in a non-pregnant state have significantly higher risk (odds ratio 11.67 and 20.0 respectively) of obstetric complications during their pregnancies. The authors conclude that this observation can be taken into account when deciding to use fibrinogen replacement therapy, especially in previously asymptomatic patients. It has to be emphasized that until now, the use of fibrinogen replacement therapy in pregnant woman has been considered based on fibrinogen concentration, bleeding complications or positive pregnancy complications history.

All our patients demonstrated abnormal fibrin plasma polymerization according to the turbidimetric method. Median values of the maximal velocity of polymerization, maximal absorbance (parameter indicating the fiber thickness and the degree of crosslinking) and velocity of clot lysis were diminished as compared to controls. In fact, although the parameters listed above were below reference values in all nine patients, this decrease was especially evident in two cases (afibrinogenemia and dysFI) where no fibrin polymerization was recorded. The absence of any marked differences in plasmin amidolytic activity between studied cases and reference plasma suggest that our patients have such mutations in fibrinogen genes which did not affect the interactions between fibrin and fibrinolysis mediators [33].

The authors are fully aware of the limitations of this study. These limitations include the mainly small group of patients and the lack of the genetic analysis, which was only performed on two patients. However, it has to be emphasized that all causes of secondary hypofibrinogenemia were excluded before enrolment into the study.

In conclusion, our data suggests that both rotation thromboelastometry and fibrin plasma polymerization by turbidimetry have a high sensitivity towards the detection of different congenital fibrinogen disorders. While the assessment of ROTEM, MCF may help to discriminate patients with hypo or dysfibrinogenemia; its effectiveness has to be confirmed on larger groups of patients.

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Conflict of interest: None declared

Received: 12.01.2016
Revised: 20.09.2016
Accepted: 12.10.2016