Thrombelastography Monitoring of Platelet Substitution Therapy and rFVIIa Administration in Haemato-oncological Patients with Severe Thrombocytopenia

Salaj P., Marinov I., Marková M., Pohlreich D., Cetkovský P., Hrachovinová I.
Institute of Haematology and Blood Transfusion, Prague Czech Republic

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Abstract: Thrombocytopenic patients refractory to platelet concentrates (PC) could be treated during bleeding episodes with the recombinant activated FVII (rFVIIa). However, monitoring of administration of the rFVIIa or a response to platelet substitution therapy in thrombocytopenia patients is not well documented so far. Using of whole blood ROTEG® analysis we monitored the changes in haemostatic parameters following in vivo platelet concentrate administration compared to ex vivo rFVIIa administration in patients with a severe to mild thrombocytopenia secondary to haemato-oncological disease. We use non-activated thrombelastography (NATEG) and a mild intrinsic activation thrombelastography (INTEG). NATEG analysis was sufficiently sensitive to monitor changes following PC and rFVIIa administration. Both, platelet infusion and rFVIIa treatment induced significant shortening of clotting time (CT) and clot formation time (CFT) parameters ($p<0.05$). When we compared the effect of platelet vs. rFVIIa treated whole blood by NATEG analysis we did not found any significant difference. Analysis with INTEG system was less sensitive and changes in CT and CFT were not significant. The monitoring with thrombelastography could enable efficient application of platelet concentrate and furthermore the using of rFVIIa as an alternative treatment of patients refractory to platelet infusion or with allergic reactions.

Key words: Thrombelastography – Platelet substitution – rFVIIa – Thrombocytopenia

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Mailing address: Peter Salaj, MD., Institute of Haematology and Blood Transfusion, U nemocnice 1, 128 20 Prague 2, Czech Republic,
Phone: +420 221 977 284, Fax: +420 221 977 249, e-mail: *salaj@uhkt.cz*

Introduction

Haemato-oncological patients with a severe thrombocytopenia and bleeding episodes require platelet infusion therapy. Platelet infusion is however associated with risk of bacterial and viral infections, antibody production and anaphylactic reaction [1]. The application of recombinant factor VIIa (rFVIIa) is an effective and safe alternative for haemostatic treatment [2, 3]. However, the substantial cost of this treatment requires adequate monitoring. Whole blood thrombelastographic method was successfully used to monitor an effect of rFVIIa *in vivo* or *ex vivo* administration in haemophiliacs [4] or patients with inherited platelet function disorders [5]. No preliminary reports were so far published concerning the monitoring an effect following of platelet concentrate and rFVIIa administration to patients with acquired thrombocytopenia. However, it was found that high-dose rFVIIa increases initial thrombin generation and mediates faster platelet activation in thrombocytopenia-like conditions in a cell-based model system [6] and thrombelastography was stated as useful tool for *in vitro* evaluation of rFVIIa haemostatic effects [7].

Our study was conducted in order to investigate a possible suitability of whole blood thrombelastograph (ROTEG®) [8] for haemostatic monitoring following a platelet concentrate (PC) administration in patients with a severe to mild thrombocytopenia secondary to hemato-oncological disease. Furthermore, the study compares these results with the results following the rFVIIa administration in the same patients.

Materials and methods

Patients

For our study we obtained approval from the Ethical Board of the Institute of Hematology and Blood Transfusion. We studied eleven patients treated in our institution for hemato-oncological diseases (3 acute lymphoblastic leukaemia – ALL, 2 acute myeloid leukaemia -AML, 2 myelodysplastic syndrome – MDS, 3 following bone marrow transplantation – BMT and one prostatic cancer with bone marrow infiltration). All patients developed secondary thrombocytopenia induced by chemotherapy. The mean platelet count was $14{,}6 \pm 9 \times 10^9$/l (range 6–41×10^9). No primary coagulation disorder was diagnosed in any of them. Fibrinogen level was 4.31 ± 2.14g/l (range 2.56–6.14g/l). Patient's age was 50.5 ± 15 years (range 27–67 years).

PC infusion

Eleven PC administrations were studied. Peripheral whole blood was collected into trisodium citrate (0.129 mol/l) always before PC administration. The thrombelastography and *ex vivo* rVIIa experiment was performed immediately. Thirty minutes following the administration of approximately $4x10^{11}$ platelets second blood sample was collected, the platelet count increase was measured and thrombelastography was repeated.

rFVIIa study

We compared the haemostatic effect of rFVIIa administration in a parallel, e*x vivo* experiment. The rFVIIa in final concentration (fc) 1.5 mg/ml (theoretical concentration of 90–100μg/kg b.w) was added to a freshly drawn citrated whole blood (before PC administration) and incubated at room temperature under gentle agitation. Thrombelastography was performed in 30 min.

Dose-dependent effect of the rFVIIa on ROTEG® parameters was tested in blood samples of nine thrombocytopenic patients (0.02, 0.2 and 2 mg/ml – fc).

Thrombelastography

Analysis was performed with the whole blood haemostasis analyser, Model ROTEG® 05, Pentapharm, Munich, Germany. Clotting time (CT), clot formation time (CFT) and maximum clot firmness (MCF) were measured in whole blood after recalcification (NATEG) with 12.5mM calcium chloride solution (fc). The same parameters were measured in a mild intrinsic activation system (INTEG) with the soluble contact activator ellagic acid. CT is the time taken for the clot formation to begin (Fig. 1A). CFT is the interval between initiation of clotting and the achievement of clot strength with 20mm amplitude. MCF represents the impedance of a pin movement that is converted to the amplitude in millimetres.

For statistical evaluation of the dose-dependent effect of rFVIIa we used Fisher's r to z correlation test. For all others analysis was used pair *t*-test. P values <0.05 were considered as significant.

Table 1 – The ROTEG® parameters before and following substitution of platelets (PLT) and incubation with rFVIIa (n = 11)

	NATEG analysis		
	CT Normal range 540–720s	CFT Normal range <300s	MCF Normal range 50–65mm
Before	1568±297	1880±496	33.2±3.0
PLT	976±145	665±128	48.7±2.1
rFVIIa	919±130	906±180	33.8±2.0
	INTEG analysis		
	CT Normal range 23–173s	CFT Normal range 43–93s	MCF Normal range 55–69mm
Before	304±119	684±303	35.1±2.2
PLT	172±22	206±42	44.9±2.1
rFVIIa	174±19	574±178	31.3±1.9

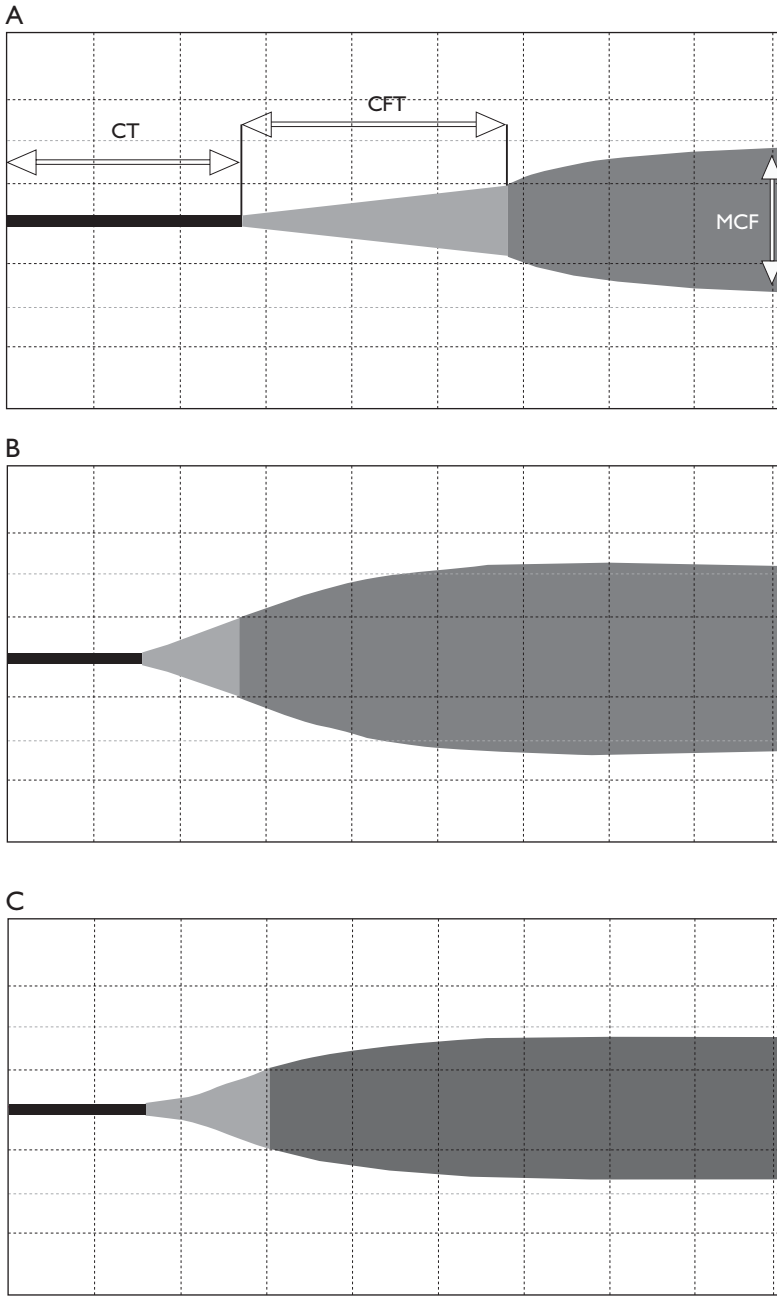

Fig. 1 – Three thrombelastogram patterns obtained as mean of values CT, CFT, MCF (n=11) of whole blood NATEG before substitution (A), 30 min after platelets substitution (B) and 30 min after in vitro incubation with rFVIIa (C).

Results

PC infusion

Platelet numbers 30min following PC infusion increased about two times to $30.5 \pm 16.6 \times 10^9$/l (range $9–65 \times 10^9$/l). The increase of platelet count varied from 1.3 to 3.3 times.

NATEG: platelet concentrate vs. rFVIIa

Mean values obtained by the NATEG analysis before PC administration and 30min after are shown in Table 1. Both, platelet infusion and rFVIIa treatment induced significant shortening of CT and CFT ($p<0.05$) as shown in Fig. 2A. MCF did not change in rFVIIa treated blood but was significantly higher ($p<0,001$) following platelet infusion. When we compared the effect of platelet vs. rFVIIa treated whole blood by NATEG analysis, we did not found any significant difference concerning CT and CFT ($p=0.17$ res. $p=0.28$), while MCF value was significantly different ($p<0.002$). We found the effect of rFVIIa in whole blood significantly dose-dependent in CT values ($p< 0.02$).

INTEG: platelet concentrate vs. rFVIIa

We did not find significant changes in CT, CFT, MCF values measured in the INTEG system, when we compared values before and following rFVIIa and platelet treatment (Fig. 2B). Only MCF parameter was different ($p<0.001$) following both kinds of treatments.

Discussion

Except for the simple count measurement, there is so far no evidence for the monitoring of the haemostatic effect following PC administration in

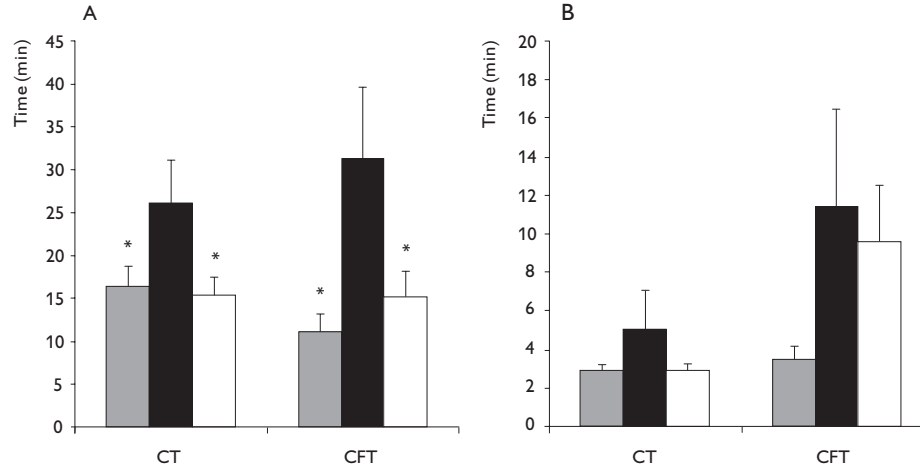

*Fig. 2 – Parameters of whole blood NATEG (A) and INTEG (B) analysis in whole blood before (black) and following of PC administration (grey) and rFVIIa substitution (no filling), * $p<0.05$.*

thrombocytopenic patients. For this purpose we decided to use the whole blood ROTEG® analysis because the kinetic of clot formation and the maximum clot firmness in this system depend on the amount [7] but especially on the function of platelets in the sample. The effect of PC infusion (approximately the same dose) was variable in our studied group of patients. The increase of platelet count varied from 1.3 to 3.3 times. In some patients, in spite a negligible platelet count increase, we observed significant improvement of haemostatic parameters in ROTEG® analysis. This observation suggests a possible favourable effect of PC. The clinical relevance of this finding remains to be investigated. However, Cammerer *et al*. [9] showed a large coincidence of pathological ROTEG® parameters with clinical bleeding and the results from the recent pilot study [10] shows that application of ROTEG® analysis may decrease the use of blood products.

On the other hand there is a group of thrombocytopenic patients that are refractory to PC administration and with no clinical benefit concerning bleeding complication. Several studies describe the use of rFVIIa infusion to avoid or stop severe bleeding complications in such cases. None of the of the previously carried studies concerning rFVIIa treatment of secondary bleeding complications in thrombocytopenic patients did describe the possibility to monitor its effect [3, 11], except by clinical evaluation [2, 12]. In our study we used two tests, NATEG and INTEG. We found the NATEG analysis as a better method for monitoring because both, PC infusion and rFVIIa treatment induced significant shortening of CT and CFT. This result is actually not surprising in case of rFVIIa administration since the NATEG is more sensitive to the intrinsic content of tissue factor what together with rFVIIa accelerates thrombin formation. Interestingly, we found a shortening of CT also following PC administration. Since the INTEG reagent also contains pro-coagulant phospholipids, this effect may be partially masked in this system. Disadvantage of using NATEG analysis is the high sensitivity and the higher variation of results. It would be interesting in future studies to try a modification of the NATEG employing the minimal tissue factor activation [13].

It was published that already 10 to 30×10^9 /l platelets are sufficient for the haemostatic effect of rFVIIa (6, 7). Our results support these *in vitro* studies. We found significant shortening of CT and CFT ($p < 0.05$) in the patients with less than 25×10^9/l platelet. We compared *ex vivo* and *in vivo* effect of the rFVIIa on changes in haemostasis in samples of two patients treated with the rFVIIa for a severe bleeding. We found the same changes in CT, CFT and MCF.

In conclusion, we have demonstrated the usefulness of the ROTEG® system in monitoring of the haemostatic changes following PC infusion. Based on our experience (ex vivo application of rFVIIa) we recommend thrombelastography as a good test for evaluation the effect of rFVIIa application in thrombocytopenic patients.

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