

ORIGINAL ARTICLE *Inhibitors*

Thrombelastographic monitoring of recombinant factor VIIa in acquired haemophilia

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Summary. Monitoring of the global haemostatic capacity is desired to optimize the treatment with bypassing agents in inhibitor patients. Thrombelastographic methods have been used in *ex vivo* studies and were suggested useful to evaluate the individual response to bypassing agents. This study aimed at assessing changes in thrombelastographic profiles and their association to clinical outcome in patients treated with recombinant factor VIIa (rFVIIa). Ten patients with acquired haemophilia were treated with rFVIIa for acute bleeding. Thrombelastography was performed after activation with a small amount of tissue factor in samples obtained before and after *in vivo* administration of rFVIIa. In patients studied before and after a first dose, correction of the thrombelastographic profile was observed but did not predict cessation of bleeding. During steady-state dosing, the median Alpha angle tended to be higher in patients with a good clinical treatment response as

compared with patients with a partial or poor response. Similar trends were observed for clotting time and clot formation time. A good clinical treatment response was more frequent in patients with a fully corrected trough-level thrombelastographic profile as compared with patients with an abnormal profile. However, a poor treatment response was observed also in a surgical patient with a normal thrombelastographic profile during steady-state dosing. In conclusion, thrombelastographic monitoring was sensitive to haemostatic changes in response to treatment with rFVIIa. In the limited number of patients studied here, a better clotting profile during steady-state dosing was associated with a better clinical treatment response.

Keywords: acquired haemophilia, factor VIII, inhibitor, pharmacodynamic monitoring, recombinant factor VIIa (NovoSeven)

Introduction

Bypassing agents enhance thrombin formation independent of factor VIII (FVIII) and are used to treat bleeding episodes in patients with neutralizing anti-FVIII antibodies, so-called inhibitors. The two currently available agents are recombinant FVIIa (rFVIIa) and plasma-derived activated prothrombin complex concentrate (APCC) [1,2]. Treatment with these agents is usually guided by clinical observation, sometimes in combination with imaging and monitoring of the haemoglobin concentration. Laboratory monitoring of haemostasis can potentially

support clinical assessment, especially in difficult situations such as deep, occult or refractory bleeding. Monitoring may also help to secure adequate haemostasis before surgery and may guide the development of continuous infusion and prophylactic treatment strategies. The development of monitoring assays is challenging as bypassing agents have multiple modes of action. APCC contains vitamin K dependent coagulation factors and their activated forms enhancing coagulation at different steps [3]. rFVIIa activates FX in two different pathways: after binding to tissue factor (TF), and TF-independently on the surface of activated platelets [4].

Factor VII one-stage activity assay (FVII:C) and modifications of this assay have been used to monitor rFVIIa [5,6]. A treatment dose of 90 $\mu\text{g kg}^{-1}$ results in a FVII:C peak of 50–70 U mL^{-1} and a trough of 6–8 U mL^{-1} attained two hours after administration [7]. FVII:C monitoring has been used in continuous

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infusion studies, but clinical efficacy did not correlate with FVII:C levels obtained [8–10].

FVII:C as well as more global assays such as prothrombin time and activated partial thromboplastin time (APTT) only detect the initiation of coagulation. In contrast, assays have been developed that record a continuous profile throughout the coagulation process. Examples include thrombelastographic methods determining the whole blood clot elasticity [11–13] and various thrombin generation assays using chromogenic substrates in platelet-poor plasma (PPP) or fluorogenic substrates in either PPP or platelet-rich plasma (PRP) [14,15]. Thrombelastographic methods have the potential advantage of recording the coagulation process under conditions in the patient's own blood without pre-analytical manipulation. This may be relevant because the platelet count in PRP preparations affects thrombin generation assays and may introduce a pre-analytical error. Moreover, the role of fibrinogen is disregarded in any thrombin generation assay; likewise the role of platelets is disregarded in any assay performed in PPP. Another potential advantage of thrombelastographic methods is rapid availability as a point of care test.

Sorensen *et al.* [12] first introduced a whole-blood thrombelastographic method using activation by small amounts of TF. This method allowed for the analysis of samples from haemophilia patients with sufficient sensitivity and precision. The assay has been used to study the effects of *ex vivo* supplementation of inhibitor patients' blood with rFVIIa and APCC [13]. Patterns of response were similar for both agents, but the extent of normalization appeared to be dose-dependent and differed among the patients. These results encouraged us to use this method during rFVIIa treatment *in vivo*. This report documents our experience in patients with acquired haemophilia treated with rFVIIa for acute bleeding episodes and correlates the results obtained to the clinical response of treatment.

Patients, materials and methods

Inclusion criteria and study design

This was a single-centre, non-interventional observation study. Approval was obtained from the local Ethics Committee. Patients were included if they were at least 18 years of age, had acquired haemophilia and were treated with rFVIIa for a bleeding episode. Patients studied during administration of the first dose were required not to be treated with any bypass agent within the previous 72 h. Patients

studied in steady state had been on the same dose and dosing interval for at least five doses. For both first dose and steady-state measurements, blood was drawn immediately before and 20 min after intravenous injection of rFVIIa.

Clinical evaluation

The clinical response to treatment was assessed for the first 48 h after each measurement and a short narrative description is provided. In the lack of validated instruments to evaluate the diverse bleeding situations in inhibitor patients, we defined criteria to categorize the treatment response retrospectively as follows. A good response was defined as (i) no dose escalation or switch of bypass product, (ii) improvement of bleeding-related symptoms, (iii) absence of new bleeding sites or events, (iv) maximum haemoglobin decrease of 2 g dL^{-1} , and (v) minimum haemoglobin increase of 1.5 g dL^{-1} for every 2 U of red blood cells (RBC). A partial response was stated if one of these criteria was not met, but bleeding symptoms did not worsen. A poor response was stated if more than one of the criteria were not met or if bleeding symptoms worsened during the initial treatment.

Blood sampling and standard laboratory assays

Blood was collected at the indicated time points into a Sarstedt Monovette containing 1/10 volume 0.106 M sodium citrate. For plasma-based assays, blood was centrifuged at 1500 g for 15 min. The FVIII one-stage clotting activity (FVIII:C) was determined using reconstituted lyophilized human FVIII-deficient plasma (Dade Behring, Marburg, Germany) and APTT reagent (Pathromtin SL; Dade Behring) on a Schnitger & Gross coagulometre. The inhibitor titre was determined using the Nijmegen modified Bethesda assay and the same reagents as for the one-stage assay.

Thrombelastographic method and reference ranges

Citrated whole blood obtained as described above was subjected to thrombelastography after a respite time of 30–60 min. Coagulation profiles were recorded using a ROTEM[®] thromboelastometry analyser (Pentapharm, Munich, Germany). The numerical parameters describing the profile were: CT (clotting time: time from addition of calcium to the start of clot formation); CFT (clot formation time: time from start of clot formation to a clot firmness of 20 mm); Alpha (angle of tangent at

20 mm); and MCF (maximum clot firmness reached during analysis).

To activate clotting, a minute amount of tissue factor (TF) was added. We used the manufacturer's TF reagent (ex-TEM) serially diluted 1:1000 in diethyl-barbital acetate buffer, pH 7.4, immediately before use. This dilution was determined in preliminary experiments. Using the manufacturer's exTEM pipetting protocol, 20 µL diluted TF reagent (1:1000, final dilution 1:17 000), 20 µL of 200 mM CaCl₂, and 300 µL blood were added to a pre-warmed cup, mixed and subjected to recording for at least 60 min. Reference ranges were determined for this method in 18 healthy volunteers: CT 275–606 s; CFT 97–259 s; alpha 43–71°; MCF 49–61 mm.

Statistics

Descriptive statistics were used to analyse the data. Data are reported by their median and range if not otherwise stated.

Results

Patient characteristics

Ten patients with acquired haemophilia were enrolled. Demographics, baseline characteristics and treatment information are given in Table 1. All patients were treated with rFVIIa for an acute bleeding episode. Information on the bleeding type and response to treatment is shown in Table 2. Along with rFVIIa, patients 9 and 10 received tranexamic acid.

First dose

Five patients (patient nos 1–5 according to Table 1) were studied before and after a first dose of rFVIIa.

Their median FVIII:C was <1 IU dL⁻¹ (range <1–15); fibrinogen and platelets were normal or slightly increased.

Treatment-related changes in thrombelastographic variables are shown in Fig. 1. Before dosing, clot formation was highly variable ranging from no detectable clot after >1 h to only slightly prolonged clot formation as observed in patient 4. The CT (median 1246 s, range 792 to >3000 s) and CFT (median 1039 s, range 274 to >3000 s) were prolonged in all patients to a variable degree. Alpha was normal in one patient and reduced in the others (median <15°, range <15–49°). The MCF (median 51 mm, range 0–68 mm) was normal or increased in three of five patients; in the remaining two patients clotting did not start or was not complete after 1 h of analysis. There was no association between the pretreatment thrombelastographic results and the severity of bleeding symptoms or the response to treatment during the first 48 h.

Twenty minutes after administration of the first dose, the CT (median 567 s, range 256 to >3000 s) and CFT (median 249 s, range 145 to >3000 s) were shorter compared with those before administration in four of five patients. In three patients, values within or even below the reference range were obtained. Similarly, Alpha (median 48°, range <15–65°) increased in four of five patients and was normal in three of them. The MCF slightly increased in most patients indicating that the maximum clot strength had not been reached after 1 h of analysis in the pretreatment samples.

In patients with a complete correction of the thrombelastographic profile, the dose of rFVIIa tended to be higher (96, 103 and 126 µg kg⁻¹) when compared with that of patients, who showed no or only partial correction of their clotting profile (96 and 99 µg kg⁻¹). The clinical response to treatment is given in Table 2. Patient 1, whose

Table 1. Demographics, baseline characteristics and rFVIIa dosing.

No.	Age (year), sex	APTT (s)	FVIII:C (IU dL ⁻¹)	Inhibitor (BU mL ⁻¹)	Fibrinogen (g L ⁻¹)	Platelets (10 ⁹ L ⁻¹)	rFVIIa dose (µg kg ⁻¹)	Dosing interval (h)
1	68, M	60	15	1	5.3	289	96	2
2	62, M	115	<1	371	5.8	705	99	4
3	83, F	78	<1	18	4.7	264	126	2
4	73, F	119	3	26	3.4	359	96	2
5	84, M	82	<1	35	3.0	144	103	2
6	72, F	60	5	7	1.5	363	150	6
7	80, F	31	65	0.5	2.1	138	107	6
8	80, F	68	1	13	3.7	237	88	2
9	86, F	48	1	7	3.0	197	144	2
10	52, M	55	8	2	5.0	443	128	6
Median (range)	76 (52–86)	64 (31–119)	2 (<1–65)	10 (0.5–371)	3.6 (1.5–5.8)	277 (138–705)	105 (88–150)	2 (2–6)

Table 2. Clinical bleeding phenotype and evaluation of treatment response.

No.	Type of bleeding	Baseline Hb (g dL ⁻¹)*	Max. Hb decrease (g dL ⁻¹)**	RBC units (N)**	Treatment response (narrative)	Treatment response category
1	Haematuria, gastrointestinal	8.8	2.9	4	Haematuria continued; resolved after rFVIIa dose escalation	Poor
2	Occult	7.6	0.3	2	Bleeding stopped	Good
3	Skin, muscle	11.7	0.9	0	Bleeding stopped	Good
4	Haematuria, muscle	10.0	2.7	0	New haematomas while on treatment; resolved after rFVIIa dose escalation	Poor
5	Postsurgery	6.7	0.0	12	Bleeding continued; poorly controlled after rFVIIa dose escalation	Poor
6	Skin, muscle	10.3	0.5	0	Bleeding stopped	Good
7	Skin, muscle, haematuria	11.1	0.4	0	New haematomas while on treatment	Partial
8	Muscle	9.1	1.0	0	Bleeding stopped	Good
9	Skin, muscle	9.6	2.5	5	New occult bleeding while on treatment	Partial
10	After femoral artery puncture	10.9	0.0	0	After initial improvement, new bleeding from the wound	Partial

*Last value before start of rFVIIa treatment.

**Within 48 h after start of rFVIIa treatment.

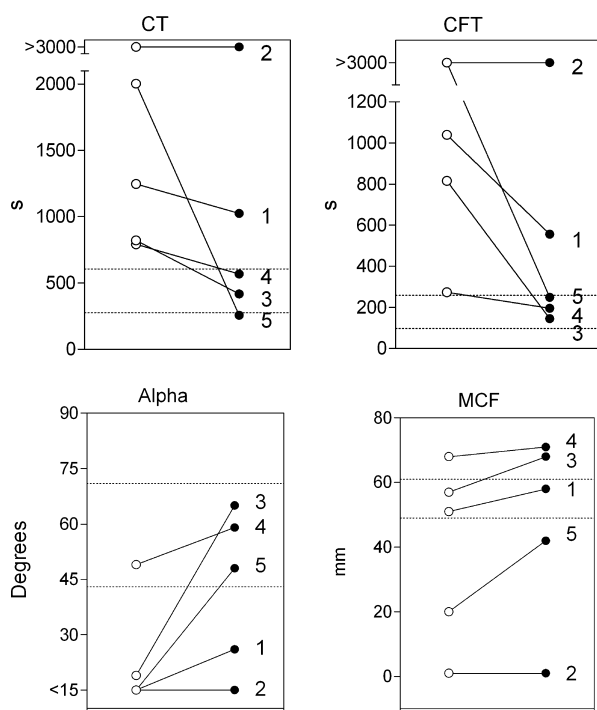


Fig. 1. Changes in thrombelastographic variables before (open circles) and after (closed circles) a first dose of rFVIIa. Patient numbers are given according to Tabel 1. The reference range is indicated by horizontal lines.

clotting profile was partially corrected, suffered from severe urogenital bleeding that was controlled only after dose escalation and treatment for many

days. Patient 2 had apparently no improvement of his clotting profile, but the bleeding episode rapidly resolved without further intervention. Patient 3, whose clotting profile was normalized after the first dose, responded very well to treatment. In contrast, patients 4 and 5, whose profile was similarly corrected, showed a poor treatment response. In summary, one of three patients with a normal clotting profile after dosing showed a good treatment response. One of two patients without a normalization of the thrombelastographic profile also had a good treatment response. A poor treatment response was not preceded by an incomplete or absent normalization of the clotting profile.

Steady state

As baseline thrombelastographic profiles and changes after a first dose did not seem to correlate with clinical outcome, we also studied patients during steady state. Seven patients were included (patient nos 4–10 according to Table 1) receiving rFVIIa in intervals of 2 h (*n* = 4), or 6 h (*n* = 3). The median FVIII:C was 3 IU dL⁻¹ (range <1–65). Fibrinogen was decreased in patient 6, but normal or increased in the others. Platelets were slightly decreased in patient 7, but normal in the others.

Before dosing, more than half of the patients had values within the reference range for CT (four of seven), CFT (five of seven), and Alpha (six of seven,

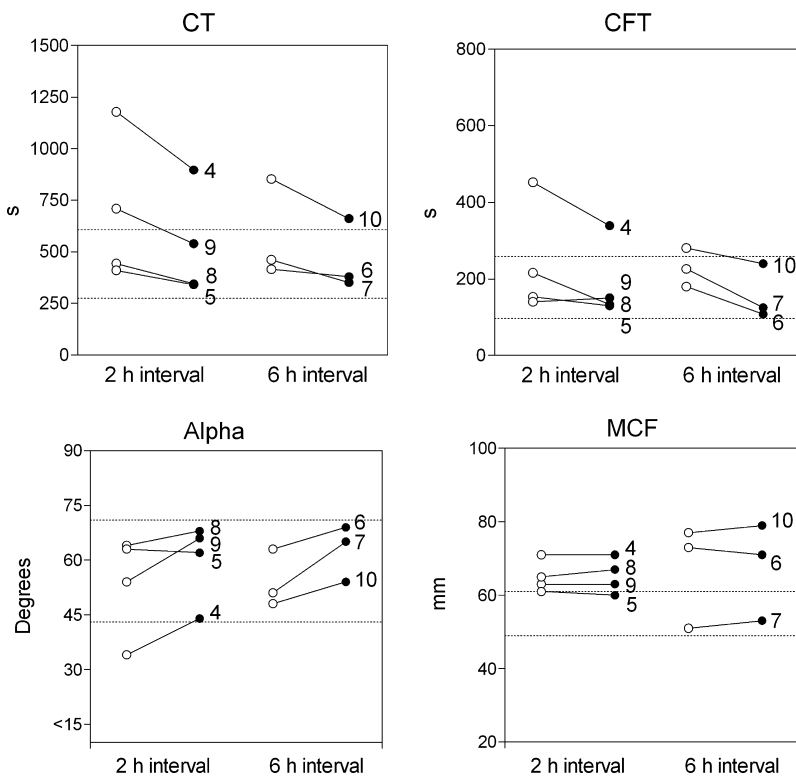


Fig. 2. Changes in thrombelastographic variables before (open circles) and after (closed circles) rFVIIa in patients during steady state dosing of rFVIIa in intervals of 2 h or 6 h. Patient numbers are given according to Table 1. The reference range is indicated by horizontal lines.

Fig. 2). After dosing, the CT and CFT shortened further in most cases, but to a lesser extent in patients who were within the reference range before dosing.

Patients with a fully corrected clotting profile before dosing (CT and CFT within or below the reference range, Alpha and MCF within or above the reference range), as compared to the remaining patients, were not different with respect to dose, dosing interval or any other base line variable. However, their outcome seemed to be more favourable (two good and two partial/poor responses) when compared with that in patients without a fully corrected clotting profile (zero good and three partial/poor responses, Table 2). The median Alpha angle before dosing was higher in patients with a good clinical response (64°) when compared with that in patients with a partial (51°) or poor (48°) clinical response. Similar trends were observed for the CT (good: 430 s, partial: 709 s, poor: 794 s) and the CFT (good: 166 s, partial: 226 s, poor: 296 s). The MCF was not different between the groups.

Discussion

This study addressed the question how thrombelastographic variables change in response to treatment with rFVIIa *in vivo* and whether they correlate with

the clinical response to rFVIIa in patients with acquired haemophilia. Rotation thrombelastography of citrated blood samples was used in this study. Variables according to classical thrombelastography were used to describe the clotting curves: CT (corresponding to the r time), CFT (k time) and Alpha (α) [11]. These variables correlate with recently used variables derived from the first derivative of the curve ([12], data not shown) and appear to be appropriate describing curves of prolonged clot formation. CFT and Alpha can be indeterminate if clotting is severely prolonged as sometimes observed before and after the first dose, but not during steady-state dosing.

We found that administration of rFVIIa accelerates clotting as indicated by a decrease of CT and CFT and an increase of Alpha. In contrast, there was no direct effect on the MCF, which was mainly determined by platelet count and fibrinogen concentration (data not shown). Improvement of thrombelastographic variables after first dosing did not correlate with the clinical response to treatment. During steady-state dosing, however, patients with a good clinical response tended to have better thrombelastographic variables as compared with those with a partial or poor clinical response. Therefore, studying patients in steady state may be more useful for future clinical studies.

The clinical response to treatment was assessed for the first 48 h after measurement. In the five patients studied before and after a first dose, correction of the thrombelastographic profile did not predict cessation of bleeding. During steady-state dosing, a good response was more frequent in patients with a fully corrected trough-level clotting profile. Accordingly, patients with a good response tended to have a shorter CT and CFT as well as a higher Alpha. These results are encouraging. However, we also experienced a poor clinical response in a surgical patient whose thrombelastographic profile was completely normalized even after a first dose and continued to be normal during steady-state dosing (patient 5). In summary of these findings, we conclude that thrombelastographic monitoring remains a promising tool but requires further study before it can be used for clinical decision making.

We preferred a single-centre over a multi-centre study mostly because treatment patterns and technical aspects are more easily standardized. Evaluation of the treatment response is also more accurate under conditions of a single-centre study. Cessation of bleeding as an endpoint is difficult to assess, especially in cohorts with a diverse bleeding pattern such as inhibitor patients. Prospectively defined, objective criteria hardly exist and clinical studies used subjective assessments made by the treating physicians [16–19]. In our study, the treatment response was assessed by chart review using criteria defined retrospectively after critical appraisal of the data. Thereby we confirmed that all available information was considered. As many of our patients suffered from major bleeding requiring transfusion, we also included the haemoglobin count and the haemoglobin increase after RBC transfusion as additional objective criteria. It should be noted that the resulting categorization cannot be compared with other studies without caution: because of the different criteria, the proportion of 'poor' treatment results is higher in our study (30%) when compared with the literature (8–10%) [16,18,19].

Our results fit with two recent reports on the use of thrombelastographic methods in smaller cohorts of inhibitor patients. Johansen *et al.* [20] studied two patients after *in vivo* administration of rFVIIa. Both had a complete normalization of their thrombelastographic profile recorded after activation with a small amount of TF. One of these patients required 2.5 weeks of rFVIIa treatment, which may indicate that bleeding did not resolve easily despite of a full correction of the thrombelastographic profile. Young *et al.* [21] administered rFVIIa to three patients with

congenital haemophilia and high-titre inhibitors outside of clinical bleeding events. Kaolin-activated thrombelastography was recorded before and 15 min after administration and demonstrated improvement in two of three patients. Intriguingly, the lack of response in one patient was consistent with a poor clinical response to rFVIIa. These and our own results need to be interpreted with caution, in particular because our study is the first and a very small single-centre trial. Our study was not powered to detect smaller effects of thrombelastographic variables on clinical outcome or on the influence of additional characteristics such as treatment with tranexamic acid. Considering the difference in the proportion of good treatment responses in patients with a normalized clotting profile vs. not normalized profile, a sample size of 24 patients will be required to demonstrate statistical significance using Fisher's exact test and error margins of $\alpha = 0.05$ and $\beta = 0.8$. Recruiting this number of patients within a reasonable time is only feasible in a multicentre clinical trial.

Conclusion

Thrombelastography after activation with a small amount of TF is sensitive to haemostatic changes in response to treatment with rFVIIa. Favourable treatment responses seem to be more frequent in patients with a normalized thrombelastographic profile during steady-state dosing, but not after first dosing. However, this association is incomplete and further clinical studies are required before thrombelastography can be used for treatment monitoring in routine clinical settings.

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Disclosures

The authors stated that they had no interests which might be perceived as posing a conflict or bias.

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