Prolonged Prothrombin Time After Recombinant Activated Factor VII Therapy in Critically Bleeding Trauma Patients Is Associated With Adverse Outcomes

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Background: In trauma patients with significant hemorrhage, it is hypothesized that failure to normalize prothrombin time (PT) after recombinant activated factor VII (rFVIIa) treatment predicts poor clinical outcomes and potentially indicates a need for additional therapeutic interventions.

Methods: To assess the value of PT to predict outcomes after rFVIIa or placebo therapy, we performed a post hoc analysis of data from 169 severely injured, critically bleeding trauma patients who had 1-hour postdose PT measurements from two randomized clinical trials. Baseline characteristics and outcome parameters were compared between subjects with 1-hour postdose PT ≥18 seconds and PT <18 seconds.

Results: In rFVIIa-treated subjects, prolonged postdose PT values ≥18 seconds were associated with significantly higher 24-hour mortality (60% vs. 3%; $p < 0.001$) and 30-day mortality, increased incidence of massive transfusion, and fewer intensive care unit-free days compared with postdose PT values <18 seconds. Recombinant rFVIIa-treated subjects with postdose PT ≥18 seconds had significantly lower baseline hemoglobin levels, fibrinogen levels, and platelet counts than subjects with postdose PT values <18 seconds even though they received similar amounts of blood products before rFVIIa dosing. Placebo-treated subjects with postdose PT ≥18 seconds had significantly increased incidence of massive transfusion, significantly decreased intensive care unit-free days, and significantly lower levels of fibrinogen and platelets at baseline compared with subjects with postdose PT values <18 seconds.

Conclusions: The presence of prolonged PT after rFVIIa or placebo therapy was associated with poor clinical outcomes. Because subjects with postdosing PT ≥18 seconds had low levels of hemoglobin, fibrinogen, and platelets, this group may benefit from additional blood component therapy.

Key Words: Trauma; Prothrombin time; Recombinant activated factor VII; rFVIIa.

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The first two international, multicenter, randomized, placebo-controlled, double-blind studies of recombinant activated factor VII (rFVIIa; NovoSeven/NiaStase; Novo Nordisk A/S, Bagsvaerd, Denmark) in trauma demonstrated that rFVIIa significantly reduced transfusion requirements and showed trends toward a reduction in mortality and critical complications in blunt trauma, with similar trends being observed in penetrating trauma.[1] This randomized phase 2 study generated much discussion about the optimal use of the rFVIIa, and more generally, the early coagulopathy associated with trauma patients.

Although normalization of prothrombin time (PT) and activated partial thromboplastin time (aPTT) are believed to indicate evidence of coagulopathy reversal after rFVIIa administration, improvement of these parameters may not necessarily reflect clinical efficacy, but simply the interaction between rFVIIa-laden blood and reagents used in the PT/aPTT test.[2–4] However, in a series of 18 patients with traumatic or surgical bleeding receiving a mean rFVIIa dose of 100 μg/kg, McMullin et al.[5] observed that the 17 patients who responded clinically to rFVIIa therapy all experienced posttreatment reductions in PT into the normal range (12.0–14.3 seconds). The single patient who did not respond to rFVIIa therapy only experienced a marginal reduction in PT (from 19 seconds to 18 seconds). The authors hypothesized that patients who fail to normalize their PT after administration of rFVIIa therapy may be deficient of other coagulation factors and may require additional blood component therapy. Because there was only one PT nonresponder, characteristics of responders and nonresponders could not be compared.

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This exploratory investigation sought to determine whether failure to normalize PT after either rFVIIa therapy or placebo therapy is associated with poor clinical outcomes and whether this is associated with deficiencies of key components of the coagulation cascade. A post hoc analysis of data from two international, multicenter, randomized, placebo-controlled, double-blind trials evaluating rFVIIa in trauma[1] was performed to test whether there was a significant difference in one or more baseline hematology and coagulation parameters and clinical outcomes between rFVIIa- or placebo-treated subjects with 1-hour postdose PT values ≥18 seconds and 1-hour postdose PT values <18 seconds.

PATIENTS AND METHODS

Design

The design of both studies has been previously described.[1] Briefly, to be eligible for randomization, patients had to have received six units of red blood cells (RBC) within a 4-hour period, were between 16 years (or of legal of age according to local law) and 65 years of age at screening. Key exclusion criteria included cardiac arrest (prehospital or in the emergency or operating room before trial drug administration), gunshot wound to the head, a Glasgow Coma Scale score <8 (unless accompanied by a normal head computed tomography scan), base deficit >15 mEq/L or severe acidosis (pH <7.0), transfusion of 8 or more units RBC before arrival at the trauma center, and an injury that occurred 12 or more hours before randomization. The initial study protocols were approved by the Ethics Committee of each participating institution, and the trial was conducted according to the International Conference on Harmonization guidelines for Good Clinical Practice and the Declaration of Helsinki.

On receiving six units of RBC within a 4-hour period, eligible patients were randomized to receive three intravenous injections of either rFVIIa (200 μg/kg, 100 μg/kg, and 100 μg/kg) or placebo. The first dose of rFVIIa or placebo was to be administered immediately after transfusion of the eighth unit of RBC, if the patient was expected to require additional transfusions. The second and third doses were administered 1 hour and 3 hours after the first dose, respectively. Placebo or rFVIIa were administered in addition to standard treatment for injuries and bleeding. To reduce the differences in standards of care between countries and institutions, each participating trauma center developed specific transfusion guidelines in line with the transfusion guidelines provided in the study protocol.

Coagulation and fibrinolysis parameters PT, aPTT, fibrinogen concentration, platelet count, thrombin-antithrombin complex (TAT), prothrombin fragment 1 + 2 (F1 + F2), antithrombin-III (AT-III), and D-dimer levels were to be measured at baseline (predosing) and at 1, 4, 8, 12, 24, 36, and 48 hours after the first dose of rFVIIa or placebo. Samples from each country were analyzed at their respective regional central laboratories (Quintiles Laboratories in East Asia, South Africa, Europe, and United States), with the exception of TAT and F1 + F2 samples, which were all analyzed at Quintiles Laboratories South Africa. PT (normal reference range, ≤13 seconds) was measured using standard assays. Factor VII levels were analyzed at a central laboratory using the FVII coagulant activity (FVII:C) assay, a one-stage assay using thromboplastin tissue factor, which quantifies FVII clotting activity in plasma (Capio Diagnostik A/S, Denmark).[6]

In this post hoc analysis, several cutoff values for 1-hour postdosing PT were initially considered, receiver operator curves were constructed, and area under the curve values were also calculated (Fig. 1). The PT cutoff value of 18 seconds was selected. Once 18 seconds was chosen as the PT cutoff value, outcome parameters were then compared between the 1-hour postdose PT ≥18 seconds group versus the 1-hour postdose PT <18 seconds group. Separate PT comparisons were performed for subjects treated with rFVIIa and those treated with placebo. Intensive care unit-free days and hospital-free days were calculated as the number of days not in the intensive care unit (ICU) or in the hospital in the period from the initial dose of rFVIIa or placebo to 30 days postdose.

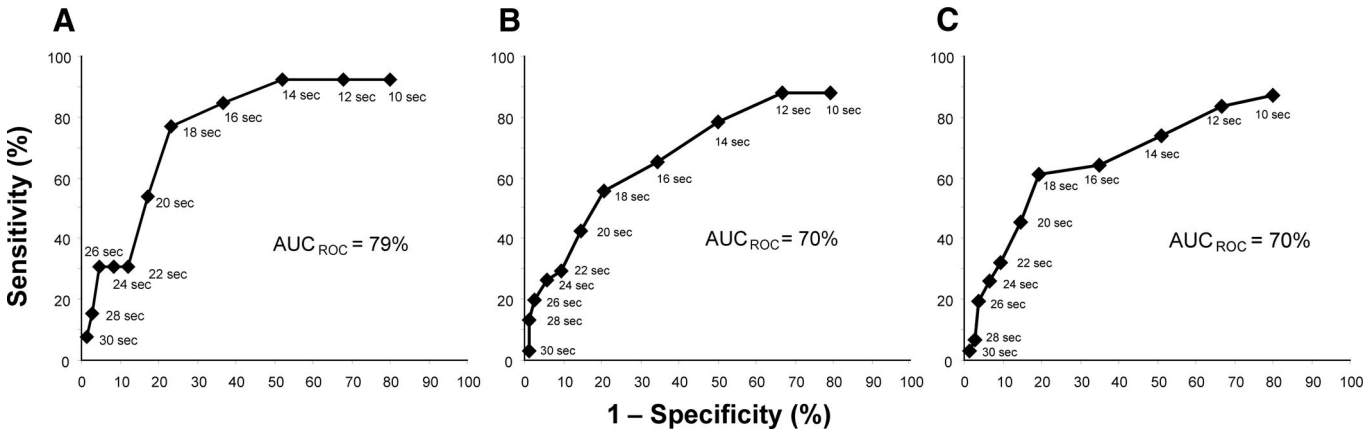

Figure 1. Receiver operator curves were constructed for al three dichotomous patient outcomes: (*A*) 24-hour mortality, (*B*) 30-day mortality, and (*C*) Massive transfusion. Area under the curve values are also labeled by their respective charts. The data indicate that 18 seconds is a reasonable cutoff value for PT in these studies.

To assess whether patients who failed to normalize their PT after rFVIIa or placebo therapy suffered from deficiency of other components of the coagulation cascade, we performed a comparison of the baseline (predosing) hemoglobin, hematocrit, fibrinogen concentration, platelet counts, FVII:C, TAT, F1 + F2, and D-dimer concentrations, and AT-III percentage between the 1-hour postdose PT ≥18 seconds groups and the 1-hour postdose PT <18 seconds groups. We also performed a comparison of the number of units of blood products (fresh frozen plasma [FFP], RBC, and platelets) administered before rFVIIa or placebo dosing. Allogeneic and autologous RBC and whole blood volumes were converted into standard RBC units using an algorithm accounting for the center-specific hematocrit percentages. Total RBC transfused were the sum of the standard allogeneic RBC, autologous RBC, and whole blood volumes. In addition, the mean changes from dosing until 48 hours 48 PT, aPTT, fibrinogen concentration, and platelet counts were plotted for the rFVIIa and placebo postdose PT ≥18 seconds and <18 seconds groups.

Statistical Analysis

Out of the 277 subjects eligible for analysis from the two studies, the 169 subjects who had a PT value 1 hour after dosing comprised two respective analysis sets (rFVIIa, 86 subjects; placebo, 83 subjects). For each analysis set, subjects with 1-hour PT values ≥18 seconds were compared with subjects with 1-hour PT values <18 seconds. The change in PT from baseline to 1 hour after rFVIIa or placebo dose was only presented for the 134 subjects who had a PT values both at baseline and at 1 hour.

Age, Injury Severity Score, body temperature, systolic blood pressure, and pH, all baseline hematology and coagulation parameters, ICU- and hospital-free days, and number of units of blood products administered before rFVIIa or placebo administration were expressed by PT group as mean values (±standard deviations [SD]). Values of zero ICU- and hospital-free days were imputed for subjects who died during the study period.[7] Gender, 24-hour mortality, 30-day mortality, and massive transfusion (MT) were expressed by group as number of subjects with percentages. Baseline characteristics, baseline hematology and coagulation parameters, blood products administered before rFVIIa or placebo dosing, and clinical outcomes were compared between PT groups using a two-tailed *t* test for continuous variables and a two-tailed Fisher's exact test for categorical variables (e.g., gender, 24-hour mortality, 30-day mortality, and MT). In addition, median (interquartile range) values were obtained for units of blood products administered before dosing, ICU- and hospital-free days, and were compared between PT groups using a Wilcoxon two-sample test. Statistical significance was defined as a p value <0.05. Separate PT group comparisons were performed for rFVIIa-treated subjects and placebo-treated subjects. No between-treatment group statistical comparisons were performed.

Subjects were grouped into three categories based upon the ratio of FFP to RBC that they received before treatment with either rFVIIa or placebo. Category 1:1 included subjects who had a FFP:RBC ratio of >0.5. Category 1:2 included subjects with a FFP:RBC ratio of ≤0.5 but >0.25. Category 1:4 included subjects with a FFP:RBC ratio ≤0.25 (including those who received 0 units of FFP). The distribution of FFP:RBC ratio before treatment was plotted by category (1:1, 1:2, 1:4), PT group, and treatment (rFVIIa or placebo) and presented as a percentages and numbers of subjects.

RESULTS

As shown in Figure 1, a PT cutoff value of 18 seconds was chosen because it provided an optimal combination of sensitivity and specificity in both the 24 mortality and MT (defined as >20 units of RBC within 48 hours of admission) data sets (both treatment groups). Although 16 seconds provided a better combination of sensitivity and specificity for 30-day mortality, it was followed very closely by the 18 seconds data point. Thus, 18 seconds seemed to be the most appropriate point for PT to discriminate between positive and negative dichotomous clinical patient outcomes (24-hour mortality, 30-day mortality, incidence of MT). The 18-second cutoff value provided significantly better ability to predict 24-hour mortality in the rFVIIa group compared with the placebo group ($p = 0.0025$).

For the 277 subjects eligible for analysis in the blunt and penetrating trauma trials from March 2002 to September 2003, postdosing PT values obtained 1 hour after dosing were available for 169 subjects (rFVIIa, 86 subjects; placebo, 83 subjects). One hour after treatment, 10 of 86 (12%) rFVIIa-treated subjects and 38 of 83 (46%) placebo-treated subjects had PT values ≥18 seconds.

Baseline (predosing) and 1-hour PT values were available for 72 of 86 (84%) rFVIIa-treated subjects and 62 of 83 (75%) placebo-treated subjects. Table 1 summarizes the change in PT response from baseline to 1 hour for these subjects. None of the rFVIIa-treated subjects with baseline PT values <18 seconds experienced a worsening of coagulopathy (PT ≥18 seconds) 1 hour after receiving rFVIIa. Of the 66 rFVIIa-treated subjects in the 1-hour PT <18 seconds group that also had baseline values, in 42 (64%) PT remained <18 seconds 1-hour postdose, and in 24 (36%) PT decreased to <18 seconds at 1-hour postdose (Table 1). For the 66 subjects in the 1-hour PT <18 seconds group, mean PT values decreased from baseline by 6.12 seconds 1-hour after rFVIIa dosing. For the six rFVIIa-treated subjects who had 1-hour PT values ≥18 seconds, PT decreased from baseline (mean decrease of 7.07 seconds), but never below the bench-

TABLE 1. Number of Subjects Presenting With No Change or a Change From Baseline to 1-h Postdose in PT Classification (≥18 s, <18 s) for rFVIIa and Placebo-Treated Subjects Having PT Measurements at Both Time Points

Baseline Status	1-h PT	rFVIIa (n = 72)	Placebo (n =62)
Coagulopathic (PT ≥18 s)	PT <18 s	24	3
	PT ≥18 s	6	22
Noncoagulopathic (PT <18 s)	PT <18 s	42	26
	PT ≥18 s	0	11

TABLE 2. Baseline Characteristics for rFVIIa- and Placebo-Treated Subjects With 1-h PT Values

	rFVIIa Treatment (N = 86)					Placebo Treatment (N = 83)				
	1-h PT ≥18 s Group (n = 10)		1-h PT <18 s Group (n = 76)			1-h PT ≥18 s Group (n = 38)		1-h PT <18 s Group (n = 45)		
	No. Observed	Values	No. Observed	Values	*p*	No. Observed	Values	No. Observed	Values	*p*
Age (yr)	10	30 ± 8.9	76	31 ± 11.9	0.879	38	33 ± 11.6	45	32 ± 12.3	0.953
Gender, n (%)										
Female	10	0 (0)	76	11 (15)	0.348	38	8 (21)	45	6 (13)	0.390
Male		10 (100)		65 (86)			30 (79)		39 (87)	
ISS	10	36 ± 11.6	75	28 ± 14.3	0.068	38	27 ± 8.8	43	25 ± 10.0	0.461
Body temp. (°C)	6	35.0 ± 1.52	44	35.3 ± 1.41	0.450	30	35.1 ± 1.45	28	35.3 ± 1.46	0.479
SBP (mm Hg)	8	93 ± 13.9	73	113 ± 21.0	0.009	36	112 ± 24.7	44	118 ± 25.8	0.312
pH	10	7.21 ± 0.10	75	7.27 ± 0.11	0.109	37	7.28 ± 0.098	44	7.29 ± 0.099	0.435

ISS, injury severity score; SBP, systolic blood pressure.
Data are mean values ± SD or n (%) where indicated.

TABLE 3. Baseline Hematology and Coagulation Parameters for rFVIIa- and Placebo-Treated Subjects With 1-h PT Values

	rFVIIa Treatment (N = 86)					Placebo Treatment (N = 83)				
	1-h PT ≥18 s Group (n = 10)		1-h PT <18 s Group (n = 76)			1-h PT ≥18 s Group (n = 38)		1-h PT <18 s Group (n = 45)		
	No. Observed	Values	No. Observed	Values	*p*	No. Observed	Values	No. Observed	Values	*p*
PT (s)	6	28 ± 4.0	66	17 ± 6.0	<0.001	25	24 ± 4.9	37	18 ± 5.2	<0.001
aPTT (s)	5	85 ± 29.9	62	47 ± 24.1	0.001	21	62 ± 32.2	33	44 ± 18.6	0.022
Hemoglobin (g/dL)	9	7.7 ± 1.85	69	9.5 ± 2.41	0.034	36	8.5 ± 3.02	42	9.3 ± 2.40	0.179
Hematocrit (%)	7	22.8 ± 5.43	61	27.9 ± 7.23	0.072	33	25.2 ± 9.35	36	27.1 ± 7.26	0.353
Fibrinogen level (g/L)	6	0.7 ± 0.18	66	1.4 ± 1.16	<0.001	25	0.8 ± 0.37	37	1.4 ± 0.57	<0.001
Platelet count ($\times 10^9$/L)	9	52 ± 17.6	69	82 ± 39.6	0.001	36	54 ± 27.4	42	84 ± 58.0	0.004
FVII:C concentration (U/dL)	7	23 ± 24.7	51	25 ± 36.6	0.858	23	24 ± 20.4	29	38 ± 13.3	0.008
TAT level (μg/L)	2	33 ± 33.1	37	31 ± 14.2	0.945	12	35 ± 14.6	21	32 ± 15.2	0.541
F1 + F2 level (nmol/L)	10	4.8 ± 2.46	68	3.5 ± 2.14	0.072	38	3.9 ± 1.98	43	3.4 ± 1.93	0.251
AT-III level (%)	10	24 ± 7.2	66	41 ± 15.8	<0.001	38	27 ± 10.7	39	43 ± 18.0	<0.001
D-dimer concentration (ng/L)	10	1052 ± 467.8	72	1137 ± 438.5	0.568	38	1235 ± 410.1	42	1262 ± 460.0	0.779

Data are mean values ± SD.

mark value of 18 seconds. Eleven placebo-treated subjects became more coagulopathic (PT <18 seconds at baseline but PT ≥18 seconds at 1-hour postdose).

PT group comparisons of additional baseline characteristics that were obtained before rFVIIa or placebo administration are shown in Table 2. With the exception of systolic blood pressure for rFVIIa-treated subjects, there were no other significant differences between the baseline characteristics of the two PT groups. As expected for this critically bleeding trauma population, subjects were hypothermic, acidotic, had decreased systolic blood pressure (more pronounced in the rFVIIa-treated 1-hour PT ≥18 seconds group), and high Injury Severity Scores at baseline, indicating the severity of the injuries sustained by subjects enrolled in this study.

Mean (±SD) PT and aPTT values at baseline were significantly higher in the groups of subjects with 1-hour PT values ≥18 seconds than in the groups of subjects with 1-hour PT values <18 seconds for both rFVIIa and placebo (Table 3). For rFVIIa, mean baseline hemoglobin, fibrinogen, and platelet levels were significantly lower in the 1-hour PT ≥18 seconds group compared with the 1-hour PT <18 seconds group (Table 3). Baseline levels of FVII:C, TAT, F1 + F2, and D-dimers were similar between groups; however, baseline AT-III levels were significantly lower in the 1-hour PT ≥18 seconds groups ($p < 0.001$) (Table 3). Similar results were attained for the PT-group comparisons of baseline coagulation and hematology parameters for placebo-treated subjects. The only exceptions were that placebo-treated subjects with 1-hour PT values ≥18 seconds did not have significantly lower hemoglobin concentrations but had significantly lower concentrations of FVII:C than placebo-treated subjects with 1-hour PT values <18 seconds (Table 3).

Changes in PT, aPTT, fibrinogen concentration, and platelet counts over time (up to 48 hours post-rFVIIa dose) are presented in Figure 2 for rFVIIa (closed squares) and placebo (open circles).

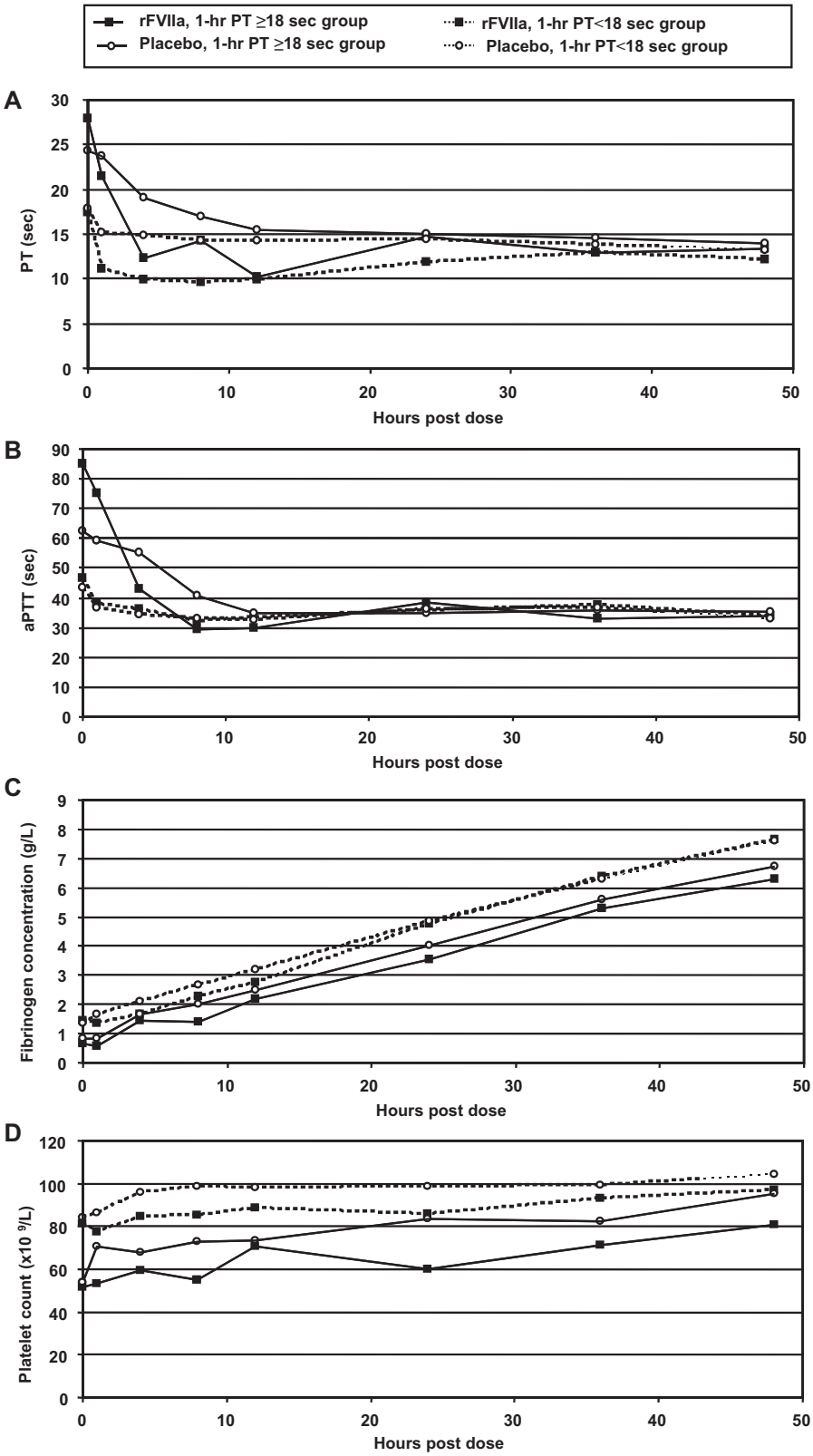

Figure 2. Changes in coagulation parameters over time by treatment and PT group (*A*) PT, (*B*) aPTT, (*C*) fibrinogen concentration, and (*D*) platelet count. ■ with a solid line = rFVIIa treatment, 1-hour PT ≥18 seconds group (n = 10); ■ with a dotted line = rFVIIa treatment, 1-hour PT <18 seconds group (n = 76); ○ with a solid line = placebo treatment, 1-hour PT ≥18 seconds group (n = 38); and ○ with a dotted line = placebo treatment, 1-hour PT <18 seconds group (n = 45).

TABLE 4. Blood Product Administration Before Dosing for rFVIIa- and Placebo-Treated Subjects With 1-h PT Values

	rFVIIa Treatment (N = 86)					Placebo Treatment (N = 83)				
	1-h PT ≥18 s Group (n = 10)		1-h PT <18 s Group (n = 76)			1-h PT ≥18 s Group (n = 38)		1-h PT <18 s Group (n = 45)		
	No. Observed	Values	No. Observed	Values	*p*	No. Observed	Values	No. Observed	Values	*p*
No. units FFP before dose	8	1 ± 1.8	59	2 ± 2.4	0.193	30	2 ± 2.1	37	3 ± 2.7	0.061
No. standard units RBC before dose	9	9 ± 1.2	74	8 ± 1.2	0.419	36	9 ± 1.8	44	8 ± 1.4	0.081
No. units platelets before dose	8	0 ± 0.4	71	1 ± 1.7	0.074	38	1 ± 1.5	43	1 ± 1.6	0.481

Data are mean values ± SD.

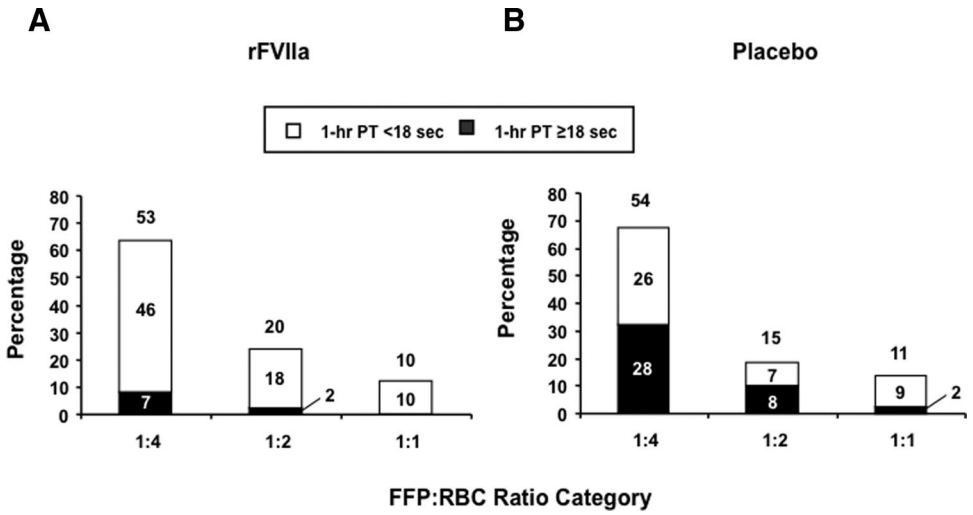

Figure 3. Percentages and numbers of subjects treated with (*A*) rFVIIa or (*B*) placebo by PT group categorized by the FFP:RBC ratio category administered before dosing. Category 1:1 included subjects that had a FFP:RBC ratio >0.5, category 1:2 included subjects with a FFP:RBC ratio ≤0.5 but >0.25, and category 1:4 included subjects with a FFP:RBC ratio ≤0.25 (included subjects with 0 units of FFP). Percentages are graphed, and the numbers provided are numbers of subjects in each category.

As shown in Table 4, there were no statistically significant differences between PT groups in mean (±SD) units of blood products administered before rFVIIa or placebo dosing in both rFVIIa- and placebo-treated subjects. In addition, a comparison of median (interquartile range) values of blood products demonstrated no significant differences between PT groups for the majority of comparisons: FFP (rFVIIa, $p = 0.155$; placebo, $p = 0.040$), RBC (rFVIIa, $p = 0.593$; placebo, $p = 0.399$), and platelets (rFVIIa, $p = 0.586$; placebo, $p = 0.155$). Therefore, it was unlikely that the lower baseline levels of hemoglobin, fibrinogen, and platelets in the 1-hour PT ≥18 seconds groups compared with the 1-hour PT <18 seconds groups in Table 3 could be explained by differences in amounts of blood products transfused before dosing.

After the original prospective analysis was completed, data became available that showed improved outcomes associated with increased ratios of blood products administered before dosing, in particular the FFP to RBC ratio.[8–19] The numbers and percentages of subjects in each of the three FFP:RBC categories (1:1, 1:2, and 1:4) are presented by PT group and by treatment in Figure 3. The majority of subjects were in the 1:4 FFP:RBC ratio categories, meaning that they had a FFP:RBC ratio ≤0.25. Many of these subjects received no FFP before study drug administration, and therefore, had a FFP:RBC ratio of zero before dosing (rFVIIa, 44 of 53 [83%] subjects in the 1:4 FFP:RBC category; placebo, 34 of 54 [63%] subjects in the 1:4 FFP:RBC category). Only 10 of 83 subjects (12%) treated with rFVIIa and 11 of 80 subjects (14%) receiving placebo were in the 1:1 FFP:RBC category. Three subjects in each group were not categorized because they had 0 units of RBCs reported before administration of rFVIIa or placebo. Although the percentages of subjects in each of the three FFP:RBC categories seemed to be similar between rFVIIa and placebo, indicating similar pre-treatment resuscitation, more subjects in the rFVIIa-treatment group had a 1-hour PT <18 seconds.

The PT comparison of clinical outcomes showed that rFVIIa-treated subjects in the 1-hour PT ≥18 seconds group had a 23-fold higher 24-hour mortality rate, a nearly 6-fold higher 30-day mortality rate, a 4-fold higher rate of MT, 3-fold fewer ICU-free days, and 1.8-fold fewer hospital-free days compared with the PT >18 seconds group (Table 5). All

TABLE 5. Clinical Outcomes for rFVIIa- and Placebo-Treated Subjects With 1-h PT Values

	rFVIIa Treatment (N = 86)					Placebo Treatment (N = 83)				
	1-h PT ≥18 s Group (n = 10)		1-h PT <18 s Group (n = 76)			1-h PT ≥18 s Group (n = 38)		1-h PT <18 s Group (n = 45)		
	No. Observed	Values	No. Observed	Values	*p*	No. Observed	Values	No. Observed	Values	*p*
24-hr mortality	10	6 (60)	76	2 (3)	<0.001	38	4 (11)	45	1 (2)	0.174
30-d mortality	10	6 (60)	73	8 (11)	0.001	37	11 (30)	44	6 (14)	0.102
MT*	9	4 (44)	74	8 (11)	0.022	36	14 (39)	44	3 (7)	<0.001
ICU-free days†	9	6 ± 11.6	72	18 ± 10.9	0.002	37	9 ± 11.5	43	17 ± 11.2	0.003
Hospital-free days†	10	4 ± 8.9	73	7 ± 9.5	0.330	37	4 ± 8.2	44	7 ± 9.1	0.200

Data are n (%) for 24-hr and 30-d mortality, and data are mean values ± SD for ICU- and hospital-free days.
* Massive transfusion was defined as >20 units of RBC within 48 hr of admission.
† Through day 30.

outcome comparisons between PT groups were statistically significant with the exception of hospital free days. Comparisons of median (interquartile range) values also showed significant differences between the rFVIIa 1-hour PT ≥18 seconds group and the rFVIIa 1-hour PT <18 seconds group, respectively, for ICU-free days (0 [0–1] days vs. 22 [9–28] days; $p = 0.005$) and were nonsignificant for hospital-free days (0 [0–0] days vs. 0 [0–14] days; $p = 0.066$).

The PT comparison of clinical outcomes showed that placebo-treated subjects in the 1-hour PT ≥18 seconds group had a nearly 6-fold higher 24-hour mortality rate, a 2-fold higher 30-day mortality rate, a 5-fold higher rate of MT, 1.9-fold fewer ICU-free days, and 1.8-fold fewer hospital-free days compared with the PT >18 seconds group (Table 5). For subjects treated with placebo, differences in clinical outcomes between PT groups were statistically significant for MT and ICU-free days and were not significant for 24-hour mortality, 30-day mortality, and hospital-free days (Table 5). Comparisons of median (interquartile range) values also showed significant differences between the placebo 1-hour PT ≥18 seconds group and the placebo 1-hour PT <18 seconds group, respectively, for ICU-free days (0 [0–21] days vs. 21 [5–27] days; $p = 0.003$) but not for hospital-free days (0 [0–0] days vs. 0 [0–16] days; $p = 0.091$).

DISCUSSION

Of the five clinical outcomes evaluated, prolonged postdose PT values ≥18 seconds were associated with significantly higher 24-hour and 30-day mortality, a significantly increased incidence of massive transfusion and significantly fewer ICU-free days compared with postdose PT values <18 seconds in trauma subjects treated with rFVIIa. Subjects with postdose PT ≥18 seconds had significantly lower baseline hemoglobin, fibrinogen, and platelet counts than subjects with postdose PT values <18 seconds even though they had been administered similar numbers of blood products before rFVIIa dosing.

The etiology of traumatic coagulopathy is multifactorial and involves loss of coagulation factors due to bleeding and consumption due to clot formation at the injury sites.[10–12,20,21] Coagulopathy in trauma may further be aggravated by dilution caused by fluid and RBC administration and by hypothermia and acidosis, which may adversely impact coagulation.[22,23] Twenty-five percent to 35% of trauma patients have biochemical signs of coagulopathy at the time of arrival in the emergency department, and up to 50% of massively transfused patients develop coagulopathy.[24–28] Four clinical studies have shown that coagulopathy is a strong, independent risk factor for mortality.[25–28]

Currently, no definitive laboratory test is available to gauge the efficacy of rFVIIa. The PT assay is an in vitro measurement of time to clot formation by the tissue factor (TF) dependent pathway, and it is used to screen for hemostatic disorders. PT is measured as the time from the addition of calcium chloride and thromboplastin (a phospholipid source) and tissue factor, to the time of clot formation, as measured by a mechanical or visual endpoint. The assay is now performed using automated instruments in clinical laboratories. The PT reflects a deficiency of factor VII, V, X, II (prothrombin), and I (fibrinogen) with varying degrees of prolongation to the result. It is commonly used to monitor the effects of warfarin sodium and the resultant production of dysfunctional factors II, VII, and X.

This study focused on PT specifically because this measure examines the TF dependent pathway, it is sensitive to exogenous rFVIIa administration, and is commonly done in trauma settings. Although PT is affected by rFVIIa, it is classically not considered a reliable indicator of clinical efficacy. Dutton et al.[29] and Eikelboom et al.[30] noted that all patients treated with rFVIIa had improved PT, even those who did not respond clinically.

Conversely, others have shown that shortening of the PT post-rFVIIa dosing may nevertheless correlate with clinical effect.[2] Although PT shortened in all patients after rFVIIa therapy, Dutton et al.[29] also found that the postdosing PT was shorter in patients who responded clinically than in those who did not (10.2 ± 3.6 vs. 12.7 ± 3.2, $p = 0.008$). Similarly, Martinowitz and Michaelson[31] reported lower postdosing PT values in rFVIIa clinical responders compared with nonresponders (10.7 ± 2 seconds vs. 20 ± 9.7 seconds). McMullin et al.[5] observed that lack of normalization of PT in a rFVIIa-treated patient was associated with lack of clinical response

and a poor outcome, and these data suggested that even though a simple reduction in PT is not useful in predicting clinical responders to rFVIIa therapy, the lack of an adequate reduction in PT may identify patients with persistent coagulopathy despite rFVIIa therapy. In a recent review, Pusateri and Park[2] discussed possible explanations for lack of normalization of PT and hypothesized that when hemodilution/consumption reaches a critical point at which a component other than FVIIa becomes rate limiting, rFVIIa efficacy will be compromised without replacement of other components, such as FII, FV, FX, fibrinogen, and platelets.

In our post hoc analysis of a large population of blunt and penetrating trauma subjects, a PT threshold of 18 seconds 1-hour after rFVIIa administration provided a significant difference in clinical outcomes. In this study, 12% of the rFVIIa subjects still had prolonged PT values (PT $\geq$18 seconds) 1-hour after dosing, and in accordance with the findings by McMullin et al.,[5] high postdosing PT values were associated with adverse outcomes. Delayed PT normalization in the first few hours after rFVIIa dosing seemed to be most predictive of clinical outcome, because PT eventually normalized in survivors over time for both of the PT groups (Fig. 2). The rFVIIa dosing regimen (3 doses: 200 μg/kg, 100 μg/kg, and 100 μg/kg) used in this study was based on levels effective for hemophilia patients with inhibitors; therefore, it was unlikely that underdosing of rFVIIa contributed to the compromised clinical response associated with prolonged PT.

The finding that rFVIIa-treated subjects with prolonged postdosing PT had low baseline levels of hemoglobin, fibrinogen, and platelet levels suggests that these factors may have been rate-limiting for clot formation and that subjects who receive rFVIIa therapy but still have a PT value $\geq$18 seconds 1-hour after dosing may require additional blood component therapy. In line with this supposition, no significant differences were observed between groups in the transfusion of blood products before rFVIIa dosing. The recommendations in recent clinically derived guidelines[32,33] to correct hemoglobin, fibrinogen, and platelet counts before rFVIIa therapy are consistent with the current understanding of rFVIIa mechanisms of action and are supported by the current findings. Further research is needed to define more clearly the limitations of rFVIIa efficacy under the multifactorial coagulopathic conditions associated with trauma and to investigate whether correction of factor and cell deficiencies improves the response to rFVIIa.

The placebo-treatment group enabled us to analyze the relationship between 1-hour PT and clinical outcomes with an additional data set that included even more subjects with a prolonged 1-hour PT after dosing. Comparisons of placebo-treated subjects by 1-hour PT group yielded similar findings. Namely, although baseline characteristics (Table 2) and units of blood products administered before dosing (Table 4) were similar between 1-hour PT groups, the presence of a prolonged PT value of $\geq$18 seconds 1-hour after dosing was associated with a significantly higher rate of MT and fewer ICU-free days (Table 5). Similar to what was observed for rFVIIa-treated subjects, placebo-treated subjects with a prolonged 1-hour PT had significantly lower fibrinogen levels and platelet counts and lower levels of hemoglobin than subjects with a shorter PT. These findings underscore the importance of early monitoring of PT and treatment with additional blood products to reverse coagulopathy.

In recent years, early use of FFP has been advocated for severely injured trauma patients to correct coagulopathy and improve clinical outcomes.[12,34–36] Since coagulation factors can be diluted as a result of MT of RBCs, it is thought that early administration of FFP (which contains clotting factors and fibrinogen) can reduce the risk of coagulopathy.[37] Thus, the use of FFP as a primary resuscitation fluid in a 1:1 or 1:2 ratio with RBC has been recommended by some, while noting that no prospective studies have been conducted to address this issue.[34,35,37–39] It is possible that early use of a plasma-based resuscitation regimen in these severely injured patients would minimize dilution and improve outcomes.[8–19]

Subjects in this analysis were severely injured trauma patients who were to receive either rFVIIa or placebo after infusion of the eighth unit of RBC. As shown in Figure 3, the majority of subjects did not have a high ratio of FFP to RBC and were in the category 1:4, suggesting that the primary resuscitation strategies used in this study may have been inadequate by current standards. Seventy-eight subjects (46%) did not receive any plasma, despite receiving eight or more units of RBCs. Although similar numbers of rFVIIa- and placebo-treated subjects were administered little to no FFP before dosing, fewer subjects had a prolonged PT 1-hour after dosing in the rFVIIa treatment group. It remains to be seen if primary resuscitation with a 1:1 ratio of FFP to RBC and/or treatment with rFVIIa can further reduce coagulopathy and provide additional improvements in clinical outcomes.

Study Limitations

Key limitations of our study are the retrospective nature and the relatively large number of patients with missing postdosing PT values. The large number of missing PT values reflects the difficulty in obtaining samples in the midst of critical trauma resuscitation. This was a multicenter study, and even though guidelines were provided, it was limited by differences in the management of coagulopathy among the centers. Furthermore, the levels of FII, FV, FX, protein C, and thrombomodulin were not determined; hence, the potential impact deficiencies of these factors may have on outcome that could not be assessed. It should also be noted that our retrospective analyses simply describe an association between 1-hour postdose PT and clinical outcomes and should not be interpreted as a causal link between the two variables. In addition, these data are not adequate to conclude that rFVIIa can improve clinical outcomes (more than placebo) as a result of its effect on PT. A larger placebo-controlled study of rFVIIa with mortality or another clinical outcome as the primary endpoint should be conducted to more clearly define the relationship between rFVIIa, PT, and clinical outcomes. It is also important to note that the age of the RBC products used was not recorded; and therefore, could not be incorporated into the analysis. There may be

a relationship between the age of the RBC transfusion and observed patient outcomes; therefore, future experimentation should include these criteria in the study protocol.

CONCLUSION

In this post hoc analysis of data from two randomized clinical trials evaluating rFVIIa or placebo treatment in severely bleeding trauma patients, marked and persistent abnormalities of several coagulation parameters were observed. A prolonged 1-hour PT with rFVIIa was associated with a significantly higher rate of MT and higher 24-hour and 30-day mortality rates, and a significantly fewer ICU-free days. The low hemoglobin, fibrinogen, and platelet levels at baseline (before rFVIIa treatment) observed in patients with persistently prolonged postdosing PT indicate that this group may require resuscitation with additional blood component therapy. Although the PT is likely not the "best" evaluation of coagulation, it is currently widely and rapidly available as a point-of-care test. The results of this analysis support the utility of PT as a laboratory measurement that allows clinicians to rapidly identify subjects who may benefit from infusion of additional blood products to prevent trauma-related mortality and morbidity.

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