Research

Pharmacokinetics of recombinant activated factor VII in trauma patients with severe bleeding

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Abstract

Introduction Recombinant activated factor VII (rFVIIa) has been used as adjunctive therapy in trauma patients with severe bleeding. However, its pharmacokinetics profile remains unknown.

Methods In two placebo-controlled studies in patients with blunt and penetrating trauma, the pharmacokinetics of rFVIIa given at an initial dose of 200 µg.kg⁻¹ after transfusion of eight red blood cell units, followed by additional doses of 100 µg.kg⁻¹, one and three hours later, have been studied, based on the FVII coagulant activity assay. Both non-compartment and population pharmacokinetic analyses were performed. A two-compartment, population pharmacokinetic model was used to estimate a population profile for the rFVIIa dosing regimen. Data are population means (percent coefficient of variation (CV)).

Results Based on the two-compartment population model, the estimated pharmacokinetic parameters were: clearance 40 (30% CV) ml.kg⁻¹.h⁻¹; central volume of distribution 89 (32% CV) ml.kg⁻¹; inter-compartmental clearance 24 ml.kg⁻¹.h⁻¹; and peripheral compartment volume 31 ml.kg⁻¹. Baseline FVII coagulant activity was estimated at 0.29 (39% CV) U.ml⁻¹, initial half-life was 0.6 (34% CV) hours, and terminal half-life 2.4 (50% CV) hours. High intra- and inter-patient variability was noted in volume of distribution and clearance, which was in part correlated with the transfusion requirements as the single significant covariate. The non-compartmental analysis led to almost identical estimates of key parameters.

Conclusion A high intra- and inter-patient variability was noted in the volume of distribution and clearance of rFVIIa in trauma patients with severe bleeding, mainly related with the transfusion requirements and thus blood loss and/or bleeding rate.

Introduction

Recombinant activated factor VII (rFVIIa, NovoSeven®/Nia-stase®, Novo Nordisk A/S, Bagsvaerd, Denmark) is an effective first-line hemostatic agent for the management of acute and surgical bleeds in patients with hemophilia A or B and inhibitors to factor VIII or factor IX [1]. The ability of rFVIIa to provide effective hemostasis in patients with a variety of clinical conditions associated with bleeding can be attributed to its capacity to increase thrombin generation on the surface of activated platelets accumulated at the site of injury to the ves-

AUC = area under the curve; Cmax = maximum concentration; CV = coefficient of variation; RBC = red blood cell; rFVIIa = recombinant factor VIIa; Tmax = time to maximum concentration.
that can reliably predict the clinical outcome of a patient with severe
bleeding. Moreover, our aim was also to identify significant and
clinically relevant covariates affecting pharmacokinetics in this
population. The present study is an ancillary pharmacokinetic
study of the large clinical trial that has been recently published
in a significant reduction in red blood cell (RBC) transfusion
requirements and in the occurrence of massive transfusion
in the trauma population it is difficult to measure the
amount of blood loss accurately. Accordingly, units of RBC
transfused was chosen as a surrogate endpoint for bleeding,
and units of RBC transfused during the 48 hour observation
period following the initial dose of trial product was chosen as
the primary endpoint to indicate the ability of rFVIIa to control
bleeding [10]. Among the patients with blunt trauma and sur-
viving the initial 48 hours after first dose, RBC transfusion was
significantly reduced with rFVIIa relative to placebo (estimated
reduction of 2.6 RBC units, \(P = 0.02\)), and the need for mas-
sive transfusion (\(\geq 20\) RBC), a post hoc analysis, was also sig-
nificantly reduced (14% versus 33% of patients, \(P = 0.03\)). In
penetrating trauma, similar analyses showed trends towards
rFVIIa reducing RBC transfusion (estimated reduction of 1.0
RBC units, \(P = 0.10\)), and massive transfusion (7% versus
19% of patients, \(P = 0.08\)) [10].

Clinical studies have not identified pharmacodynamic markers
that can reliably predict the \textit{in vivo} hemostatic effect of rFVIIa. Instead,
dose selection is guided by \textit{in vitro} studies with human biomaterials, clinical experience, among other from
studies in patients with hemophilia [3,11-14], and an under-
standing of the time course of the drug levels with dose, that
is, the pharmacokinetics.

The objectives of our study were to address the latter issue,
using two different analytical approaches to describe the phar-
amacokinetic profile of rFVIIa in trauma patients with severe
bleeding. Moreover, our aim was also to identify significant and
clinically relevant covariates affecting pharmacokinetics in this
population. The present study is an ancillary pharmacokinetic
study of the large clinical trial that has been recently published
[10].

Materials and methods
The study protocol was approved by the ethics committee of
each participating institution, and the trial was conducted
according to Good Clinical Practice standards and the Hel-
sinki Declaration. Written, informed consent was obtained
from all patients, or, where applicable, from a legally authorized
representative. Due to the emergency conditions and the pos-
sible absence of relatives at enrolment into the trial, waived
informed consent was authorized by the ethical committees.
However, whenever a patient was included without written
informed consent, such consent was promptly searched from
a legally authorized representative and subsequently from the
patient himself. When adequate confirmation of consent was
not obtained, data were excluded from analysis.

The methods of the study have been previously detailed [10].
Briefly, to be eligible for inclusion, patients were to have
received six units of RBC within a four-hour period, and to be
of known age \(= 16\) years (or legally of age according to local
law) and \(<85\) years. Key exclusion criteria comprised: cardiac
arrest pre-hospital or in the emergency or operating room prior
to trial drug administration; gunshot wound to the head; Glas-
gow coma scale \(<8\) unless in the presence of a normal head
CT scan; base deficit of \(>15\) mEq.\(1\) or severe acidosis with
\(pH <7.00\); transfusion of eight units or more of RBC prior to
arrival at trauma center; and injury sustained \(= 12\) hours before
randomization.

This was a randomized, placebo-controlled, double-blind trial
with two parallel treatment arms in two separate trauma pop-
ulations. Patients were evaluated for inclusion into the trial on
admission to the trauma center, and eligible patients were
assigned to either the blunt or penetrating trauma trial arm.
Upon receiving six units of RBC within a four-hour period, eli-
gible patients within each trauma population were equally ran-
domized to receive either three intravenous injections of rFVIIa
(200, 100 and 100 \(\mu\)g.kg\(^{-1}\)) or three placebo injections. The first
dose of trial product was to be administered immediately
after transfusion of the eighth unit of RBC, given that the
patient, in the opinion of the attending physician, would require
additional transfusions. The second and third doses followed
one and three hours after the first dose, respectively. This dose
regimen was chosen to target an average concentration of
FVIIa >40 U.ml\(^{-1}\), based on \textit{in vitro} studies and clinical studies
conducted in hemophilic patients. Trial product was adminis-
tered in addition to standard treatment for injuries and bleed-
ning at the participating hospitals, and no restrictions were
imposed on procedures deemed necessary by the attending
physician, including surgical interventions, resuscitation strat-
egies, and use of blood products. However, before patient
enrolment, each participating trauma center developed spe-
cific transfusion guidelines in line with the transfusion guide-
lines provided in the study protocol.

FVII coagulant activity assay
All pharmacokinetic assessments in this study were based on
the FVII coagulant activity assay, a one-stage clotting assay
using thromboplastin tissue factor, which forms complexes
with both FVIIa and FVII zymogen to quantify FVII clotting activ-
ity in plasma (Capio Diagnostik A/S, Copenhagen, Denmark)
[15]. The lower limit of quantification for the assay is 0.06 U.ml\(^{-1}\).
1. The assay involves use of diluted samples of plasma that
were then mixed with FVII-deficient plasma. Temperature was then adjusted to 37°C, and coagulation was initiated by the addition of thromboplastin tissue factor and calcium chloride. The time until fibrin formation was measured and related to the time observed for this reaction in normal plasma. Due to the temperature adjustment and the sample dilution in buffer and FVII-deficient plasma (which has a high buffer capacity), no effect of hypothermia or acidosis on assay results are expected. Since the FVII coagulant activity assay does not distinguish endogenous FVII/FVIIa from rFVIIa, baseline plasma activity (that is to say, before administration of rFVIIa) was taken into account in the pharmacokinetic analyses. The therapeutic doses of rFVIIa used in the study were expected to give peak plasma concentrations at least 30-fold greater than the normal endogenous FVII/FVIIa level in non-coagulopathic patients.

**Pharmacokinetic sampling**

Within the two study populations of blunt and penetrating trauma, subjects were allocated to two groups for pharmacokinetic analysis, one group to frequent blood sampling and another to sparse blood sampling for determination of FVII coagulant activity assay. The frequent sampling group comprised approximately 50 patients from whom plasma was sampled before first dose of trial product and 30 minutes and 1, 2, 3, 4, 6, 8 and 12 hours after the first dose. The remaining patients formed the sparse sampling group, from whom one sample was taken in each of at least two of the following four time intervals: 0 to 1 hour (after the first dose but before the second dose); 1 to 3 hours (after the second dose but before the third dose); 3 to 8 hours (after the third dose); and 8 to 12 hours.

**Pharmacokinetic analyses**

Data from patients with frequent sampling were analyzed non-compartmentally, whereas data from both patients with frequent and patients with sparse sampling were used for population pharmacokinetic analysis. The latter approach – population analysis – is suitable for this type of data. In contrast to the non-compartmental analysis – which is based upon separate analysis of each individual profile and requires that enough data be available for each individual to actually estimate the individual pharmacokinetics profiles – the population approach does not have this constraint. Instead, even very sparsely sampled individual profiles can be included in the dataset, in addition to the more richly sampled profiles. Eventually, an overall ‘population pharmacokinetic profile’ is estimated, as are estimates of how the parameters describing this profile vary between individuals. Using this approach, the effect of a number of covariates on individual parameters may also be analyzed [16,17].

As mentioned, the FVIIa coagulant assay does not distinguish endogenous FVII/FVIIa from rFVIIa. Although very small in comparison with the levels obtained during FVIIa treatment, a baseline FVIIa coagulant activity level was estimated for each individual and adjusted for in the analysis. In the non-compartmental analysis, the level was estimated based on pre-dose FVIIa coagulant activity levels and subtracted from the post-dose measurements; in the population analysis, the level was included as a subject specific random effect and estimated as part of the model estimation procedure. The mean baseline level was estimated at 0.30 and 0.29 U.ml⁻¹ for the placebo and the treatment group, respectively (non-compartmental estimates).

**Non-compartmental analysis**

The following parameters were estimated: maximum plasma FVII coagulant activity from time of first dose (time zero) to 12 hours after first dose (Cₘₚ); time to maximum plasma FVII coagulant activity (Tₘₚ); area under the plasma FVII coagulant activity-time profile from time of first dose (time zero) to 12 hours after first dose (AUC₀–₁₂ₙ); and volume of distribution and clearance. For calculation of AUC₀–₁₂ₙ, adjusted activities below zero were substituted by zero.

**Population analysis**

In this analysis, both a one- and a two-compartment population model were explored. Since the latter type was found to better describe the data, the final model was a two-compartment population model with first order elimination from the central compartment and baseline FVII coagulant activity assay to account for endogenous production of FVIIa. The influence of the covariates RBC transfusion, body weight, type of injury (blunt or penetrating), injury severity score, sex, age, and ethnicity on the model parameters were tested during development of the model. These covariates, based on the available data, were a priori identified as clinically relevant in collaboration with the medical team. Results were compared to population analysis results obtained in a previous study in hemophilia patients (Novo Nordisk data on file). In this analysis, pharmacokinetic data previously analyzed non-compartmentally [18] were analyzed with a two-compartment population pharmacokinetic model similar to the one presented here to allow for simulation of the population pharmacokinetic profile in hemophilia patients.

The final population pharmacokinetic model was used to simulate the predicted mean profile for the trauma population, at different levels of post-dose RBC transfusion requirements, as clearance was found to correlate with this covariate.

**Statistical analysis**

Data are expressed as geometric and population means (non-compartmental and population analysis, respectively), percent coefficient of variation (%CV = standard deviation × 100/mean) for pharmacokinetic parameters and arithmetic mean ± standard deviation for other variables. All statistical comparisons were based on analysis of variance methods, using two-tailed tests and a significance level of 0.05.
corresponding to the difference in number of parameters [19].

approximately Chi-square distributed, with degrees of freedom
statistical theory that the change in objective function value is

modeling was performed with NONMEM version 5.11
Corporation, Seattle, WA, USA); non-linear mixed effects

Version 8.2 and S-PLUS v. 6.0 for Trial Simulator (Insightful
Institute Inc., Cary, NC, USA). For the population pharmacoki-

analysis, data file preparation was performed with SAS

version 8.2 (SAS

Institute Inc., Cary, NC, USA). For the non-compartmental analysis, data file preparation and

compiling and clinical trial simulation was performed with the

5.1 (RDPP, Montpellier, France). Visual Fortran Version 6.1
(GoboMax, Hanover, MD, USA), run under Visual-NM Version
5.1 (Hewlet-Packard Company, Palo Alto, CA, USA) was used for

were calculated on the log-scale, as estimates ± 1.96 standard deviation.

For the non-compartmental analysis, data file preparation and statistical analysis was performed with SAS version 8.2 (SAS Institute Inc., Cary, NC, USA). For the population pharmacokinetic analysis, data file preparation was performed with SAS Version 8.2 and S-PLUS v. 6.0 for Trial Simulator (Insightful Corporation, Seattle, WA, USA); non-linear mixed effects modeling was performed with NONMEM version 5.11 (GoboMax, Hanover, MD, USA), run under Visual-NM Version 5.1 (Hewlet-Packard Company, Palo Alto, CA, USA). Visual Fortran Version 6.1 (Hewlet-Packard Company, Palo Alto, CA, USA) was used for compiling and clinical trial simulation was performed with the Pharsight Trial Simulator v. 2.1.2 (Pharsight Corporation, Mountain View, CA, USA). Software was installed according to vendor instructions. In addition, NONMEM functionality was verified with a test script executed before modeling.

NONMEM's first order conditional estimation method with interaction was used for model development. Evaluation and discrimination of intermediate models was based on standard statistical theory that the change in objective function value is approximately Chi-square distributed, with degrees of freedom corresponding to the difference in number of parameters [19]. Covariates were included in a forward inclusion approach, if reduction in objective function value was significant at a \( P \) value of 0.05. Subsequently, covariates were excluded by backwards elimination from the full model, if the associated increase in objective function value was not significant at a \( P \) value of 0.001. This method ensured inclusion of the relevant covariates, and the latter highly conservative significance level was employed to retain only the most essential covariates, leading to a more robust model.

Between and within patient variability was estimated as part of the model estimation procedure in NONMEM. The between-

patient variation of parameter estimates was based upon the assumption of individual parameter estimates being log-normal distributed around a population mean and is based on the inclusion of a random subject effect for the parameter. The number of parameters in the model must be balanced with the amount of data. Thus, to avoid over-parametrization of the final model, a random effect was not included for the inter-compartmental clearance and peripheral compartment volume. For these parameters, between patient variability is hence not reported. Within patient variation was estimated from the log normal distribution of individual observations around the individually predicted pharmacokinetic profiles. Both were expressed as %CV. Confidence intervals for the parameter estimates were calculated on the log-scale, as estimates ± 1.96 standard deviation.

Results

A total of 301 patients were randomized in the two studies, 158 in the blunt trauma arm and 143 in the penetrating trauma arm. Of these, 18 were withdrawn before administration of trial product, and waived informed consent was not confirmed for 6 patients. Of the remaining 277 patients, the pharmacokinetic profiles from a total of 47 patients were excluded from the dataset for the following reasons: no recording of actual sampling time \( n = 15 \); no FVII coagulant activity recordings \( n = 13 \); apparently artifactual pre-dose FVII coagulant activity levels or other aberrant data values \( n = 3 \); and lack of information on potentially relevant covariates in the population model \( n = 16 \). There were no significant differences between excluded and included patients in age, sex, Injury Severity Score, RBC requirement and survival (data not shown), suggesting that our analysis was not affected by patient exclusion bias. In the final pharmacokinetic dataset of 230 patients, 107 had been treated with placebo while the remaining 123 patients had been treated with rFVIIa. The population was characterized by the following characteristics: mean age was 32 ± 11 years; mean Injury Severity Score was 28 ± 13; there were 191 (83%) men and 39 (17%) women; and there were 110 (48%) patients with blunt trauma and 120 (52%) patients with penetrating trauma.

Before performing the actual pharmacokinetic analyses, the baseline level of FVIIa activity was explored, by plotting the activity versus time for the 107 subjects treated with placebo. The level appeared to be relatively constant in time, with random fluctuations around an average level of 0.3 U.ml-1 (range, 0.07 to 1.4 U.ml-1). Based on this, baseline levels were assumed individual specific, but constant in time.

Non-compartmental analysis

The frequent sampling group, for which the non-compartmental pharmacokinetic analysis was performed, comprised 43 patients, of which 18 patients were treated with placebo and 25 patients were treated with rFVIIa. Analysis was performed only on rFVIIa-treated patients with at least five plasma FVII
coagulant activity measurements yielding data from six blunt and 15 penetrating trauma patients (Figure 1). Results of the non-compartmental analysis are summarized in Table 1. No significant differences were noted in key pharmacokinetic parameters between patients with blunt and penetrating trauma. The uncertainty of the estimates of C\text{max}, T\text{max}, and volume of distribution was rather high, in particular for the group of patients with blunt trauma, which was not unexpected, since only a few patients contributed to these. Mean FVII coagulant activity (blunt and penetrating groups) are shown in Figure 2.

### Population analysis

In this analysis, data from 230 patients with frequent sampling and sparse sampling were used, of whom 123 were treated with rFVIIa. Diagnostics plots for the final population pharmacokinetic model indicated an acceptable fit of the model to the data, considering the amount of and variation in data (data not shown). The parameter estimates for the final population pharmacokinetics model are presented in Table 2. Population mean parameters for central compartment volume of distribution, clearance, and baseline are estimated with good precision and with estimates of the between-patient variation. Population mean parameters for inter-compartmental clearance and peripheral compartment volume are estimated with somewhat lower precision. For this latter set of parameters, the data did not allow for the estimation of inter-individual variation. Residual intra-individual variation was estimated at 32%.

For clearance, a significant part of the variation (P < 0.001) was attributable to differences in RBC transfusion requirements of the patients (Figure 2). Consequently, this clinically relevant correlation was included in the final model by the equation:

\[
CL = 40.45 \times 10^{(RBC-8.7)}
\]

where CL is the clearance, RBC is the RBC requirement after the first dose of rFVIIa and 8.7 is the average post-dose RBC requirement of the trauma population, indicating that the clearance increases with increasing RBC transfusion requirement.

### Application of the model

The final model was used to simulate the population pharmacokinetics profile (Figure 3). The observed data were found to be quite variable. Nevertheless, most patients, based on data from the trauma population and the estimated population pharmacokinetics profile, achieved FVII coagulant activity at least equal to or above the pharmacokinetics profile reached in hemophilia patient populations given a single dose of 90 µg.kg\(^{-1}\). Within the first four hours after the first dose, only a few patients (n = 10, 12%) had plasma concentrations below the hemophilia profile (Figure 3), and 30 (75%), 40 (63%), and 32 (54%) patients achieved rFVIIa plasma concentrations

### Table 1

| Pharmacokinetic parameters of factor VII coagulant activity assessed by non-compartmental analysis in blunt and penetrating trauma patients with frequent sampling |
|-------------------------------|-----------------|-----------------|
|                               | Blunt (n = 6)   | Penetrating (n = 15)   |
| C\text{max} (U.ml\(^{-1}\))  | 81 (47–241) [76%] | 58 (41–102) [27%] |
| T\text{max} (h)              | 0.9 (0.5–4.0) [110%] | 1.2 (0.5–4.0) [86%] |
| AUC\text{0–12h} (U.h.ml\(^{-1}\)) | 305 (216–362) [17%] | 311 [182–520] [26%] |
| Clearance (ml.kg\(^{-1}\).h\(^{-1}\)) | 41 (34–58) [21%] | 40 (25–79) [30%] |
| Volume of distribution (ml.kg\(^{-1}\)) | 83 (28–143) [45%] | 112 (66–165) [24%] |

Data are geometric means (min-max) and coefficient of variation [%]. There is no significant difference between the two groups. AUC\text{0–12h}, area under the plasma concentration-time profile from time of first dose (time zero) to 12 hours after first dose; C\text{max}, maximum plasma concentration; T\text{max}, time to maximum plasma concentration.
above 40 U.ml\(^{-1}\) after the first, second, and third boluses, respectively.

As mentioned above, RBC transfusion requirement was the single significant covariate in the model. To illustrate the impact of this covariate, estimates of population clearance and terminal half-life at various post-dose RBC requirements were calculated. Increased RBC requirements were associated with increased clearance, and consequently with shortening of the terminal elimination half-life (Table 3). For comparison, estimates of clearance and half-life for patients with hemophilia are also presented. To further explore the effect of the covariate, population profiles were simulated for various levels of post-dose RBC transfusion requirements (Figure 4). Consistent with the observed data, the results indicated that for the average trauma population with a post-dose requirement of 8.7 units of RBC (population average), peak plasma levels of rFVIIa activity equivalent to approximately 65 U.ml\(^{-1}\) (equal to approximately 43 nM) may be expected. Moreover, the average level appeared to remain above 40 U.ml\(^{-1}\) (approximately 26 nM) for most of the four hours after the initial dose. In comparison with this, for subjects with an estimated post-dose RBC transfusion requirement of 40 units, the predicted level of coagulant activity displayed a significantly faster decline and approached, but did not fall below, the profile for the hemophilia population (Figure 4).

### Table 2

| Variable                          | Estimate (95% CI) | Between patient CV |
|-----------------------------------|------------------|--------------------|
| Clearance (ml.kg\(^{-1}.h\(^{-1}\))  | 40 (37–44)       | 30%                |
| Central compartment volume (ml.kg\(^{-1}\)) | 89 (60–132)     | 32%                |
| Inter-compartmental clearance (ml.kg\(^{-1}.h\(^{-1}\)) | 24 (8–76)       | NA                 |
| Peripheral compartment volume (ml.kg\(^{-1}\)) | 31 (13–74)      | NA                 |
| Baseline concentration (U.ml\(^{-1}\)) | 0.29 (0.27–0.31) | 39%                |
| ∆Clearance/RBC                   | 1.014 (1.003–1.0025) | NA                |
| Initial half-life (h)            | 0.6 (0.2–1.3)    | NA                 |
| Terminal half-life (h)           | 2.4 (1.8–5.5)    | NA                 |

The within-patient variability (random error) was estimated to be 32%. ∆Clearance/RBC is the change in clearance per unit of red blood cells based on the model-specified potency function. CI, confidence interval; CV, coefficient of variation; NA, not applicable.

Figure 3

Population factor VII (FVII) coagulant activity profile modeled from the study dosing regimen. Dots represent the observed FVII coagulant activities from both frequent and sparse sampling while the solid line is trauma average population profile for multiple dosing. This model shows dosing in an adult hemophilia population superimposed for comparison (see Materials and methods).

Figure 4

Population pharmacokinetics profiles simulated at various red blood cell (RBC) transfusion requirements (20, 30, and 40 units after dosing) – increasing transfusion requirement linked with increasing clearance. The full line depicts the global trauma population (Trauma, mean of 8.7 RBC units) Data for a hemophilia population has been superimposed for comparison (see Materials and methods).
The variation in FVIIa coagulant activity in the trauma population was considerable. Part of this variation was attributable to differences in RBC transfusion requirement, resulting in significantly different predicted pharmacokinetic profiles depending on this covariate. As reflected in the data (Figure 3) and the population pharmacokinetic model parameters (Table 2), the remaining variation not accounted for was still considerable; however, with the dosing schedule used in this study, it appears that even trauma subjects with high distribution volumes and clearance (compared with population average) will achieve plasma levels of rFVIIa activity that do not fall below levels seen in the hemophilia populations managed with a single dose of 90 mg.kg⁻¹.

**Discussion**

In this large prospective study evaluating the pharmacokinetic properties of rFVIIa in trauma patients with severe bleeding, we mainly observed that: the mean clearance was 40 ml.kg⁻¹.h⁻¹ and the terminal half-life 2.4 hours; a high intra- and inter-patient variability was noted in the volume of distribution and clearance; and this high variability was significantly correlated with the transfusion requirements and thus blood loss. The pharmacokinetic analyses reported here complete our previous report on the clinical efficacy and safety data, which suggested that, in severe blunt trauma patients, a dosing schedule for rFVIIa of 200 mg.kg⁻¹ followed one and three hours later by additional doses of 100 mg.kg⁻¹ in patients with severe bleeding is an effective hemostatic therapy [10].

Although definite conclusions could not be drawn from the non-compartmental pharmacokinetic analysis due to the small number of patients analyzed, profiles derived using this method of analysis were valuable in giving an essentially model-free interpretation to the population data. The observed variability in the results of $C_{\text{max}}$ was expected, due the variation in distribution volume and clearance. The mean clearance of approximately 40 ml.kg⁻¹.h⁻¹ noted in the non-compartmental analysis was almost identical to that seen in the population pharmacokinetics analysis. Pharmacokinetic population modeling and analysis was successful in describing the profile of rFVIIa pharmacokinetics in trauma patients, in terms of a two-compartment model. For a few parameters in the model (inter-compartmental clearance and peripheral compartment volume), inter-subjects variation was not estimated. Moreover, the precision of the corresponding population mean parameter estimates was relatively low, compared with the other parameters in the model. However, considering the amount and quality of the data available, this was not unexpected and was considered satisfactory.

The estimated population pharmacokinetics profile at a post-dose RBC requirement of 8.7 units (the population average) indicated that, after three doses of rFVIIa, the peak plasma FVII coagulant activity of the population pharmacokinetics profile was approximately 65 U.ml⁻¹ (43 nM). Furthermore, the plasma level appeared to remain above 40 U.ml⁻¹ (approximately 26 nM) for most of the time over the four hours after the initial dose (Figure 3). The pharmacokinetic modeling and analysis as described here highlight that the trauma population constitute a group of patients with very high intra- and inter-patient variation in terms of rFVIIa kinetics. This may well reflect more than just differences in RBC transfusion requirements and underline the difficulties in attempting to treat such a diverse group of patients using a one-regimen-for-all approach to treatment. The population pharmacokinetic analysis illustrates the variation between trauma patients in terms of the rFVIIa pharmacokinetics, a variation that, in turn, has an effect on which dose will be required for obtaining and maintaining an effective FVII coagulant activity. Our analyses suggest that the chosen dose regimen will yield adequate plasma FVII coagulant activity during the crucial treatment period even when volume of distribution and plasma clearance are elevated.

### Table 3

| Population  | RBC requirements (Units) | Estimated clearance (ml.kg⁻¹.h⁻¹) | Terminal half-life (h) |
|-------------|--------------------------|-----------------------------------|-----------------------|
| Hemophiliaa | NA                       | 29                                | 4.4                   |
| Trauma      | 0                        | 36                                | 2.6                   |
| 8.7b        | 40                       | 2.6                               |
| 20          | 47                       | 2.1                               |
| 30          | 54                       | 1.9                               |
| 40          | 62                       | 1.7                               |

- Obtained in patients with hemophilia (see Materials and methods).
- This value corresponds to the trauma population average.

RBC, red blood cell.
volume replacement. In theory, if a trauma patient had no post-dose RBC requirement, the estimated clearance of rFVIIa would be 36 ml.kg\(^{-1}\).h\(^{-1}\) and the terminal half-life would be 2.6 hours. These estimates are in good agreement with non-compartment and population analysis results reported in (non-bleeding) healthy volunteers [20,21]. However with an RBC requirement of 40 units, clearance almost doubles to 62 ml.kg\(^{-1}\).h\(^{-1}\) while the half life is shortened by nearly one hour to 1.7 hours. When population pharmacokinetic profiles were simulated at various post-dose RBC requirements, it was found that an increase in RBC requirement correlated with a more rapid decrease in the predicted FVII coagulant activity. Furthermore, predicted peak plasma activities after second and third doses of rFVIIa were reduced as RBC requirements increased, and overall exposure to rFVIIa – as assessed by area under the FVII coagulant activity-time profile – was also reduced. These results are not surprising since hemorrhage is well known to markedly modify pharmacokinetic parameters [22,23]. Although not based on baseline information – since the RBC is measured after the first dose of rFVIIa – this correlation reflects a clinically relevant interplay between the measured FVII coagulant activity and the RBC transfusion volume and may help clinicians in choosing an appropriate dose according to the clinically estimated blood loss and/or bleeding rate. Therefore, our pharmacokinetic model may help to target appropriate rFVIIa concentrations in future randomized trials in other clinical conditions, such as postoperative bleeding [24,25]. Based on simulations using various post-dose RBC transfusion requirements and the observed individual levels of FVIIa coagulant activity in the study, it can, however, be anticipated that with the dosing schedule employed in this study, even patients with high distribution volumes and/or high RBC transfusion requirements due to severe bleeding will achieve a FVIIa coagulant activity at least equal to that known to be clinically effective in hemophilia settings.

Clinical studies with rFVIIa have not identified pharmacodynamic markers that reliably predict the in vivo hemostatic effect of rFVIIa; thus, measures such as prothrombin time and activated partial thromboplastin time are poor indicators of bleeding control in the coagulopathic patient [8]. It is important, therefore, to establish, from existing clinical data derived from trauma cohorts, that the total dose and dosing schedules for rFVIIa as evaluated in controlled studies are effective in achieving a pharmacokinetic profile of plasma FVII coagulant activity that supports the observed clinical efficacy of this hemostatic agent in trauma patients. The analysis presented here reveals some important aspects of the pharmacokinetics in this population, in terms of how variable it is and which factors may help in explaining some of this variation. But it also demonstrates that the dosing regimen chosen leads to FVIIa coagulant activity levels known to be efficient in the hemophilia population, for most subjects in the population, even those with above-average volumes of distribution and plasma clearance. This result is important since considerable variation in the range of doses of rFVIIa (from 40 to 300 µg.kg\(^{-1}\)) has been noted in previously reported case series in trauma [7,8,26,27].

Some limitations in our study deserve consideration. First, only one dosing schedule for rFVIIa was studied and the question remains of whether the same level of efficacy could be achieved with lower total doses or a different regimen of rFVIIa dosing [10]. The pharmacokinetic analysis described here is obviously limited by this fact; but it does demonstrate that a satisfactorily high level of FVII coagulant activity is obtained with the chosen regimen, compared with the levels seen in the hemophilia population, when managed with 90 µg/kg. Therefore, based on the results of the pharmacokinetic analysis described in this study, and the safety and efficacy outcomes as described by Boffard and colleagues [10], a phase III multicenter randomized placebo-controlled clinical trial investigating rFVIIa in severely injured trauma patients with bleeding refractory to standard treatment is currently ongoing with exactly the same dosing regimen as described here (that is to say, 200 + 100 + 100 µg.kg\(^{-1}\) at hours 0, 1, and 3, respectively), in order to achieve appropriate levels of rFVIIa in the study population.

Second, there are no clear data concerning the optimal duration of adequate rFVIIa concentrations required in bleeding trauma patients. This concept is difficult to test in the context of a multi-center randomized placebo-controlled clinical trial, as the clinician decision that 'bleeding has stopped' is likely to be highly subjective. In the case series of 81 patients described by Dutton and colleagues [8], among the 46 subjects with acute hemorrhagic coagulopathy, doses of rFVIIa employed ranged from 48 to 148 µg.kg\(^{-1}\) and patients in the series received an average of 1.2 (range 1 to 3) doses of rFVIIa. However, it should also be emphasized that, in this series, 25% of patients did not adequately respond to rFVIIa administration [8]. Therefore, the need for re-injection in selected patients remains a matter of debate in trauma patients and may ultimately depend on whether bleeding control has been achieved in a given patient.

Third, the average concentration targeted in our study (>40 U.mL\(^{-1}\)) was based on in vitro studies and clinical studies conducted in hemophilic patients. Further evidence is required to confirm this average target concentration in severely bleeding patients. Nevertheless, it is notable that the only available randomized study that proved the efficacy of rFVIIa in trauma patients targeted this concentration [10].

Fourth, a larger amount of data would have enabled a more precise estimation of the model parameters and possibly also inclusion of intra-subject variance on all primary parameters. Lastly, pharmacokinetic models usually assume the volume and rate constants remain fixed for the duration of the experiments and, in our study, we modeled rFVIIa in a non-stationary system. We have tried to account for this by including RBC
transfusion requirement as a covariate. However, the variability inherent in this clinical setting probably still adds to the intra- and inter-patient variabilities [28].

Conclusion
In trauma patients with severe bleeding, the mean clearance of rFVIIa was 40 ml.kg⁻¹.h⁻¹ and its terminal half-life 2.4 hours. A high intra- and inter-patient variability was observed in the volume of distribution and clearance of rFVIIa, mainly related with the transfusion requirements. This pharmacokinetic analysis completes our previous report on the clinical efficacy and safety of rFVIIa in trauma patients with severe bleeding [10] and may help to determine the precise, appropriate dosing regimen in future trials and clinical practice. Our study suggests that dosing might be adapted to the clinically estimated blood loss and/or bleeding rate.

Key messages
- In trauma patients with severe bleeding, the mean clearance of recombinant factor VIIa is 40 ml.kg⁻¹.h⁻¹ and its terminal half-life 2.4 hours.
- A high intra- and inter-patient variability occurs in the volume of distribution and clearance of recombinant factor VIIa in trauma patients with severe bleeding.
- This high variability was mainly related to the transfusion requirements and thus the blood loss and/or bleeding rate.
- Our study suggests that dosing might be adapted to the clinically estimated blood loss and/or bleeding rate in future trials and clinical practice.

Competing interests
KB, SR, YK, and BR have received consultancy fees and lecture sponsorships from Novo Nordisk. RR has received lecture sponsorship from Novo Nordisk. TK and RTyP are employed by Novo Nordisk A/S.

Authors’ contributions
TK and RTyP designed and performed the pharmacokinetic analyses. TK, RTyP, and BR drafted the manuscript. All authors participated in the design and coordination of the study, and read and approved the final manuscript.

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References
1. Abshire T, Kenet G: Recombinant factor VIIa: review of efficacy, dosing regimens and safety in patients with congenital and acquired factor VIII or IX inhibitors. J Thromb Haemost 2004, 2:899-909.
2. Hoffman M, Monroe DM: A cell-based model of haemostasis. Thromb Haemost 2001, 85:958-965.
3. Monroe DM, Hoffman M, Oliver JA, Roberts HR: Platelet activity of high dose factor VIIa is independent of tissue factor. Br J Haematol 1997, 99:542-547.
4. Carr ME, Martin EJ: Recombinant factor VIIa: clinical applications for an intravenous haemostatic agent with broad-spectrum potential. Expert Rev Cardiovasc Ther 2004, 2:861-874.
5. Roberts HR, Monroe DM, Escobar MA: Current concepts of hemostasis: implications for therapy. Anesthesiology 2004, 100:885-893.
6. Malherbe S, Taus BC, Stobart K, Koller J: Argatroban as anticoagulant in cardiopulmonary bypass in an infant and attempted reversal with recombinant activated factor VII. Anesthesiology 2004, 101:1048-1049.
7. Martinowitz U, Kenet G, Segal E, Luboshitz J, Lubetsky A, Ingerslev J, Lynn M: Recombinant activated factor VII for adjunctive hemorrhage control in trauma. J Trauma 2001, 51:431-439.
8. Dutton RP, McCunn M, Hyder M, D’Angelo M, O’Connor JO, Hess JR, Scalea TM: Factor VIIa for correction of traumatic coagulopathy. J Trauma 2004, 57:709-718.
9. Pottage JP, Jonas S, Oussoultzoglou E, Malago M, Jay C, Cherqui D, Anthuber M, Mirza DF, Juulman L, Bechstein WO, et al.: Recombinant coagulation factor VIIa in major liver resection: a randomized, placebo-controlled, double blind clinical trial. Anesthesiology 2005, 102:269-275.
10. Bohr KD, Riou B, Warren B, Iau P, Rizoli S, Kluger Y, Rossaint R, Axelsen M, The NovoSeven® Trauma Study Group: Recombinant Factor VIIa as adjunctive therapy for bleeding control in severely injured trauma patients. A randomized, placebo-controlled, double-blind clinical trial. J Trauma 2005, 59:8-18.
11. Allen GA, Monroe III, Roberts HR, Hoffman M: The effect of factor X level on thrombin generation and the procoagulant effect of activated factor VII in a cell-based model of coagulation. Blood Coagul Fibrinolysis 2000, 11(Suppl 1):S3-S7.
12. Santagostino E, Morfini M, Rocino A, Baudo F, Scaraggi FA, Gringeri A: Relationship between factor VII activity and clinical efficacy of recombinant factor VIIa given by continuous infusion in patients with factor VIII inhibitors. Thromb Haemost 2001, 86:954-958.
13. Villar A, Aronis S, Morfini M, Santagostino E, Auerswald G, Thomsen HF, Erhardtse E, Giangrande PC: Pharmacokinetics of activated recombinant coagulation factor VII (NovoSeven) in children vs adults with haemophilia A. Haemophilia 2004, 10:352-359.
14. Erhardtse E: Pharmacokinetics of recombinant activated factor VII (rFVIIa). Semin Thromb Hemost 2000, 26:385-391.
15. Morrissey JH, Macik BG, Neunenschwerder PF, Comp PC: Quantification of activated FVII levels in plasma using a tissue factor mutant selectively deficient in promoting factor VII activation. Blood 1993, 81:734-744.
16. Aarons L: Population pharmacokinetics: Theory and practice. Br J Clin Pharmacol 1991, 32:669-670.
17. Ette EI, Williams PJ: Population pharmacokinetics. I: Background, concepts, and models. Ann Pharmacother 2004, 38:1702-1706.
18. Lindley CM, Sawyer WT, Macik BG, Lusher J, Harrison JF, Baird-Cox K, Birch K, Glazer S, Roberts HR: Pharmacokinetics and pharmacodynamics of recombinant factor VIIa. Clin Pharmacol Ther 1994, 55:638-648.
19. The NONMEM Project Group: NONMEM Users Guide, November 1994, Part V: Introductory Guide San Francisco; University of California.
20. Fridberg MJ, Hedner U, Roberts HR, Erhardtse E: A study of the pharmacokinetics and safety of recombinant activated factor VII in healthy Caucasian and Japanese subjects. Blood Coagul Fibrinolysis 2005, 16:259-266.
21. Girard P, Nony P, Erhardtse E, Delair F, French P, Dechavanne M, Boissel JP: Population pharmacokinetics of recombinant factor VIIa in volunteers anticoagulated with acenocoumarol. Thromb Haemost 1998, 80:109-113.
22. Kurita T, Kazama T, Morita K, Fujiy S, Uraoka M, Takata K, Sato S: Influence of fluid infusion associated with high-volume blood loss on plasma procoisol concentrations. Anesthesiology 2004, 100:871-878.
23. Johnson KB, Egan TD, Kern SE, McJames SW, Cluff ML, Pace NL: Influence of hemorrhagic shock followed by crystalloid resus-
citation on propofol: pharmacokinetic and pharmacodynamic analysis. *Anesthesiology* 2004, 101:647-659.

24. Karkouti K, Beattie WS, Wijeysundera DN, Yau TM, Mccluskey SA, Ghannam M, Sutton D, van Rensburg A, Karski J: Recombinant factor VIIa for intractable blood loss after cardiac surgery: a propensity score-matched case-control analysis. *Transfusion* 2005, 45:26-34.

25. Weiskopf RB: Intraoperative use of recombinant activated coagulation factor VII. *Anesthesiology* 2002, 96:1287-1289.

26. Martinowitz U, Kenet G, Lubetski A, Luboshitz J, Segal E: Possible role of recombinant activated factor VII (rFVIIa) in the control of hemorrhage associated with massive trauma. *Can J Anaesth* 2002, 49(Suppl):S15-S20.

27. Barletta JF, Ahrens CL, Tyburski JG, Wilson RF: A review of recombinant factor VII for refractory bleeding in non-hemophilic trauma patients. *J Trauma* 2005, 58:646-651.

28. Kurita T, Kazama T, Morita K, Fuji S, Uraoka M, Takata K, Sato S: Influence of fluid infusion associated with high-volume blood loss on plasma propofol concentrations. *Anesthesiology* 2004, 100:871-878.