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ORIGINAL ARTICLE

Combining factor VIII levels and thrombin/plasmin generation: A population pharmacokinetic-pharmacodynamic model for patients with haemophilia A

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Aims: Prophylactic treatment of haemophilia A patients with factor VIII (FVIII) concentrate focuses on maintaining a minimal trough FVIII activity level to prevent bleeding. However, due to differences in bleeding tendency, the pharmacokinetic (PK)-guided dosing approach may be suboptimal. An alternative approach could be the addition of haemostatic pharmacodynamic (PD) parameters, reflecting a patient's unique haemostatic balance. Our aim was to develop a population PK/PD model, based on FVIII activity levels and Nijmegen Haemostasis Assay (NHA) patterns, a global haemostatic assay that measures thrombin/plasmin generation simultaneously.

Methods: PK/PD measurements were collected from 30 patients treated with standard half-life FVIII concentrate. The relationship between FVIII activity levels and the thrombin/plasmin generation parameters (thrombin potential, thrombin peak height and plasmin peak height), were described by sigmoidal E_{max} functions.

Results: The obtained EC_{50} value was smallest for the normalized thrombin potential (11.6 IU/dL), followed by normalized thrombin peak height (56.6 IU/dL) and normalized plasmin peak height (593 IU/dL), demonstrating that normalized thrombin potential showed 50% of the maximal effect at lower FVIII activity levels. Substantial inter-individual variability in the PD parameters, such as EC_{50} of thrombin potential (86.9%) was observed, indicating that, despite similar FVIII activity levels, haemostatic capacity varies significantly between patients.

Conclusion: These data suggest that dosing based on patients' individual PK/PD parameters may be beneficial over dosing solely on individual PK parameters. This model could be used as proof-of-principle to examine the application of PK/PD-guided dosing. However, the relation between the PD parameters and bleeding has to be better defined.

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The authors confirm that the Principal Investigators for this article are Britta A.P. Laros-van Gorkom and Saskia E.M. Schols, who both had direct clinical responsibility for patients.

KEYWORDS
factor VIII, haemophilia A, pharmacodynamics, pharmacokinetics, plasmin generation, thrombin generation, treatment individualization

1 | INTRODUCTION

Patients with haemophilia A suffer from recurrent spontaneous and trauma-related bleeding due to **coagulation factor VIII** (FVIII) deficiency.[1] During recent decades, treatment has altered from on-demand treatment to prophylactic therapy for patients with severe haemophilia.[2] However, a large inter-individual variability in the pharmacokinetics (PK) of FVIII is observed when dosing is based on bodyweight.[3,4] To overcome this variability, it is recommended to perform PK-guided dosing to optimize dosing regimens.[5–10] Generally, dosing regimens are calculated to maintain coagulation factor activity levels above a certain trough level (traditionally >1 IU/dL).[11,12] Interestingly, however, some patients do not experience bleeding at all with observed trough levels <1 IU/dL, while others require higher targets depending on bleeding tendency, level of physical activity and joint status.[13,14]

An alternative approach could be to use population PK/pharmacodynamic (PD) models to determine personalized prophylactic dosing schemes for haemophilia A patients, by the addition of PD parameters to the standard population PK models. PD can be defined by a variety of physiological effects following drug administration. Objective bleeding events are regarded as the optimal clinical outcome parameter for haemophilia treatment. But they require long-term follow-up. Therefore, alternative parameters, such as the haemostatic potential illustrated by a global haemostatic test, may be an alternative. Global haemostatic assays such as the **thrombin** generation assay (TGA) are able to measure the individual's haemostatic potential, as they strive to measure the combined effect of all coagulation factors, instead of only FVIII activity levels.[15] Therefore, global assays may be able to identify individuals with a higher bleeding tendency. For example, several studies show that thrombin generation can distinguish different bleeding phenotypes in severe haemophilia A patients.[16,17] Furthermore, a study by Lewis et al. demonstrated significant inter-individual variability in the correlation between FVIII levels and thrombin generation. The relation between FVIII levels and thrombin generation within a patient was predictable, as the intra-individual variability was small.[18] Therefore, there is clinical potential to use TGAs to monitor FVIII replacement therapy and predict bleeding risk. A previous study by Delavenne et al. already developed a population PK/PD model for a single human-cl rhFVIII concentrate (Nuwiq®), relating FVIII activity levels to endogenous thrombin potential and bleeding risk.[19] However, this model was developed with data from one FVIII concentrate and only looked at thrombin generation.

The Nijmegen Haemostasis Assay (NHA) is a global assay that measures thrombin generation and plasmin generation simultaneously in a single assay using a fluorimeter.[20] We have previously described the baseline thrombin and plasmin generation measured by NHA and the results after a single bolus of FVIII replacement therapy in a cohort of 25 severe, moderate and mild haemophilia A patients.[21] In that study we concluded that thrombin peak height, thrombin potential and, if available, plasmin peak height best represent the haemostatic balance in patients with haemophilia A.

In this study, our aim was to develop a population PK/PD model for a range of standard half-life (SHL) FVIII concentrates, relating FVIII dosage to FVIII activity level and haemostatic outcome as defined by NHA parameters in patients with haemophilia A. This population PK/PD model may lead to a better understanding of the dose–concentration–effect relation of FVIII replacement therapy and may aid in improving prophylactic dosing based on a patient's FVIII activity level and thrombin/plasmin generation.

What is already known about this subject

- Due to differences in bleeding tendency, pharmacokinetic (PK)-guided dosing for haemophilia A patients may be suboptimal.
- Thrombin generation assays (TGA) are able to measure the individual's haemostatic potential, as they strive to measure the combined effect of many coagulation factors.
- An alternative approach to the current PK-guided dosing could be the addition of haemostatic pharmacodynamic (PD) parameters, reflecting a patient's unique haemostatic balance.

What this study adds

- A population pharmacokinetic/pharmacodynamic model was developed, relating FVIII activity levels to thrombin generation as defined by Nijmegen Haemostasis Assay parameters.
- Substantial inter-individual variability in thrombin generation was observed, confirming that patients with similar FVIII activity levels present with varying overall haemostatic capacity.
- This model could be used to explore the application of PK/PD-FVIII-guided dosing.

2 | METHODS

2.1 | Patients

Patients with severe (FVIII activity level <1 IU/dL), moderate (FVIII activity level 1–5 IU/dL) and mild haemophilia A (FVIII activity level >5–40 IU/dL) were eligible to enrol in this single centre study (Radboud University Medical Center, Nijmegen, The Netherlands). The patients were enrolled if a PK profile was required, either because of switching of FVIII concentrate or suspicion of an FVIII alloantibody inhibitor. Exclusion criteria of the study can be found in a previous publication of the data.[21] The Medical Ethical Committee of the Radboud University Medical Center approved the study and all participating patients, or parents in case of underaged children (<12 year), gave written informed consent. The study was conducted in accordance with the Declaration of Helsinki and is registered in the Dutch Trial Registry (#NL2808).

The concentrate and dosage of SHL FVIII concentrates (preferably 25–50 IU/kg) were determined by the treating physician. Blood samples were collected by venepuncture in 3.2% buffered sodium citrate siliconized blood collecting tubes (Becton Dickenson, Plymouth, UK) at baseline (before FVIII administration), and at 3, 5, 15, 30 minutes, 1, 3, 6, 9 and 24 hours after the bolus. Sampling schemes were similar for adults and children. At baseline haemoglobin level, haematocrit, von Willebrand factor ristocetin cofactor activity level and inhibitor titres were determined. Inhibitor titre was analysed with the Nijmegen Bethesda Assay (NBA)[22] and Nijmegen Low Titre Inhibitor Assay (NLTIA),[23] both as described before.

2.2 | FVIII activity level and NHA measurement

FVIII activity was measured in fresh samples immediately after collection with the FVIII one-stage clotting assay (OSA) (Cephascreen reagents and STA Evolution, both Stago Group, Asnières sur Seine, France) according to manufacturer's instructions and the FVIII chromogenic assay (CSA) according to manufacturer's instruction (Biophen FVIII:C assay, HYPHEN Biomed SAS, Neuville-sur-Oise, France), at the STA Evolution (Stago Group).

For NHA measurements, platelet poor plasma (PPP) was obtained, which was directly stored at −80 °C until analysis. It was defrosted only once to measure thrombin and plasmin generation with the NHA, as described previously,[20] and described in detail in the Supplementary Methods section. The essential parameters that were obtained are shown in Figure 1. The legend of Figure 1 includes a detailed description of the parameters. For all results, the mean of two NHA measurements were used. With each NHA test, normal control measurements (normal pooled plasma [NPP], see Supplementary Methods section) are determined as quality control and to be able to normalize the NHA parameter values to a percentage of normal. This normalization was done by dividing the absolute parameter of the NHA by the mean of the NPP samples that were used along the specific patient's assay run. The normalized parameters can be compared more easily with other TGAs than the absolute NHA parameter values.[24] The coefficient of variation of the NHA was determined to be 6–25%, depending on the studied parameter.[20] The thrombin potential had the lowest inter-assay variation (5.9%), and thrombin peak height had an inter-assay variation of 19%.

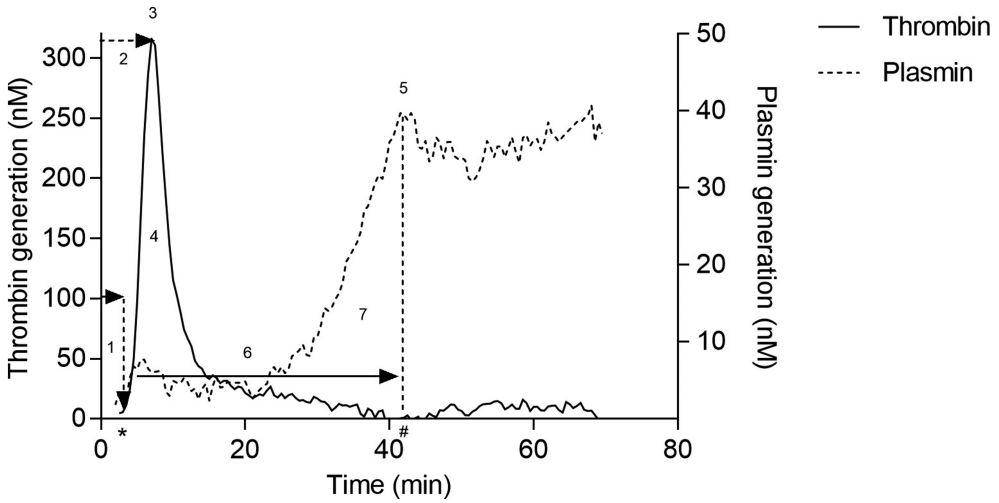

FIGURE 1 Standard curve and characteristic parameters of the Nijmegen Haemostasis Assay (NHA). The following parameters are obtained with the NHA: (1) lag time (in minutes): the time until the thrombin generation signal increases by two standard deviations from baseline, which represents initial thrombin formation via the extrinsic route; (2) time to thrombin peak (in minutes): the time after initiation when thrombin production reaches maximal velocity, it represents thrombin formation during the propagation phase; (3) thrombin peak height (in nM): the maximal velocity of thrombin production; (4) area under the curve (AUC, also called thrombin potential, in nM·min): the total amount of thrombin generated; (5) plasmin peak height (in nM): the maximal concentration of plasmin generation, at the moment where the curve shifts from a convex rate to a linear one, representing the point of lysis of the clot by plasmin; (6) fibrin lysis time (FLT, in minutes): the time between the plasmin peak time (marked with #) and the surrogate peak time (marked with *); and (7) plasmin potential (in nM·min): the surface under the plasmin curve during the FLT, this represents the total amount of plasmin generated

2.3 | Model development

The population PK/PD model developed for this study was built using nonlinear mixed effect modelling, using NONMEM (version 7.4.2, ICON Development Solution, Gaithersburg, MD, USA) and was developed sequentially. Since all included patients used a SHL FVIII concentrate as specified in Table 1, the performance of published population PK models for SHL FVIII was evaluated first.[4,25] When these population PK models did not perform adequately, indicated by goodness-of-fits (GOF) plots and/or a visual predictive check, a new population PK model was developed based on the collected study population. During development of this new population PK model, a structural model was obtained by evaluating the number of compartments, the residual error, the introduction of inter-individual variability (IIV) and inter-occasion variability (IOV). PK parameters were a priori scaled to bodyweight (allometric scaling), as both children and adults were represented in the data. To identify patient or treatment characteristics that explain part of the inter-individual variability in the PK parameters, a covariate analysis with forward inclusion and backward elimination was performed. During forward inclusion an objective function value (OFV) drop of >3.84 ($P < .05$, Chi-square distribution with 1 degree of freedom) was considered as a significant improvement of the model, while during backwards elimination an OFV increase of >6.64 ($P < .01$, Chi-square distribution with 1 degree of freedom) was considered as a deterioration of the model. The following covariates were examined: age, haemophilia severity (severe, moderate or mild), von Willebrand factor ristocetin cofactor activity level (VWF:Act), haematocrit, haemoglobin, presence of an inhibitor titre defined by the NBA or NLTIA, FVIII DNA genotype and type of FVIII concentrate (plasma, full-length recombinant or B-domain deleted recombinant) that was administered.

From the population PK model, the individual empirical Bayes estimates for the PK parameters were obtained. These individual PK parameters were used as input for the population PD model. Linear, maximal effect (E_{max}) and sigmoid E_{max} relationships between FVIII activity level (PK) and haemostatic effect defined by normalized thrombin peak height, normalized thrombin potential and normalized plasmin peak height (PD) were evaluated (see Supplementary Methods section for further explanations). To evaluate which patient and treatment characteristics explain the difference in PD parameters, a covariate analysis on the final PD model was performed. The approach was similar as for the PK part of the model.

Goodness-of-fits plots, parameter estimations and the objective function were used to evaluate the developed models. Thereafter, the models were internally validated by a visual predictive check. Additionally, a bootstrap analysis was performed to evaluate the robustness of the newly developed PK/PD model. Additional information on the modelling process can be found in the Supplementary files.

2.4 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, and are permanently archived in the Concise Guide to PHARMACOLOGY 2021/22.[26]

3 | RESULTS

3.1 | Data

Data from 30 patients with haemophilia A, aged between 2 and 77 years, collected between 1 February 2003 and 1 July 2013, were used for the development of the population PK/PD model. The dataset included five children <12 years. No patients between 12 and 18 years of age were included. Characteristics of the included patients are presented in Table 1. From the 30 included patients, 466 FVIII activity levels measured by OSA and 386 FVIII activity levels measured by CSA were available for the PK part of the model. In some of the patients, a washout period of 72 hours was applied. Therefore, 27 out of 44 pre-administration FVIII activity levels measured with OSA and 13 out of 38 pre-administration FVIII activity levels measured with CSA were below the quantification limit (BQL) of <1 IU/dL. In the full dataset 5.8% (OSA) and 3.4% (CSA) of the samples were BQL; these samples were excluded. When a washout period was not applied, the last three prophylactic FVIII concentration administrations were included in the dataset for correction of the residual FVIII activity levels of these FVIII concentrate doses. In addition, the lowest FVIII activity level ever measured was regarded as the endogenous baseline and subtracted from the observed FVIII concentration in the modelling process. For 24 out of the 30 patients, a PD profile, consting of NHA measurements expressing thrombin and plasmin generation, was determined. For both thrombin peak height and plasmin peak, a total of 252 measurements was available. Thrombin potential could sometimes not be determined when FVIII activity levels were very low, as fluorescence in the assay does not exceed background fluorescence. Therefore, this parameter was missing in eight cases (3.2%) and these were excluded from analysis. The median and ranges of pre- and post-infusion values of the FVIII activity levels and NHA parameters are given in Supplementary Table S1.

3.2 | Pharmacokinetic model

Firstly, the predictive performance of the previously published SHL FVIII population PK models was tested.[4,25,27] When these models were applied to the newly collected dataset using Bayesian estimation, GOF plots showed large deviations for some observations (conditional weighted residuals [CWRES] > 5) and a trend was visible in the population prediction versus observed FVIII activity level (data not shown). The observations causing these deviations were from patients in which an FVIII inhibitor titre was measured. Therefore, a novel population PK model was developed to describe our population more adequately. The final model was a two-compartment model with linear elimination and inter-individual variability on clearance and central volume of distribution. The FVIII activity levels measured with OSA

TABLE 1 Demographic characteristics

	PK study	PD study
Number of patients	30	24
Number of determined profiles[a]	44	25
Number of profiles per patient, median (range)[a]	1 (1–4)	1 (1–2)
Total number of samples	466[c]	252[d]
Number of samples per patient	10 (8–45)[c]	10 (8–25)[d]
Haemophilia severity, number of patients (%)		
Severe	20 (67)	15 (63)
Moderate	4 (13)	3 (13)
Mild	6 (20)	6 (25)
Age, median (range)	40 (2–77)	51 (2–77)
Weight, median (range)	75 (11–134)	85 (11–134)
VWF activity in %, median (range)[b]	100 (28–216)	97 (55–142)
Haemoglobin in mmol/l, median (range)[b]	8.7 (6.0–10.4)	9.1 (6.0 – 10.4)
FVIII DNA variant, number of patients (%)[b]		
Inversion intron 1	2 (7)	1 (4)
Inversion intron 22	8 (27)	6 (25)
Exon 10 mutation	2 (7)	1 (4)
Exon 11 mutation	2 (7)	2 (8)
Exon 12 mutation	1 (3)	1 (4)
Exon 14 mutation	7 (23)	6 (25)
Exon 23 mutation	2 (7)	2 (8)
Exon 25 mutation	3 (10)	3 (13)
Unknown mutation	3 (10)	2 (8)
Inhibitor titre		
Positive NBA, number of patients (%)	4 (13)	2 (8)
NBA titre in NBU/mL, median (range) [b]	0.3 (0.3–0.3)	0.3 (0.3–0.3)
Positive NLTIA, number of patients (%)	7 (23)	4 (16)
NLTIA titre in NLTIU/mL, median (range)[b]	0.05 (0.01–0.15)	0.09 (0.04–0.15)
Treatment		
Dosage in IU/kg, median (range)	29.0 (10.9–90.9)	32.1 (12.8–90.9)
Product, number of profiles (%)[a]		
Aafact (plasma)	8 (18)	5 (20)
Advate (recombinant full-length)	17 (39)	10 (40)
Haemate P/Humate P (plasma)	3 (7)	1 (4)
Helixate (recombinant full-length)	8 (18)	5 (20)
Kogenate (recombinant full-length)	5 (11)	3 (12)
Novo-eight (recombinant BDD)	2 (5)	-
Refacto (recombinant BDD)	1 (2)	1 (4)

Note: Data from 30 patients in total was collected. For 24 out of these 30 patients, PD data was collected. A PK/PD profile is defined as the samples that are taken after one FVIII administration (mostly 10 samples). For time-varying covariates, baseline characteristics of every pharmacokinetic (PK)/pharmacodynamic (PD) profile, even if performed in the same patient, are included in this table.

Abbreviation: BDD, B-domain deleted/truncated.

[a]For several patients, multiple PK/PD profiles were determined, as a new profile was required in case of FVIII concentrate switching or suspicion of a FVIII alloantibody inhibitor.

[b]Data was missing on VWF activity level: 14 PK and 3 PD profiles; haemoglobin: 15 PK and 3 PD profiles; Nijmegen Bethesda Assay (NBA): 4 PK and 2 PD profiles; and Nijmegen Low Titre Inhibitor Assay (NLTIA): 9 PK and 3 PD profiles.

[c]Measured by one-stage assay.

[d]per NHA parameter.

and CSA were both included and a correction factor was applied to correct for the difference in assay method. In Supplementary Figure S1, the correlation between the two assay methods is displayed, which shows that the deviation between the OSA and CSA method is not similar for all observed FVIII activity levels, as is also described in the literature.[28,29] Therefore, an inter-individual variability term was additionally introduced on the correction factor, which enables patients to have a different correction factor.

Subsequently, a covariate analysis was performed. A relationship between the FVIII concentrate type, clearance and volume of distribution was found, linking full-length recombinant FVIII concentrates to a 27% higher clearance and a 17% higher central volume of distribution as compared to plasma-derived and B-domain deleted recombinant concentrates. Higher VWF:Act levels were associated with a lower FVIII clearance, as expected.[30] Finally, a positive FVIII inhibitor titre was related to a 49% higher clearance and a 14% higher central volume of distribution. The NLTIA proved to describe this relation more significantly than the NBA, indicating that low levels of inhibitors may still affect PK parameters. A visual representation of the effects of the covariates on the pharmacokinetic parameters is presented in Supplementary Figure S2. The final PK parameters estimates, including the results of the bootstrap analysis can be found in Table 2. The GOF plots of the final pharmacokinetic model, presented in Supplementary Figure S3, show that the newly developed model describes the FVIII activity levels adequately.

3.3 | Pharmacodynamic model

The individual PK parameters obtained from the PK model were used for estimation of the PD part of the model. Separate models for normalized thrombin peak height, normalized thrombin potential and normalized plasmin peak height were developed. The relationship between FVIII activity level and the PD parameters are presented in Figure 2. For all three evaluated parameters, the FVIII level–effect relation was best described by a nonlinear (sigmoid) E_{max} function, following Equation 1:

$$E_{drug} = \frac{E_{max} * C^n}{(EC_{50}^n + C^n)} \quad (1)$$

where E_{max} is the maximal effect, C is the FVIII activity level (including endogenous baseline), EC_{50} is the FVIII activity level that is associated with 50% of the maximal effect and n is the hill factor that controls the steepness of the curve. Since the normalized thrombin peak height and normalized thrombin potential increased after FVIII administration, a positive E_{max} function was used to describe the association between FVIII activity level and these effect parameters. An inhibitory E_{max} (I_{max}) function was used to describe the relation between FVIII activity level and normalized plasmin peak height, as the normalized plasmin peak decreased slightly after FVIII administration.

The obtained FVIII activity level that is associated with 50% of the maximal effect (EC_{50}) was smallest for the normalized thrombin potential (11.6 IU/dL), followed by normalized peak height (56.6 IU/dL) and then normalized plasmin peak height (593 IU/dL) (Table 2). This demonstrates that the normalized thrombin potential shows 50% of the maximal effect at lower FVIII activity levels than the other parameters. Therefore, lower FVIII levels are required to increase the thrombin potential, which is also visualized in Figure 2. This figure also depicts that FVIII levels above 25–50 IU/dL do not seem to have an additional effect on the thrombin potential.

Figure 3 illustrates that patients with similar PK profiles can exhibit different normalized thrombin potential profiles, caused by inter-individual variability in EC_{50} (86.9%) and E_{max} (15.8%). Namely, the normalized thrombin potential curves of patients 2 and 3 are prolonged due to a lower individual EC_{50} than for patient 1. This shows that factors other than FVIII PK cause inter-individual variability in the normalized thrombin potential.

To assess which patient characteristics influence the observed inter-individual variability in PD parameters, an additional covariate analysis on the PD parameters was performed. After forward inclusion and backward elimination, the weight on the E_{max} of thrombin potential was retained in the model. Patients with a bodyweight of 20 kg showed a typical maximal normalized thrombin potential effect of 127% of NPP, which is higher compared to patients of 100 kg (84% of NPP). A visual representation of the effects of the covariates on the E_{max} is presented in Supplementary Figure S4.

The GOF plots of the final model, presented in Supplementary Figure S5, show that the models adequately describe the relation between FVIII level and the normalized thrombin peak height, normalized thrombin potential and normalized plasmin peak height. The visual predictive check confirmed that the model can predict the PD parameters (Figure 4), as the lines (representing the observed data) run through the shaded areas that represent the simulated data. In addition, the bootstrap analysis confirmed the robustness of the developed model (Table 2).

4 | DISCUSSION

In this study, we present a population PK/PD model describing the association between FVIII activity levels and the haemostatic effect, characterized by thrombin and plasmin generation, after administration of various SHL FVIII concentrates in a group of haemophilia A patients with varying severity. We observed substantial inter-individual variability in the PD parameters, such as EC_{50}, indicating that patients with similar FVIII activity levels can present with a different overall haemostatic capacity, as is also observed in clinical practice.

The population PK model was built using data from 30 haemophilia A patients, receiving different SHL concentrates. The PK parameters of the newly developed model are consistent with the previously published population PK models for SHL FVIII concentrates, though different covariates such as VWF and inhibitor presence are included in our population PK model.[4,25,27] The effect of inhibitor presence on FVIII clearance was also demonstrated by Abrantes et al.[31] The PK model was developed to obtain adequate

TABLE 2 Final parameter estimates of the population pharmacokinetic (PK)/pharmacodynamic (PD) model

Parameter	Final model		Bootstrap	
	Typical estimate (RSE %)	Estimate IIV (RSE%) [Shr.]	Typical estimate (95% CI)	Estimate IIV (95% CI)
Pharmacokinetic model				
V1 (dL)	27.7 (5.8)	15.6 (16.5) [2.8]	27.7 (24.2–31.0)	15.0 (9.74–20.7)
V2 (dL)	5.63 (19.2)	–	5.75 (3.80–10.8)	–
CL (dL/h)	1.69 (11.1)	41.2 (18.9) [2.1]	1.68 (1.33–2.11)	40.6 (25.0–59.2)
Q (dL/h)	2.27 (44.5)	–	2.29 (1.20–4.87)	–
Correction factor CSA	1.20 (3.5)	18.1 (13.3) [4.7]	1.15 (1.02–1.39)	17.6 (12.5–22.2)
Correlation IIV V1 and CL (%)	–	43.6	–	44.5 (−3.96–82.4)
Covariates				
Positive NLTIA on V1 (%)	114 (3.9)	–	114 (108–130)	–
Full-length recombinant product on V1 (%)	117 (6.8)	–	115 (102–139)	–
VWF exponent on CL	−0.52 (26.6)	–	−0.50 (−0.79–0.20)	–
Positive NLTIA on CL (%)	149 (11.1)	–	146 (117–179)	–
Full-length recombinant product on CL (%)	127 (10.3)	–	128 (96.8–163)	–
Residual variability				
Proportional error OSA (%)	11.2 (21.6)	–	11.0 (4.48–15.6)	–
Additive error OSA (IU/dL)	4.15 (14.9)	–	4.06 (2.93–5.36)	–
Proportional error CSA (%)	10.5 (17.5)	–	10.2 (5.84–13.6)	–
Additive error CSA (IU/dL)	4.28 (9.7)	–	4.23 (3.24–5.18)	–
Pharmacodynamic model				
Normalized thrombin peak height				
Baseline effect (% of NPP)	15.6 (18.8)	–	15.7 (11.4–21.6)	–
EC_{50} (IU/dL)	50.1 (24.4)	55.1 (26.8) [12.5]	48.8 (36.5–83.0)	51.5 (18.7–86.5)
Maximal effect (factor of baseline)	7.05 (33.6)	37.3 (25.8) [16.8]	6.81 (3.87–12.7)	34.5 (16.9–64.0)
Hill coefficient	1.85 (25.7)	–	1.90 (1.23–3.46)	–
Additive error (% of NPP)	11.2 (8.0)	–	11.1 (9.37–12.8)	–
Normalized thrombin potential				
Baseline effect (% of NPP)	37.5 (13.1)	41.8 (25.2) [15.7]	37.5 (27.4–47.8)	40.3 (20.4–72.9)
EC_{50} (IU/dL)	13.9 (21.2)	88.0 (16.9) [15.5]	14.1 (9.56–20.5)	80.9 (49.8–148)
Maximal effect (E_{max}) (% of NPP)	72.5 (9.5)	22.9 (23.9) [17.5]	71.7 (56.7–86.2)	21.1 (8.55–31.5)
Mild haemophilia on E_{max} (% of severe)	70.9 (15.9)	–	71.9 (51.4–98.9)	–
Coefficient bodyweight on E_{max}	−0.28 (21.0)	–	−0.28 (−0.42–0.11)	–
Hill coefficient	1.62 (20.8)	–	1.65 (1.04–2.62)	–
Additive error (% of NPP)	8.62 (12.2)	–	8.39 (6.11–10.2)	–

(Continues)

individual PK parameters to relate to the PD effect as the aim of the study was to describe the PK/PD relation. Despite the fact that the included population was heterogeneous, the sample size was sufficient to estimate the PK parameters with adequate precision (relative standard error <30%) and adequate individual PK parameters were obtained. The population PK model was developed using both OSA and CSA samples. However, when this model is used for PK/PD-guided dosing, we recommend using either both OSA and CSA samples or only OSA data of an individual patient, as in this model the OSA PK parameters are related to the PD parameters.

The PD part of the model was based on the thrombin peak height, thrombin potential and plasmin peak height measured by the NHA. These PD parameters were found to best represent haemostatic balance in a previous study by our research group.[21] A study by Lewis et al. also described that thrombin potential and thrombin peak height were able to describe the thrombin generation in a patient, while Delavenne et al. only used thrombin potential as the major PD parameter of thrombin generation in their population PK/PD model for patients with haemophilia A treated with Nuwiq.[18,19] Our model, which uses all three PD parameters, may give a more complete overview of the haemostatic balance.

TABLE 2 (Continued)

Parameter	Final model		Bootstrap	
	Typical estimate (RSE %)	Estimate IIV (RSE%) [Shr.]	Typical estimate (95% CI)	Estimate IIV (95% CI)
Normalized plasmin peak height				
Baseline effect (% of NPP)	125 (8.2)	32.1 (19.0) [1.0]	124 (105–148)	31.4 (18.3–43.6)
EC_{50} (IU/dL)	614 (47.7)	–	615 (304–380)	–
Maximal effect (% of NPP)	1 FIX	–	1 (1–1)	–
Hill coefficient	1 FIX	–	1 (1–1)	–
Proportional error (%)	26.8 (6.6)	–	26.6 (23.0–30.2)	–

Note: Of the 1000 data subsets used for bootstrap analysis, 158 runs were skipped.

Abbreviations: CL, clearance; CSA, chromogenic FVIII activity assay; CV, coefficient of variation calculated as $\sqrt{(\exp[\omega 2]-1)} * 100$; EC50, the FVIII activity level that is associated with 50% of the maximal effect; IIV, inter-individual variability; NLTIA, Nijmegen Low Titre Inhibitor Assay; NPP, normal pooled plasma; OSA, one-stage FVIII activity assay; Q, intercompartment clearance; RSE, relative standard error; Shr, shrinkage; V1, central volume of distribution; VWF, von Willebrand factor activity level (%); V2, peripheral volume of distribution.

Pharmacokinetic model:

$$CL = \theta_{CL} * \left(\frac{Weight_i}{75}\right)^{0.75} * \left(\frac{VWF:Act_{ij}}{100}\right)^{-0.52} * 1.27^{recombinant\ product} * 1.49^{Positive\ NLTIA} * e^{\eta_{CL}};$$

$$V1 = \theta_{V1} * \left(\frac{Weight_i}{75}\right) * 1.17^{recombinant\ product} * 1.14^{Positive\ NLTIA} * e^{\eta_V};$$

$$Q = \theta_Q;$$

$$V2 = \theta_{V2}$$

$$CSA\ activity = OSA\ acitvity * 1.20.$$

Pharmacodynamic model:

Normalized thrombin peak height: $E = E_{base} * \left(1 + \frac{E_{max} * C^n}{(EC_{50}^n + C^n)}\right)$

Normalized thrombin potential: $E = E_{base} + \frac{E_{max} * C^n}{(EC_{50}^n + C^n)}$;

$$E_{max} = \theta_{Emax} * \left(\frac{\text{Weight}_i}{75}\right)^{-0.28} * 0.709^{\text{mild hemophilia}}$$

Normalized plasmin peak height: $E = E_{base} * \left(1 - \frac{I_{max} * C^n}{(IC_{50}^n + C^n)}\right)$.

When comparing estimations of these three PD parameters, interesting differences were observed. The EC_{50} of the thrombin potential was the lowest, which reveals that lower FVIII activity levels are associated with a higher thrombin potential. Furthermore, the thrombin potential seems to exhibit a sustained response, when the FVIII activity levels return to the endogenous baseline. On the contrary, this prolonged effect was not seen in other analyses performed with different thrombin generation assays and therefore could also be an artefact in one of the used assays.[18,19] For thrombin potential, higher inter-individual variability in the PD parameters was quantified, indicating that patients may demonstrate a more similar thrombin peak height and especially plasmin peak height response than thrombin potential response. The overall change in plasmin peak height after FVIII concentrate administration was small, as the high EC_{50} value indicates. Namely, the EC_{50} value of 593 IU/dL expresses that an FVIII activity level of 593 IU/dL is necessary to observe 50% of the maximal effect, which is an unrealistically high FVIII level. Therefore, plasmin peak height alone does not support tailoring of FVIII doses and has less value than thrombin parameters in patients with haemophilia A.

The inter-individual variability in E_{max} of normalized thrombin potential could be partly explained by bodyweight. Patients with a lower bodyweight showed higher thrombin potential E_{max} values, which implies that in younger children the thrombin potential may rise more after FVIII concentrate administration. The relationship between the presence of a low-titrr inhibitor and the PD parameters was also of interest. During forward inclusion, patients with an inhibitor measured by the NLTIA showed a 30% higher thrombin peak height EC_{50}, indicating that for this subgroup higher FVIII activity levels are necessary to create a similar thrombin peak height response, which seems to relate to clinical practice. However, during backward deletion, this relation did not prove to be significant enough ($P < .01$) to be retained in the model. Possibly, this is due to lack of data, as only four patients in the PD analysis had a positive NLTIA. For the other PD parameters, no statistically significant covariates were found in this analysis, while for some PD parameters substantial inter-individual variability was observed. This suggests that it is difficult a priori (based only on patient characteristics) to predict how the thrombin/plasmin generation of an individual patient will evolve after FVIII administration. However, PK/PD-guided dosing may aid in overcoming the difficulty of large inter-individual variability in PD parameters, as with *maximum* a posteriori Bayesian estimation, additional information from measured FVIII activity levels and NHA parameters is used to determine individual PK/PD parameters.

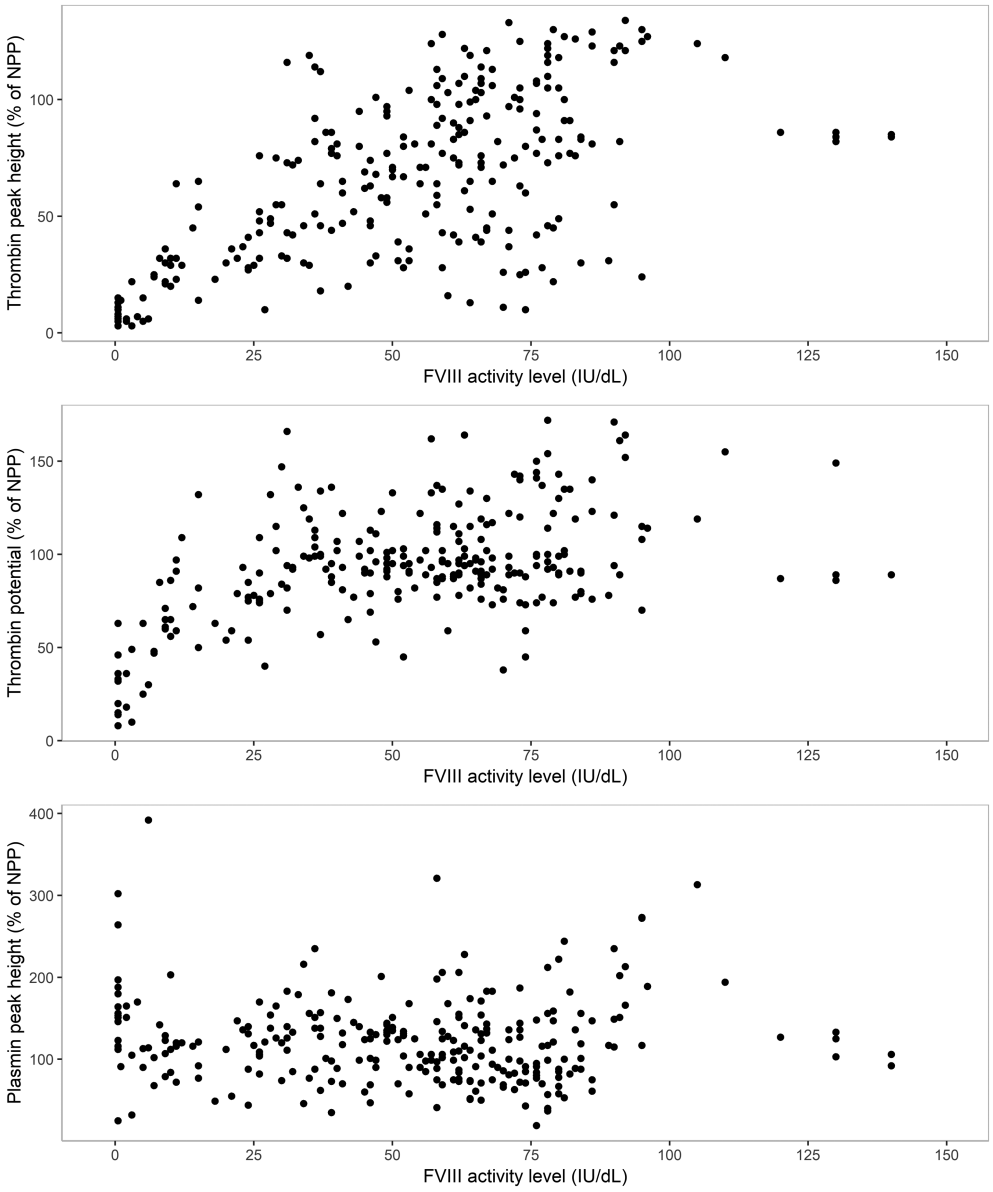

FIGURE 2 Relation between FVIII activity level and pharmacodynamic parameters. The thrombin peak height and thrombin potential increase when FVIII is administrated, following a maximal effect relationship. The relation between plasmin peak height and FVIII activity levels is less pronounced and shows only a slight decay at higher FVIII activity levels. The FVIII activity levels measured by one-stage assay and normalized pharmacodynamic (% of normal pooled plasma [NPP]) are used

One of the limitations of our study was that thrombin and plasmin generation were only measured until 24 hours after FVIII concentrate administration. Therefore, information on the course of the PD effects at lower FVIII activity levels contributed less to the developed model. Fortunately, FVIII activity levels and PD parameters were determined before FVIII administration, thus providing some information on the effects at low FVIII activity levels. Secondly, no information on bleeding phenotype, such as the annualized bleeding rate (ABR) was available for these patients, while it would be highly interesting to add the clinical bleeding phenotype as clinically important PD outcome to this PK/PD model.[19] Addition of bleeding could give more insight into which PD parameters are most relevant to use for dose tailoring and what PD effect levels we should aim for. Some studies have investigated the association between bleeding phenotype and thrombin generation and have reported a relationship between a higher frequency of spontaneous bleeding and reduced thrombin generation.[19,32] Thirdly, the thrombin and plasmin generation were measured by NHA, which measures thrombin generation at a relatively low tissue factor (TF) level of 0.3 pM compared to other TGAs. Also, tissue plasminogen activator (tPA) is added to be able to measure plasmin generation in the same well. Therefore, our current model is only applicable when this specific assay is used. Generally, the absence of standardized TGAs in most laboratories makes implementation difficult. However, to make the measurement of the PD parameters more reproducible, we decided to normalize the absolute PD parameters to a percentage of normal as also recommended by Dargaud et al. in their proposal to standardize conditions for TGAs.[24] We thus expect that the obtained thrombin generation parameters could be more easily compared with other TGAs. However, if other thrombin generation assays are used, we recommend to first validate the population PK/PD model. Finally, NHA data was collected over a long period of time (10 years), which resulted in a long storage time for some of these samples. However,

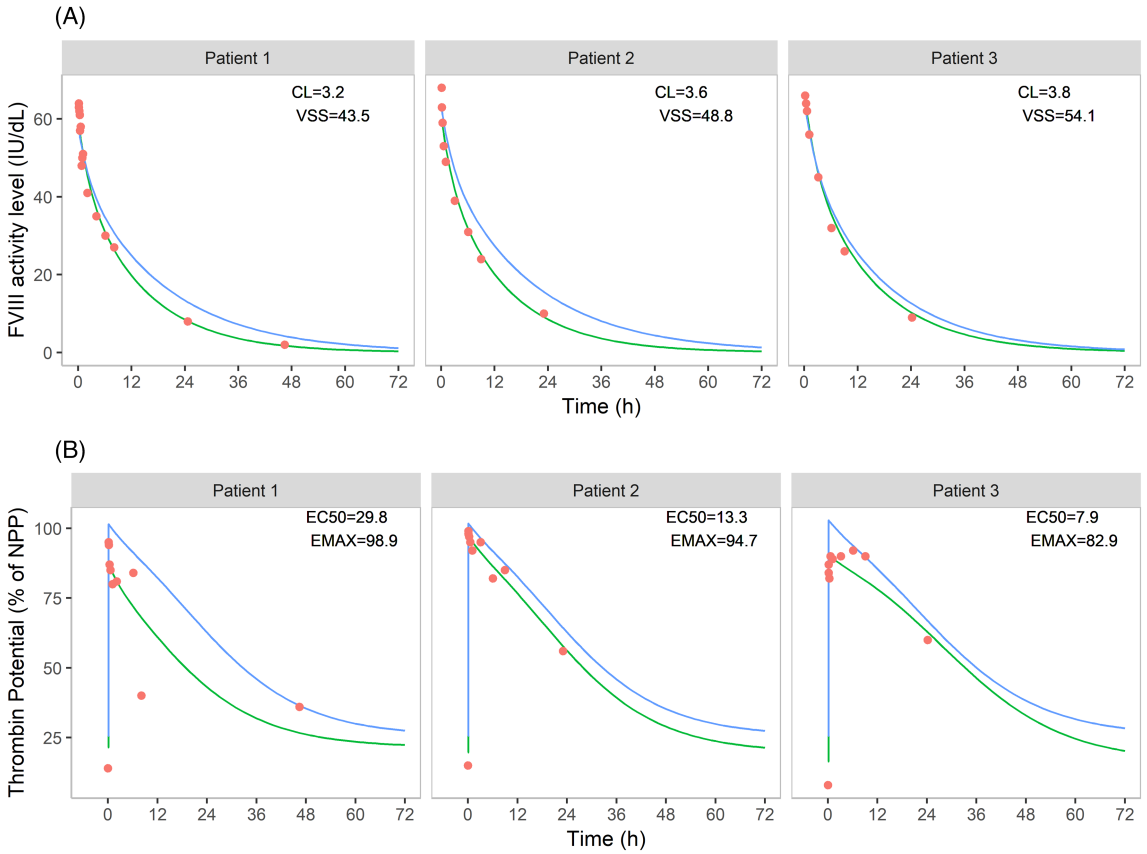

FIGURE 3 Patients with a similar pharmacokinetic profile demonstrate different normalized thrombin potential profiles after FVIII administration. The FVIII activity levels (A) and normalized thrombin potential (B) over time of three patients with comparable PK parameters from the dataset are shown. The green line indicates the individually predicted estimation, the blue line the estimation for the median patient included in our dataset and the red dots the observed data. The estimated individual clearance (CL), steady state volume of distribution (VSS), the FVIII activity level that is associated with 50% of the maximal effect (EC_{50}) and maximal effect (E_{max}) values are presented. NPP, normal pooled plasma

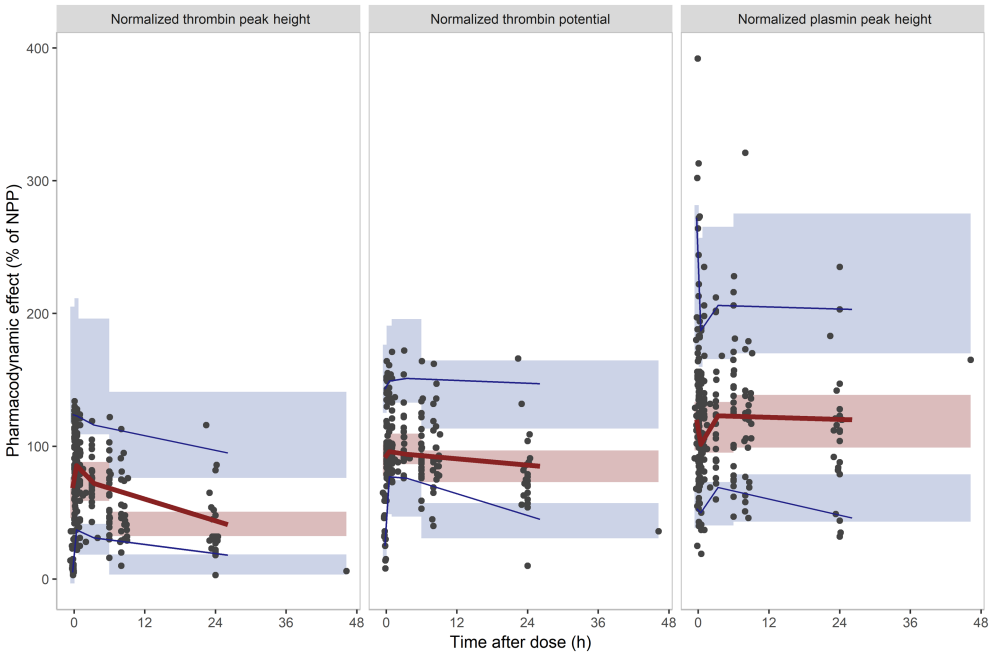

FIGURE 4 Visual predictive check of the final population pharmacodynamic model in which the coagulation effect is described by the normalized thrombin peak height, normalized thrombin potential and normalized plasmin peak height. The median and 95% confidence interval of the observed data (black dots) are summarized with the red and blue lines, respectively. These lines should run through the corresponding red and blue boxes, representing the median and 95% prediction intervals of the simulated observations ($n = 1000$)

precautionary measures were taken to prevent plasma protein degradation and evaporation during storage. An additional analysis showed no discrepancies between storage time and thrombin/plasmin generation (which was stored) and FVIII activity level (which was measured directly after collection) (data not shown).

In conclusion, the developed population PK/PD model describes the relationship between the FVIII concentrate dose, FVIII activity levels and thrombin/plasmin generation after administration of a SHL FVIII concentrate. The population PK model demonstrates that patients with comparable PK profiles show distinct individual thrombin and plasmin generation profiles. Our model could be used to explore the application of PK/PD-guided dosing for optimization of prophylactic FVIII replacement therapy in patients with haemophilia A. A clinical study involving the performance of the developed PK/PD model and the feasibility of PKPD-guided dosing based on NHA parameters is currently in preparation in Radboud University Medical Center. In this study FVIII replacement therapy of the patients will be adjusted based on their individual FVIII levels and thrombin/plasmin generation parameters. This could result in personalization of FVIII treatment based on individuals' haemostatic potential instead of factor activity level, as a better tool to represent the bleeding phenotype of the patient. This approach could also be of interest for new non-factor products such as emicizumab. However, target values for the thrombin/plasmin generation parameters have to be specified before PK-PD-guided dosing based on combined FVIII activity levels and thrombin/plasmin generation can be tested clinically. Therefore, more research into the relationship between thrombin/plasmin generation and bleeding is necessary.

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CONTRIBUTORS

L.B. and R.M. developed the population PK/PD model. L.V. collected and analysed the data. L.B. and L.V. wrote the manuscript. W.B. performed the NHA measurements. B.L.-v.G. recruited the patients. W.v.H. designed the study, oversaw execution of measurements and analysis of the data. N.B. and M.C. gave critical guidance. S.S. and R.M. supervised the study. All authors critically revised the manuscript and gave final approval for publication.

DATA AVAILABILITY STATEMENT

For data sharing, please contact r.mathot@amsterdamumc.nl.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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