European Journal of Drug Metabolism and Pharmacokinetics (2024) 49:191–205
https://doi.org/10.1007/s13318-024-00876-6

ORIGINAL RESEARCH ARTICLE

Pharmacokinetic–Pharmacodynamic Modelling in Hemophilia A: Relating Thrombin and Plasmin Generation to Factor VIII Activity After Administration of a VWF/FVIII Concentrate

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Accepted: 16 January 2024 / Published online: 17 February 2024
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Abstract

Background Hemophilia A patients are treated with factor (F) VIII prophylactically to prevent bleeding. In general, dosage and frequency are based on pharmacokinetic measurements. Ideally, an alternative dose adjustment can be based on the hemostatic potential, measured with a thrombin generation assay (TGA), like the Nijmegen hemostasis assay.

Objective The objective of this study was to investigate the predicted performance of a previously developed pharmacokinetic–pharmacodynamic model for FVIII replacement therapy, relating FVIII dose and FVIII activity levels with thrombin and plasmin generation parameters.

Methods Pharmacokinetic and pharmacodynamic measurements were obtained from 29 severe hemophilia A patients treated with pdVWF/FVIII concentrate (Haemate P®). The predictive performance of the previously developed pharmacokinetic–pharmacodynamic model was evaluated using nonlinear mixed-effects modeling (NONMEM). When predictions of FVIII activity or TGA parameters were inadequate [median prediction error (MPE) > 20%], a new model was developed.

Results The original pharmacokinetic model underestimated clearance and was refined based on a two-compartment model. The pharmacodynamic model displays no bias in the observed normalized thrombin peak height and normalized thrombin potential (MPE of 6.83% and 7.46%). After re-estimating pharmacodynamic parameters, EC_{50} and E_{max} values were relatively comparable between the original model and this group. Prediction of normalized plasmin peak height was inaccurate (MPE 58.9%).

Conclusion Our predictive performance displayed adequate thrombin pharmacodynamic predictions of the original model, but a new pharmacokinetic model was required. The pharmacodynamic model is not factor specific and applicable to multiple factor concentrates. A prospective study is needed to validate the impact of the FVIII dosing pharmacodynamic model on bleeding reduction in patients.

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Key Points

The predictive performance of a previously developed pharmacokinetic–pharmacodynamic model was investigated in a cohort of hemophilia A patients

Normalized thrombin peak height and potential pharmacodynamic models increased after FVIII dosage

Thrombin generation may be used as an additional target to normalize the hemostatic balance of a patient

Pharmacokinetic–pharmacodynamic-based dosing is a promising approach to further personalize treatment in hemophilia

1 Introduction

Hemophilia A is characterized by a deficiency of coagulation factor (F) VIII leading to recurrent spontaneous and trauma-induced bleeding [1]. Current guidelines recommend treatment with prophylactic FVIII replacement therapy [2], bypassing agents (BPAs) in case of inhibitors [3], and non-factor replacement therapies, such as emicizumab [4], for hemophilia A patients (FVIII activity level of < 1 IU/dl) aiming to reduce bleeding [5]. Prophylactic or on-demand FVIII replacement therapy is still the mainstay of treatment in much of the world, as other, more expensive therapies are not available [5].

Prophylactic FVIII replacement therapy can be dosed according to body weight and subsequently adjusted based on bleeding episodes or can be pharmacokinetic-based [3]. Increasingly, pharmacokinetic guidance is applied using population pharmacokinetic models to individualize dosing and to relate FVIII activity levels to bleeding, as bleeding risk varies significantly between persons with hemophilia [6, 7]. Both strategies exhibit disadvantages as additional bleeding episodes may occur before adequate dosing is achieved [2]. Bayesian forecasting analysis is used to apply limited sampling and to overcome a factor concentrate wash-out period [8–10]. However, this approach relies on plasma factor activity monitoring and does not consider the effect of factor replacement therapy on hemostasis (hemostatic potential or pharmacodynamics), and the inter-individual variation in bleeding tendency is not considered [11].

The thrombin generation assay (TGA) measures the amount of thrombin generated over time and is able to assess hemostasis globally [12]. It has been suggested that thrombin generation parameters are a better representation of the bleeding phenotype in hemophilia A patients than measurement of FVIII activity level [13–15]. In addition, FVIII replacement therapy can be monitored by TGA, and this global hemostatic assay may better reflect the patient's bleeding risk in the presence of similar FVIII activity levels after dosing [14]. To date, only two pharmacokinetic–pharmacodynamic models for FVIII replacement therapy have been described in the literature [16, 17], of which one suggests that bleeding can be decreased by intensifying treatment in patients presenting with low thrombin generation parameters [17].

The previously developed model [16] is based on the Nijmegen hemostasis assay (NHA), which incorporates thrombin generation with plasmin generation in one assay [18]. Multiple standard half-life (SHL) FVIII replacement concentrates were used in this model [16], but the results have not yet been replicated. In the current study, we performed a combined pharmacokinetic–pharmacodynamic analysis with the NHA of hemophilia A patients treated with only plasma-derived von Willebrand factor (VWF)/FVIII (pdVWF/FVIII) concentrate (Haemate P®). Here, we describe the thrombin and plasmin generation parameters of these patients after a single bolus. These data were used to investigate the predictive performance and eventually adapt the previously developed pharmacokinetic–pharmacodynamic model by Bukkems et al. [16].

2 Methods

2.1 Patients

Twenty-nine severe (FVIII activity level < 1 IU/dl) adult hemophilia A patients were included in this study between 1 August 2011 and 20 December 2012 in Ghaem Hospital, Mashhad University of Medical Sciences, Mashhad, Iran. This project was a sub-study of the IMPALA study (registered at the Dutch Trial Register, no. NL2808) and was approved by the Medical Ethics Committee of the Radboud University Medical Center and of the regional ethics committee of Mashhad University of Medical Sciences (number 89-88215). All patients gave written informed consent, and the study was conducted in accordance with the Declaration of Helsinki. Only adult patients (≥ 18 years) with severe hemophilia A were included. Exclusion criteria were: active bleeding, known allergy to plasma proteins, liver cirrhosis, hepatitis C treated with interferon within 6 months before inclusion, HIV infection, hemoglobin level < 8.0 mmol/l, platelet count $< 50 \times 10^9$/l, difficult venous access, and specific medications known to interact with hemostasis [non-steroidal anti-inflammatory drugs (NSAIDs), platelet aggregation inhibitors, antimicrobials, thyroid inhibitors and selective serotine reuptake inhibitors (SSRIs)].

The pharmacokinetic–pharmacodynamic study was performed with a single bolus of 25 IU/kg pdVWF/FVIII (Haemate P®, CSL Behring, King of Prussia, PA, USA). A standard 72-h wash-out period was used. Plasma samples were obtained before the bolus and at nine time points until 24 h after the bolus, as described before [19]. Blood samples were collected by venipuncture in 3.2% buffered sodium citrate siliconize blood collection tubes (Becton Dickenson, Plymouth, UK). At baseline, hemoglobin, hematocrit, and blood platelets were determined locally, and additional samples were collected for FVIII activity and VWF activity level and inhibitor level determination.

2.2 Sample Preparation

All blood samples were processed immediately after collection. Platelet-poor plasma (PPP) was obtained for NHA measurement by centrifuging the sample at 4200*g* for 15 min at 4 °C. The PPP was aliquoted into multiple 1.5-ml tubes for long-term freezer storage. All samples were frozen in liquid nitrogen, stored at − 80 °C, and shipped to the Radboud University Medical Center, Nijmegen, The Netherlands, on dry ice. Samples were stored at − 80 °C and were defrosted only once to measure FVIII activity level, VWF activity level, inhibitor levels, and the NHA.

2.3 FVIII Activity Level and Inhibitor Measurement

FVIII activity level was measured with one-stage (OSA) Cephascreen reagent in the STA Evolution (Stago Group, Asnières sur Seine, France) and chromogenic (CSA) assay (Biophen FVIII:C assay, HYPHEN Biomed SAS, Neuville-sur-Oise, France), according to the manufacturer's instructions, at STA Evolution (Stago Group).

Inhibitor titers were determined with the Nijmegen-modified Bethesda assay (NBA; cut-off for positivity ≥ 0.60 NBU/ml) [20, 21] and Nijmegen low-titer inhibitor assay (NLTIA, cut-off for positivity ≥ 0.04 NLTIU/ml) [22], as previously described.

2.4 Nijmegen Hemostasis Assay Measurement

The NHA was measured as described before and is described in detail in the supplementary methods [18, 19]. The essential parameters obtained with the NHA are shown in Supplementary Figure 1. All NHA measurements were performed in batches between 30 September until 23 November 2015. For all results, the mean of two measurements was used. Normal pooled plasma (NPP) was used as control measurement and to normalize the NHA parameter to the percentage of normal. The absolute NHA parameter of the patient was divided by the mean of the NPP samples that were used in the same run as the patient samples. The reference values of the NHA, based on healthy controls (n = 20), healthy men and women not using medication interfering with coagulation, are shown in Supplementary Table 1 and as a gray area in the figures.

2.5 Model Development

The previously described population pharmacokinetic–pharmacodynamic model was developed by Bukkems et al. [16]. The population pharmacokinetic–pharmacodynamic model was developed using nonlinear mixed-effect modeling (NONMEM, version 7.4). Their original pharmacokinetic analysis used 466 samples from 30 patients, while the pharmacodynamic analysis used 252 samples from 24 patients.

To investigate the predictive performance of the previous model in this replication study, two sequential steps were performed using the first-order estimation method in NONMEM. First, the performance in predicting the FVIII activity levels was assessed by the population pharmacokinetic model of Bukkems et al. The relative prediction error (PE%, Eq. 1) was estimated by comparing the predicted population concentrations and the corresponding observations for each subject in the dataset [23]. Here, Cpred is the model predicted value, and Cobs is the observed value. VWF activity was not measured after pdVWF/FVIII administration; therefore, this covariate effect was not included in evaluating the population pharmacokinetic model. The median prediction error (MPE) and median absolute prediction error (MAPE) were used for the evaluation bias and precision of the models. Model appropriateness was confirmed when the MPE was < 20% with the 95% CI including zero, and the MAPE was < 30% [23].

$$\mathrm{PE\%} = \frac{C_{\mathrm{PRED}} - C_{\mathrm{OBS}}}{C_{\mathrm{OBS}}} \times 100. \quad (1)$$

Second, if the population model failed to adequately predict the observations, then a novel population model was developed. During the population pharmacokinetic model development, both one- and two-compartment models were evaluated. A priori allometric scaling of pharmacokinetic parameters by body weight was included in the structural pharmacokinetic model. Inter-individual variability (IIV) was estimated and evaluated for each population pharmacokinetic model parameter. For residual error models, a proportional, an additive, and a combined error model were evaluated. Next, associations between covariates and pharmacokinetic parameters were tested to explain the IIV in the pharmacokinetic parameters. The following covariates were tested: age, NLTIA, and NBA. During the covariate analysis, stepwise forward inclusion and backward elimination approaches were used. Reductions in the objective function value (OFV) of at least 3.84 ($p < 0.05$, chi-square distribution

with 1 degree of freedom) and > 6.64 ($p<0.01$, chi-square distribution with 1 degree of freedom) were required for a covariate to be considered significant in the forward inclusion and backward elimination steps, respectively. The supplement provides further details about the development of the pharmacokinetic model. The individual pharmacokinetic parameters obtained from the pharmacokinetic model were used as input for the population pharmacodynamic model.

To investigate the predictive performance of the pharmacodynamic model by Bukkems et al., a similar approach was used as for the pharmacokinetic part of the model. When the population pharmacodynamic model failed to adequately predict observations, a novel population pharmacodynamic model was developed by using the external pharmacodynamic dataset. A maximum effect (E_{max}) and a sigmoidal E_{max} model were tested to describe the relationship between FVIII activity levels and normalized thrombin peak height, normalized thrombin potential, and normalized plasmin peak height. Evaluation of the residual error models, addition of IIV to the pharmacodynamic parameters, and performance of the covariate analysis were similar to those for the pharmacokinetic model part.

Model evaluation criteria included change in OFV, goodness-of-fit (GOF) plots, precision of parameter estimates, decreases in IIV and residual variability, condition number, shrinkage, and a successful convergence step [24]. Prediction-corrected visual predictive checks (pcVPCs) were used to assess the predictive performance of the model. The supplement contained more details on the evaluation and development of the models. A schematic overview of the predictive performance of the population pharmacokinetic–pharmacodynamic model is displayed in Fig. 1.

2.6 Statistical Analysis

The reported parameters are shown as median (interquartile range; IQR) unless otherwise stated. Descriptive statistics were used for demographic and NHA parameters. Spearman correlation was used for the correlation between FVIII OSA and CSA assay and for the correlation between FVIII activity level and thrombin generation parameters. For the analysis of differences before and after the FVIII bolus, Wilcoxon matched-pairs signed ranked tests were used.

Statistical analyses were performed using Prism GraphPad, version 9.4. All p values are two-sided, and a p value < 0.05 was considered statistically significant.

3 Results

3.1 Patient Characteristics

A total of 29 male severe hemophilia A patients were included in the study. The baseline patient characteristics are described in Table 1. Median age was 27 (range 19–53) years, mean body weight was 62 (SD: 7) kg, and median body weight was 62 (range 48–73) kg. The median pre-bolus FVIII activity level was < 1 IU/dl, as measured by both the OSA and CSA assay. The correlation of FVIII activity level measured by the OSA and CSA assay was excellent [$r=0.96$, 95% confidence interval (95% CI) 0.95–0.97, $p<0.0001$, see Fig. 2A]. Eight patients had a detectable FVIII activity level pre-bolus with the CSA (5 patients 1 IU/dl and 3 patients 2 IU/dl), while 4 were detectable with the OSA (3 patients 1 IU/dl and 1 patient 2 IU/dl).

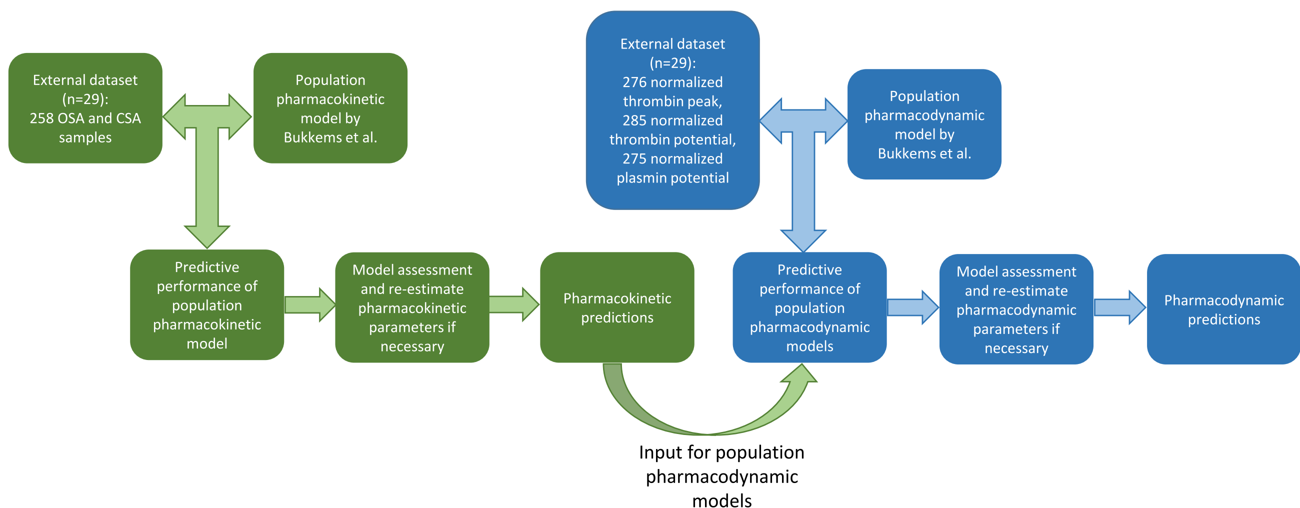

Fig. 1 Schematic workflow of predictive performance of population pharmacokinetic–pharmacodynamic models. *CSA* chromogenic substrate assay, *OSA* one-stage assay

Table 1 Baseline characteristics of the 29 included patients

Characteristic	Value
Age, median (range), years	27 (19–53)
Body weight, kg	
Mean (SD)	62 (7)
Median (range)	62 (48–73)
Baseline values	
Baseline one-stage FVIII concentration in IU/dl, median (range)	< 1 (< 1–2)
Baseline chromogenic FVIII concentration in IU/dl, median (range)	< 1 (< 1–3)
Baseline VWF concentration in %, mean (SD)	117 (46)
Total pharmacokinetic number of samples	
OSA	258
CSA	258
Total pharmacodynamic number of samples[a]	287
FVIII product and dosage	
Haemate P, *n* (%)	29 (100)
Dosage FVIII replacement therapy, median (IQR)	1600 (1500–1700)
Dosage FVIII/kg, median (IQR), IU/kg	25.0 (24.6–25.4)
FVIII half-life in hours, median (IQR)	10.6 (8.3–12.9)
Inhibitor positivity	
NBA, *n* (%)	1 (3)
NLTIA, *n* (%)	7 (24)

CSA chromogenic substrate assay, *FVIII* factor VIII, *IQR* interquartile range, *NBA* Nijmegen Bethesda assay, *NLTIA* Nijmegen low-titer inhibitor assay, *OSA* one-stage assay, *SD* standard deviation

[a]Of the normalized thrombin peak height and normalized plasmin potential, nine and ten measurements were not detectable, respectively

The mean VWF activity level before the bolus was 117% (SD 43%). All 29 patients received the same pdVWF/FVIII concentrate, with a median bolus of 1600 IU FVIII (IQR 1500–1700 IU), corresponding to 25 IU/kg FVIII (24.6–25.4). One patient had an inhibitor detected with the NBA (titer 1.1 NBU/ml), while six additional patients had an inhibitor measured with the NLTIA (titer 0.04–0.05 NLTIU/ml); all patients were included in the analysis. For the population pharmacokinetic analysis, all 258 FVIII activity levels measured with OSA and CSA were used. The FVIII activity level measured before pdVWF/FVIII concentrate administration was considered the endogenous baseline and subtracted from the observed FVIII activity levels during model development. One patient had a FVIII activity level that was not detectable at 24 h after administration. In one patient, three samples at 3, 5, and 15 min were missing. In the full dataset, 5.2% (OSA) and 4.2% (CSA) of the samples were below the detection limit of the assay, mostly samples taken before pdVWF/FVIII administration. These samples were excluded in the pharmacokinetic data analysis.

3.2 Pharmacokinetic Measurements

After the bolus injection, FVIII activity level increased to 52 IU/dl (42–62; see Fig. 3A and Supplementary Table 2) after 15 min, which was the anticipated increase with the amount of infused FVIII concentrate. At 1 h, FVIII activity level was 46 IU/dl (35–53), at 6 h 29 IU/dl (19–35), and 7 IU/dl (4–10) at 24 h after the bolus. This led to a median FVIII half-life of 10.6 (8.3–12.9) h.

3.3 Thrombin and Plasmin Generation

Thrombin generation parameters were low at baseline, but differed between patients as illustrated by the large range of obtained results (Fig. 3B, 3C). At baseline, thrombin peak height was 15 nM (undetectable: 19; Fig. 3B), thrombin potential was 280 nM-min (undetectable: 280; Fig. 3C), and plasmin peak height was within the normal range despite the low thrombin generation [median 27.4 (IQR 17.5–32.0); Fig. 3D]. The other parameters are shown in Supplementary Figure 2 and Supplementary Table 2.

After the FVIII bolus, parameters increased rapidly to near normal values (Fig. 3, Supplementary Figure 3 and Supplementary Table 2). Thrombin peak height rose to 142 nM (92–166) at 15 min and decreased slowly to 109 nM (61–144) at 6 h and to 62 nM (37–89) after 24 h. The increase in thrombin potential persisted even longer, with 1823 nM-min (1683–2028) at 15 min, 1886 nM-min (1565–2128) at 6 h, and 1440 nM-min (1044–1878) after

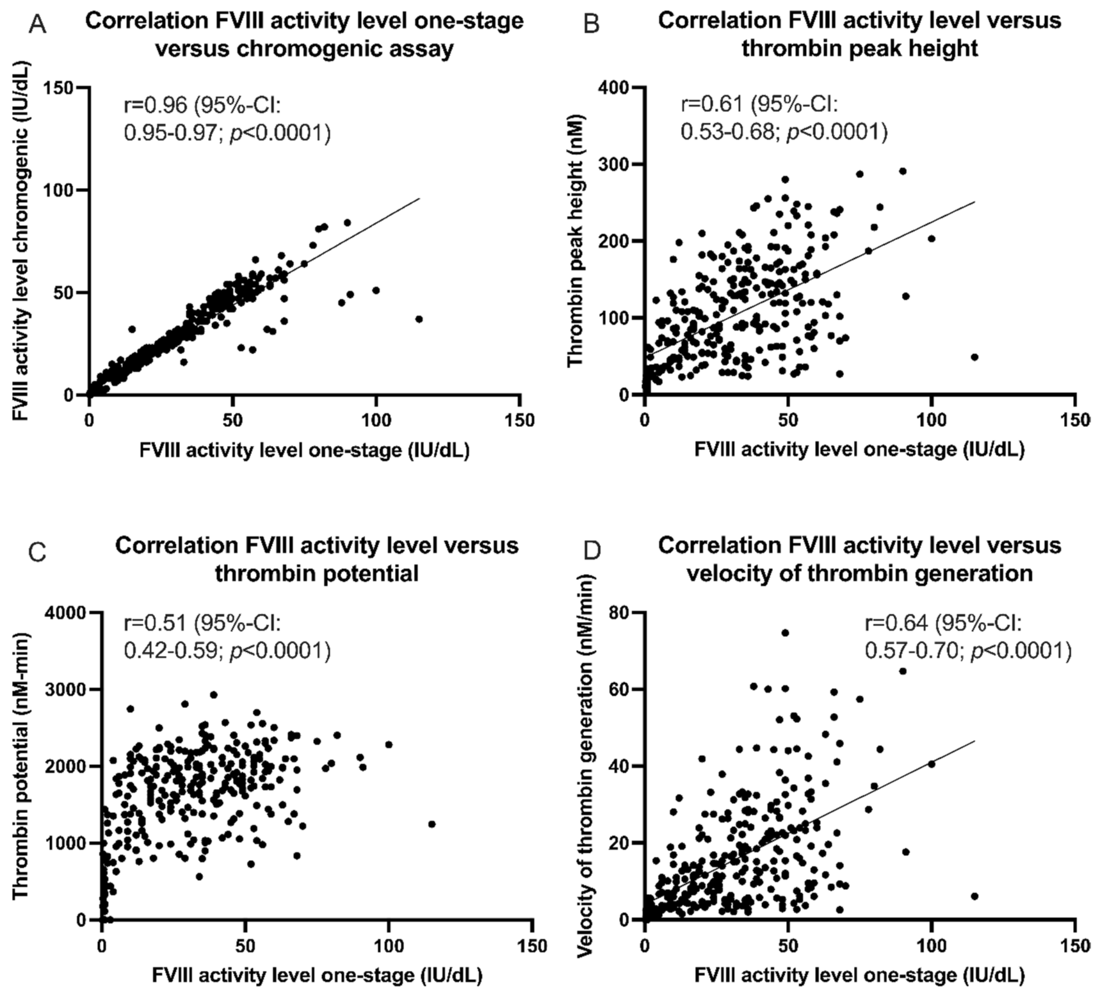

Fig. 2 Correlations of factor VIII activity level with thrombin generation parameters. **A** Correlation between factor VIII activity level determined with the one-stage assay and chromogenic assay. Correlation of factor VIII activity level with **B** thrombin peak height, **C** thrombin potential, and **D** velocity of thrombin generation

24 h. This is best illustrated with plasmin peak height, which was 21.5 nM (14.5–25.0) at 15 min after the bolus and persisted at this low level with higher thrombin generation (plasmin peak height 24.4 nM (18.9–29.0) at 6 h and 19.5 nM (13.8–23.6) after 24 h). With the increase in thrombin generation, plasmin generation decreased (Fig. 3D).

Previously, it was reported that velocity of thrombin generation is a better representation of the effect of factor supplementation in hemophilia patients [25]. The correlation between FVIII activity level and velocity of thrombin generation [$r=0.64$ (95% CI 0.57–0.70); Fig. 2D] was equal to the correlation of FVIII activity level and thrombin peak height [$r=0.61$ (95% CI 0.53–0.68); Fig. 2B] and thrombin potential [$r=0.51$ (95% CI 0.41–0.59); Fig. 2C]. Furthermore, differences in velocity of thrombin generation between patients were significant (as illustrated by the large range in Supplementary Figure 2C).

Normalized thrombin generation parameters are preferred over absolute parameters to compare results at different laboratories according to guidelines of the ISTH [26]. In Fig. 4, the normalized thrombin peak height and normalized thrombin potential are shown. The results in Fig. 4 show that T_{max} is identical for FVIII activity level and normalized thrombin peak height [normalized thrombin peak height 51% (35–71)], but it remains higher compared to FVIII activity level [at 6 h 29 IU/dl (19–35) versus 46% (26–60) and at 24 h 7 IU/dl (4–10) versus 24% (15–39), respectively]. The same holds true for normalized thrombin potential, which is 92% (79–104) after 15 min and remains 91% (78–107) at 6 h, but the T_{max} occurs at 9 h post dose with 98% (74–109) and 71% (56–94) at 24 h (see Fig. 4 and Supplementary Table 3).

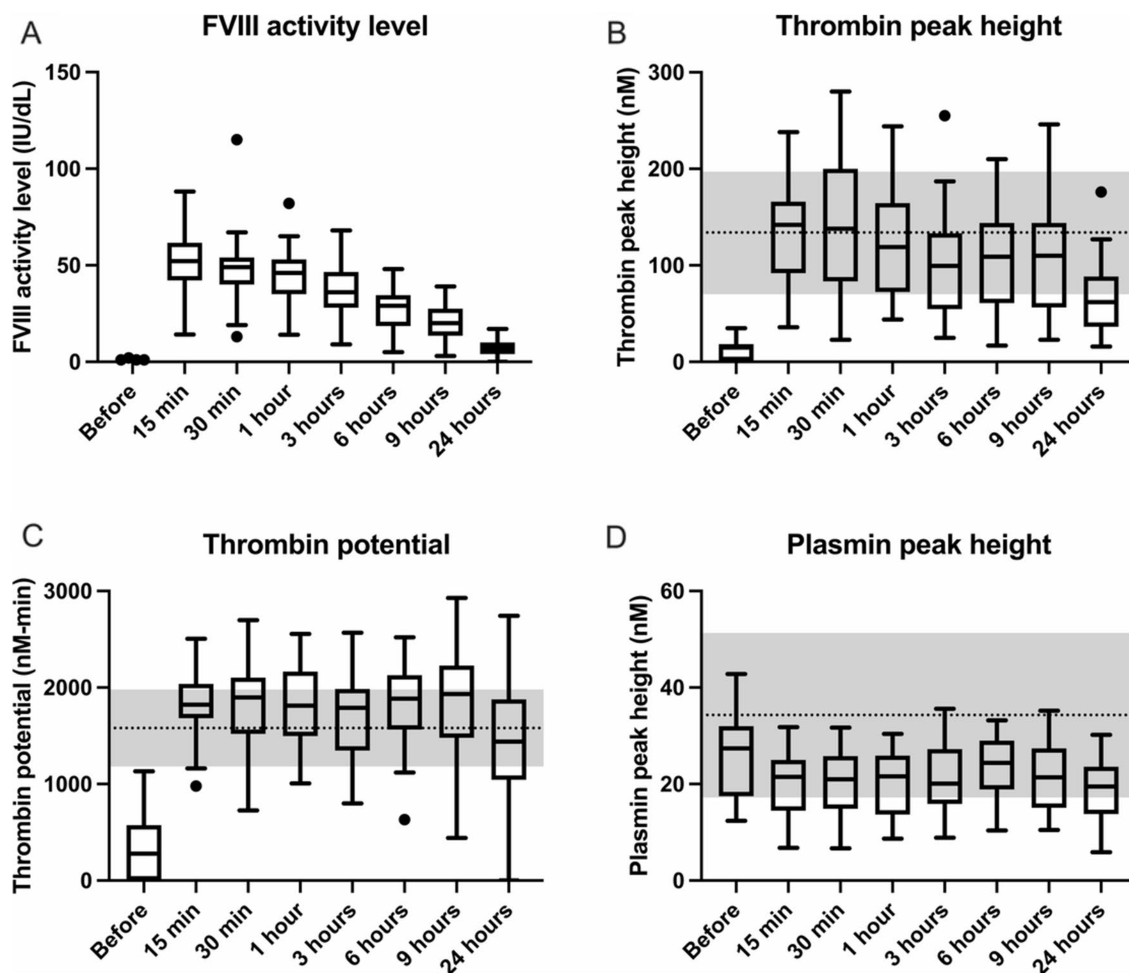

Fig. 3 Factor VIII activity level, thrombin peak height, thrombin potential, and plasmin peak height after a bolus of factor VIII replacement therapy. **A** Factor VIII activity level measured with the one-stage assay, **B** thrombin peak height, **C** thrombin potential, and **D** plasmin peak height after a standardized bolus of plasma derived von Willebrand factor/factor VIII concentrate. Box represents median with interquartile range, whiskers indicate minimum and maximum, and dots are outliers. Dotted line represents mean of individual healthy control Nijmegen hemostasis assay measurements, gray area ± 2 standard deviations

3.4 Re-Estimation of the Population Pharmacokinetic Model

The predictive performance of the population pharmacokinetic model by Bukkems et al. was evaluated with the current data [16]. The observed FVIII activity was lower than the predicted activity (Supplementary Figure 4) with MPE and MAPE values of 60.9 and 61.3% (Fig. 5). Therefore, a novel population pharmacokinetic model was developed. One- and two-compartment models were tested. The data were best described with a two-compartment model with IIV attributed on clearance and central volume of distribution. For the residual error, a combined proportional and additive error model was used. Since FVIII activity levels were measured with both OSA and CSA, a correction factor was included to correct for the difference in assay methods. Samples measured with CSA were 0.939 times lower compared to samples measured with OSA. IIV was tested on the correction factor, but including IIV on the correction factor did not significantly improve the model fit.

During the covariate analysis, a relationship was found between FVIII clearance and presence of an NBA inhibitor leading to a 153% increase in clearance. The NLTIA did not have an effect on FVIII clearance. The final pharmacokinetic parameter estimates are displayed in Table 2. The GOF plots and pcVPC of the final pharmacokinetic model are presented in Supplementary Figures 4 and 5.

3.5 Re-estimation of the Population Pharmacodynamic Models

For the population pharmacodynamic analysis, 285 values of normalized thrombin peak height and potential and normalized plasmin peak were measured. Of the normalized

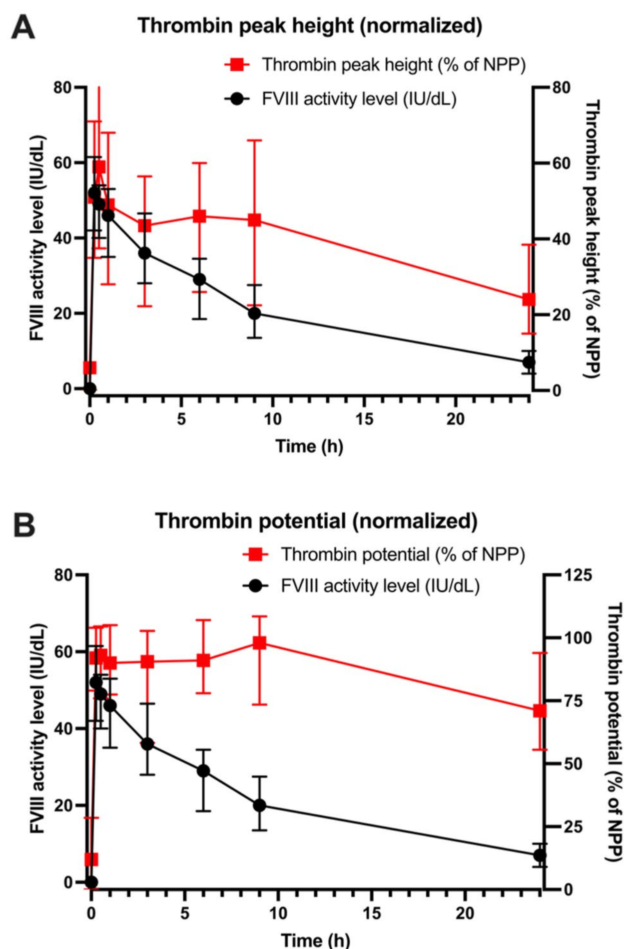

Fig. 4 Factor VIII activity level and normalized thrombin peak height and thrombin potential after a bolus of factor VIII replacement therapy. Factor VIII activity level measured with the one-stage assay (in IU/dl on left *y*-axis, in black) and **A** thrombin peak height [as percentage of normal pooled plasma (NPP)] and **B** thrombin potential (as percentage of NPP), both on right *y*-axis in red, before and after a standardized bolus of plasma derived von Willebrand factor/factor VIII concentrate. The dots represent the median with the interquartile range

thrombin peak height and normalized plasmin potential, nine and ten measurements were undetectable, respectively. These were excluded from the pharmacodynamic data analysis, since only a small portion of the samples (< 4%) was undetectable.

The individual pharmacokinetic parameters obtained from the pharmacokinetic model were used for estimation of the pharmacodynamic part of the model. The predictive performance of the previously published FVIII population pharmacodynamic models was tested. As shown in Fig. 5, differences in the predictive performance of different models were observed. For the models of Bukkems et al., the MPE and MAPE were 6.83 and 38.6, 7.46 and 18.6, and 58.9 and 58.9% for normalized thrombin peak height, normalized thrombin potential, and normalized plasmin peak, respectively. This indicates that the final pharmacodynamic parameters by Bukkems et al. for the normalized thrombin potential adequately predict our external dataset. There is no bias in prediction of the normalized thrombin peak height; however, the prediction is not accurate since the MAPE is 38.6%. Moreover, the normalized plasmin peak pharmacodynamic model showed overprediction. It should also be noted that the models have a high IIV; therefore, a high MAPE is expected. The MPE and MAPE of all models are summarized in Supplementary Table 4.

Afterwards, re-estimations of pharmacodynamic parameters were performed by using the external pharmacodynamic dataset. The final pharmacodynamic parameter estimates are displayed in Table 3. As expected, similar pharmacodynamic parameters for normalized peak height and normalized thrombin potential were obtained. For the normalized thrombin peak height an EC_{50} of 51.6 IU/dl and E_{max} (baseling factor) of 8.06 were obtained (Supplementary Eq. 9), while previously the EC_{50} and E_{max} were 50.1 IU/dl and 7.05, respectively. For the normalized thrombin potential an EC_{50} of 1.93 IU/dl and E_{max} (percentage of NPP) of 65.3 were obtained (Supplementary Eq. 10), while in Bukkems et al. the EC_{50} and E_{max} were 13.9 IU/dl and 72.5, respectively. For the normalized plasmin peak height, a baseline and EC50 of 81.2 IU/dl and 256 IU/dl were obtained (Supplementary Eq. 9), while in the original model the baseline was 125 IU/dl and EC_{50} was 614 IU/dl. In our novel pharmacodynamic models, IIV was only added to one pharmacodynamic parameter. Adding IIV to more than one pharmacodynamic parameter did not result in a significant improvement ($p = 0.05$) in model fit. Therefore, in the re-estimated pharmacodynamic models, IIV was included on the pharmacodynamic parameter with the largest drop in OFV (< 3.84, $p = 0.05$). Afterwards, a covariate analysis was performed in which age and body weight were tested. However, none of the covariates had a significant effect on the pharmacodynamic parameters. GOF plots using models of Bukkems et al. and the re-estimated models are displayed in Supplementary Figures 6–8. The pcVPC of the plasmin peak height in which parameters are re-estimated is displayed in Supplementary Figure 9. The GOF plots and pcVPC show that the final models adequately described the observed data. The pcVPC using the thrombin peak height and thrombin potential models of Bukkems et al. with the external dataset is displayed in Supplementary Figures 10–11.

Figure 6 displays patients with similar pharmacokinetic profiles but with different normalized thrombin potential profiles, caused by the IIV in the baseline, EC_{50}, and E_{max}. The third patient had a longer effect of the normalized thrombin potential due to a lower EC50 compared to the other patients. In this figure, the model of Bukkems et al. further displays sufficient predictive performance in

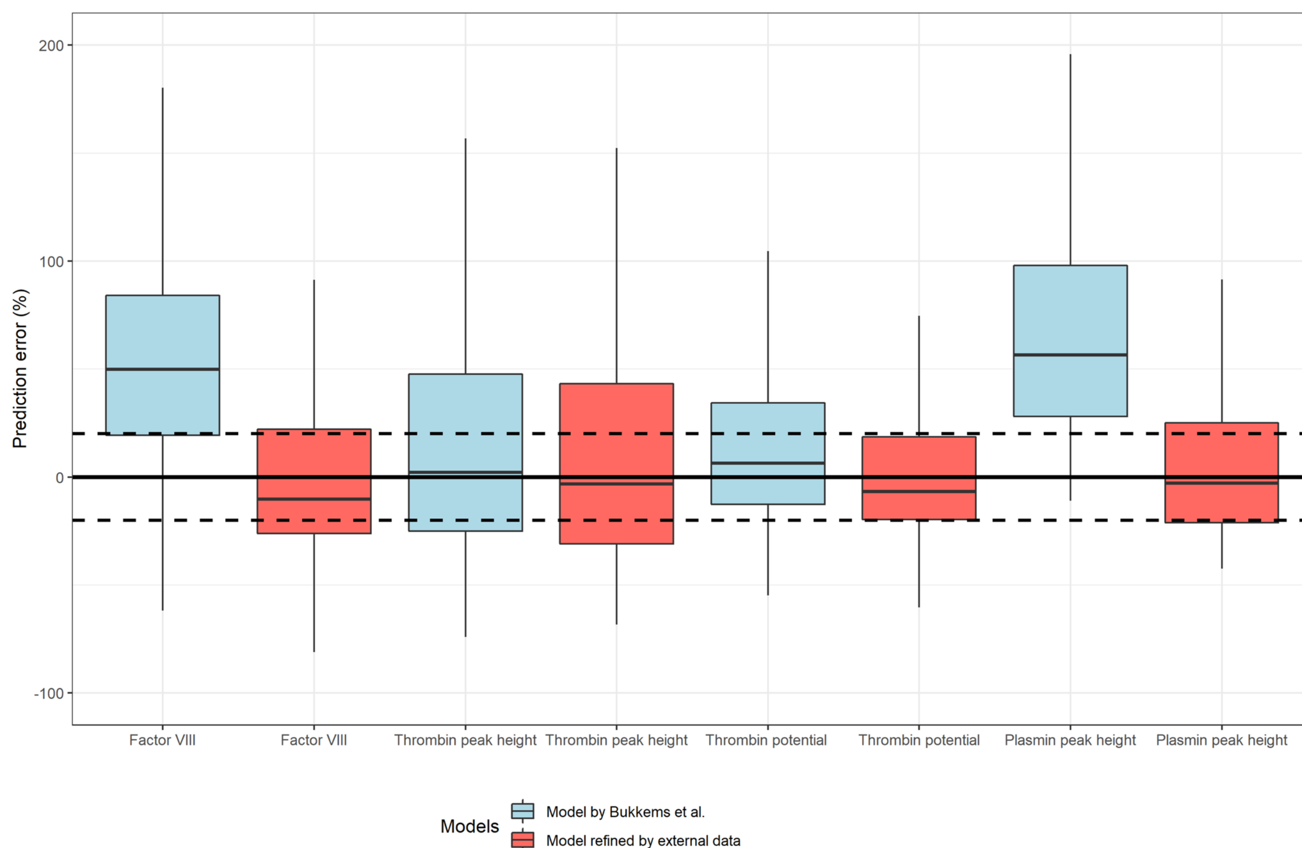

Fig. 5 Box plots of the prediction error (PE%) of the novel models vs Bukkems et al. Blue box plots indicate the PE of the models by Bukkems et al., and red box plots indicate the PE of the novel models, in which the pharmacokinetic or pharmacodynamic parameters are re-estimated. Within each box plot, horizontal black lines denote median values. Box plots extend from the first to the third quartile from each model. Black solid and dashed lines are reference lines indicating PE% of 0% or ±20%, respectively

an external dataset. Supplementary Figure 12 displays the normalized thrombin potential response after factor VIII administration in a patient with inhibitor.

4 Discussion

In this replication study, we show that our previously developed pharmacodynamic model for dosing of SHL FVIII concentrates was able to adequately describe the relationship between FVIII activity level and normalized thrombin peak height and normalized thrombin potential in another factor concentrate containing VWF, e.g., pdVWF/FVIII concentrate (Haemate P®). This finding underscores the additive value of measuring pharmacodynamics by TGA in hemophilia A patients, as thrombin generation measured by NHA subsequent to prophylactic FVIII administration was able to predict the hemostatic potential of FVIII in an individual patient. The original developed pharmacokinetic–pharmacodynamic FVIII concentrate dosing model included a wide range of plasma-derived and recombinant FVIII concentrates to secure the generalization of the model [16]. As this study shows, thrombin generation remains equal despite differences in administered factor concentrates, while the pharmacokinetic model was unable to describe the pharmacokinetic of this pdVWF/FVIII product.

In the previous study, it was difficult to compare pharmacokinetic–pharmacodynamic profiles of different patients, as whether differences in the pharmacokinetic–pharmacodynamic profile were caused by patient-related factors or were dependent on the factor VIII concentrate administered was not known. Both recombinant and plasma-derived FVIII concentrates were used, and some patients ($n = 3$) also received pdVWF/FVIII concentrate. Therefore, the use of only one FVIII/VWF product in this replication study enabled comparing generated pharmacokinetic–pharmacodynamic profiles. Subsequently, differences found between patients will be caused by patient-related factors, like body weight, pre-existent VWF concentration, and presence of (very) low-titer inhibitors.

Our data show that thrombin generation remained increased after replacement of FVIII activity during the

Table 2 Comparison population pharmacokinetic parameter estimates of Bukkems et al. [16] and external dataset in patients of this replication study

Parameter	External dataset		Bukkems et al.	
	Parameter estimation (RSE%)	Inter-individual variability (RSE%) [Shr%]	Parameter estimation (RSE%)	Inter-individual variability (RSE%) [Shr%]
Pharmacokinetic model				
CL (dl/h/70 kg)	3.07 (10)	57.3 (15) [1.4]	1.69 (11.1)	41.2 (18.9) [2.1]
V_1 (dl/70 kg)	39.1 (7.7)	38.2 (15) [0.1]	27.7 (5.80)	15.6 (16.5) [2.8]
Q (dl/h/70 kg)	1.09 (35.5)		2.27 (44.5)	-
V_2 (dl/70 kg)	9.16 (32.3)		5.63 (19.2)	-
Correction factor CSA	0.939 (2.2)		1.20 (3.50)	18.1 (13.3) [4.7]
Correlation IIV CL and V_1 (%)		77.0		43.6
Covariates				
Positive NLTIA on V_1 (%)	–		114 (3.9)	
Full-length recombinant product on V_1 (%)	–		117 (6.8)	
VWF exponent on CL	–		-0.52 (26.6)	
Positive NLTIA on CL (%)	–		149 (11.1)	
Full-length recombinant product on CL (%)	–		127 (10.3)	
Positive NBA on CL (%)	153		–	
Residual variability				
Proportional error OSA (%)	25.0 (6.6)		11.2 (21.6)	
Additive error OSA (IU/dl)	0.854 (26.5)		4.15 (14.9)	
Proportional error CSA (%)	21.0 (17.5)		10.5 (17.5)	
Additive error CSA (IU/dl)	4.28 (9.7)		2.69 (49.8)	

CL clearance, *CSA* chromogenic FVIII activity assay, *IIV* inter-individual variability, *NBA* Nijmegen-modified Bethesda assay, *NLTIA* Nijmegen low-titer inhibitor assay, *OSA* one-stage FVIII activity assay, *Q* intercompartment clearance, *RSE* relative standard error, *Shr* shrinkage, V_1 central volume of distribution, *VWF* von Willebrand factor activity level (%), V_2 peripheral volume of distribution

first 24 h, even though FVIII activity levels decrease rapidly. This is clearly illustrated by the normalized thrombin potential, which remained at 71% of normal (IQR 56–94) after 24 h while FVIII activity level was only 7 (4–10) IU/dl. This is comparable with our previous study, in which normalized thrombin potential was 75% of normal (59–87) with an associated FVIII activity level of 15 (10–26) IU/dl [19]. Notably, the FVIII activity level in the prior study was twice as high as observed in this current study because of higher dosage in the prior study, while normalized thrombin potential remained roughly equal. This indicates that FVIII-stimulated thrombin generation has a maximum capacity and only a little FVIII is necessary to stimulate thrombin generation (i.e., FVIII supplementation has a low EC50 for thrombin generation). Moreover, plasmin generation may not be suitable as a pharmacodynamic target because of the small difference in plasmin generation between healthy and hemophilia A patients.

In the current study, we observed a plasmin peak height that was within normal range compared to healthy controls. Nonetheless, plasmin peak height decreased after FVIII replacement therapy, and this decrease was present until 24 h after the bolus. This apparent hyperfibrinolysis in patients has been observed earlier and is possibly due to the reduced activation of thrombin activated fibrinolytic inhibitor (TAFI), for which a higher amount of thrombin is required than can be produced when amplification is insufficient [27, 28]. Because the NHA is the only assay that measures thrombin and plasmin generation simultaneously in a single well [29, 30], the interplay between thrombin and plasmin generation can only be investigated using this assay [18]. This observation was in accordance with our previous study, in which we also observed hyperfibrinolysis before the FVIII bolus in patients with HA, which was resolved after normalization of FVIII activity level [19].

Previously, the velocity of thrombin generation was suggested to correspond better with factor activity levels in both HA and hemophilia B (HB) [25, 31]. The correlation between FVIII activity level and velocity of thrombin generation was slightly better than the correlation with thrombin potential and thrombin peak height. Also, the curves of FVIII activity and velocity of thrombin generation correspond better with each other than the curve of thrombin potential (Supplementary Figure 3). However,

Table 3 Comparison population pharmacodynamics parameter estimates of Bukkems et al. [16] and external dataset in patients of this replication study

Parameter	External dataset		Bukkems et al.	
Pharmacodynamic model	Parameter estimation (RSE%)	Inter-individual variability (RSE%) [Shr%]	Parameter estimation (RSE%)	Inter-individual variability (RSE%) [Shr%]
Normalized thrombin peak height				
Baseline effect (% of NPP)	11.2 (26.3)	34.7 (33.6) [7.5]	15.6 (18.8)	–
EC_{50} (IU/dl)	51.6 (13.2)		50.1 (24.4)	55.1 (26.8) [12.5]
Maximal effect (factor of baseline)	8.06 (32.6)		7.05 (33.6)	37.3 (25.8) [16.8]
Hill coefficient	1 FIX		1.85 (25.7)	–
Additive error (% of NPP)	17.4 (7.4)		11.2 (8.0)	–
Normalized thrombin potential				
Baseline effect (% of NPP)	21.9 (13.6)	–	37.5 (13.1)	41.8 (25.2) [15.7]
EC_{50} (IU/dl)	1.93 (46.4)	–	13.9 (21.2)	88.0 (16.9) [15.5]
Maximal effect (E_{max}) (% of NPP)	65.3 (6.7)	33.1 (37.0) [4]	72.5 (9.5)	22.9 (23.9) [17.5]
Mild haemophilia on E_{max} (% of severe)	–		70.9 (15.9)	–
Coefficient bodyweight on E_{max}	–		−0.28 (21.0)	–
Hill coefficient	1 FIX		1.62 (20.8)	–
Additive error (% of NPP)	16.2 (6.7)		8.62 (12.2)	–
Normalized plasmin peak height				
Baseline effect (% of NPP)	81.2 (4.5)	26.4 (12.9) [0.1]	125 (8.2)	32.1 (19.0) [1.0]
EC_{50} (IU/dl)	256 (49.9)	–	614 (47.7)	
Maximal effect (% of NPP)	1 FIX	–	1 FIX	–
Hill coefficient	1 FIX	–	1 FIX	–
Proportional error (%)	16.3 (5.8)	–	26.8 (6.6)	–

EC_{50} the FVIII activity level which produces 50% of the maximal effect, *IIV* inter-individual variability, *NPP* normal pooled plasma, *RSE* relative standard error, *Shr* shrinkage

Pharmacodynamic formula external dataset

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

Normalized thrombin potential: $E = E_{base} + (1 + \frac{E_{max} \times C^n}{(EC_{50}{}^n + C^n)})$

Normalized plasmin peak height: $E = E_{base} \times (1 - \frac{E_{max} \times C^n}{(EC_{50}{}^n + C^n)})$

it remains questionable whether a thrombin generation parameter that corresponds better with FVIII activity level also reflects the hemostatic potential of the patient, especially since previous studies have shown that thrombin potential could better identify patients with an increased bleeding risk [14]. Furthermore, velocity of thrombin generation is calculated with a formula that consists of three components of the thrombin generation assay (lag time, time to thrombin peak, and thrombin peak height) and is therefore vulnerable to artifacts when one or more of these parameters are slightly aberrant. Therefore, thrombin potential could be a better parameter to use to adjust dosage of FVIII replacement therapy. It has a better correlation with bleeding phenotype and can differentiate patients into mild and severe bleeding phonotypes based on the ABR [32, 33]. Furthermore, it can improve identification of patients who will bleed while treated compared to FVIII activity level [14]. The main difficulty, however, is that trough levels of thrombin generation are not known yet. A prospective study investigating the optimal amount of thrombin generation to prevent bleeding is needed.

The previously developed population pharmacokinetic model by Bukkems et al. could not sufficiently predict the FVIII activity levels in the current study, since the predictions were higher compared to the observed FVIII activity levels. Therefore, a novel population pharmacokinetic model was developed which estimated a higher clearance (3.07 dl/h) compared to clearance of Bukkems et al. (1.69 dl/h). The volume of distribution is larger as well (39.1 dl versus 27.7 dl) [16]. Bukkems et al. previously also published a population pharmacokinetic model describing the interaction between FVIII and VWF in von Willebrand

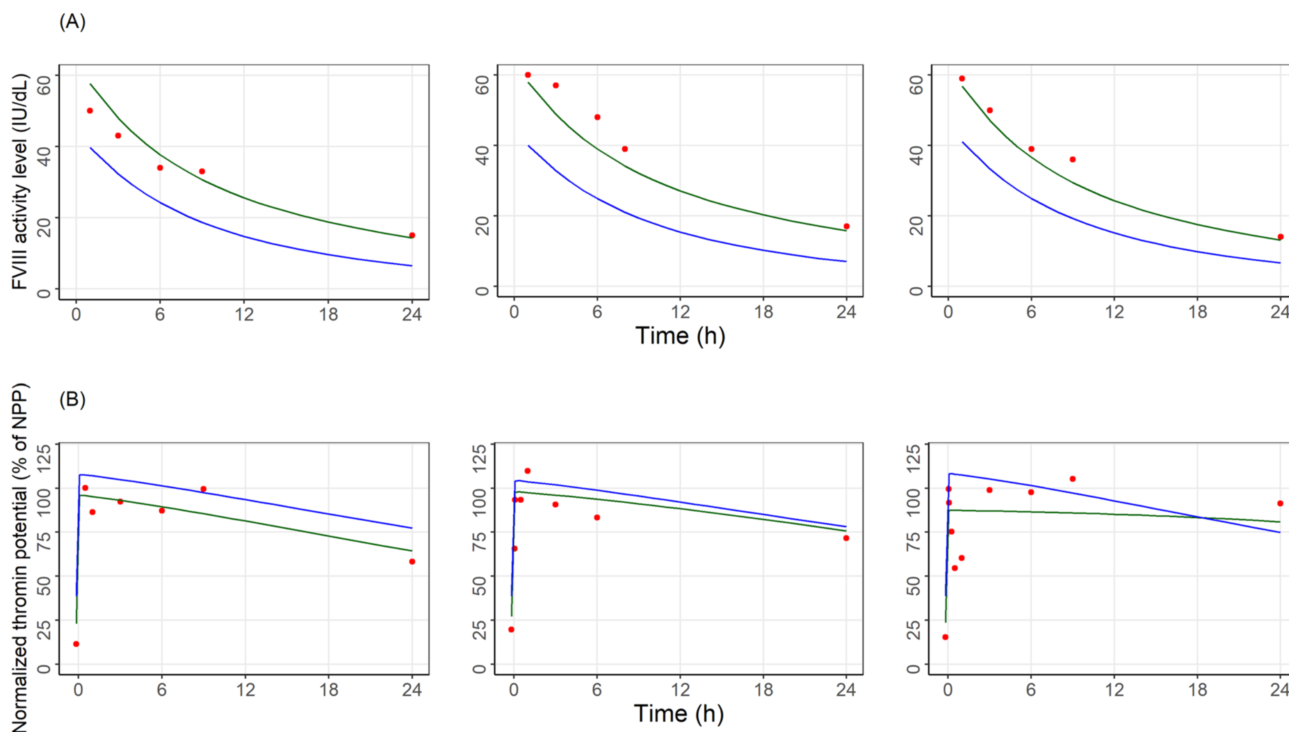

Fig. 6 Three patients from the external dataset with a similar pharmacokinetic profile displaying a different normalized thrombin potential response after factor VIII administration using the model by Bukkems et al. [16] The green lines display the individual prediction, the blue lines display the population prediction, and the red dots display the observed data

disease, in which patients received pdVWF/FVIII. The volume of distribution was estimated as 44.4 dl, which was similar to our estimate (39.1 dl), whereas the clearance was estimated as 1.17 dl/h, which was different compared to our estimate (3.07 dl/h) [31]. It is known that VWF protects FVIII from proteolysis [5]; therefore, we expected a lower clearance in the novel population. In this study, all 29 patients received pdVWF/FVIII whereas in Bukkems et al. only three received pdVWF/FVIII. Hence, the influence of pdVWF/FVIII could be underestimated in the population pharmacokinetic of Bukkems et al. Moreover, the interindividual variability in the clearance was 41.2% in Bukkems et al. In the previous population pharmacokinetic model, a significant effect between a very low-titer inhibitor and clearance and volume of distribution was found. Patients with a very low-titer inhibitor have 149% increase in clearance. In the current study, only a significant effect between a positive NBA and clearance was found.

The predictive performance of the previously developed pharmacodynamic models was also evaluated with the current dataset. Predictive performance was adequate when using the normalized thrombin potential models, but overprediction was observed when using the normalized thrombin peak height and plasmin peak height model. However, there was no bias in the predictions of the normalized thrombin peak height (MPE $< 20\%$), but the MAPE was slightly $> 30\%$, which shows inaccurate predictions. A reason for the overprediction in plasmin peak height model could be that the estimated baseline was different in our dataset (81.2% of NPP) compared to that of Bukkems et al. (125% of NPP). This difference is probably caused by an assay artifact in plasmin peak height determination. Re-estimations of the pharmacodynamic parameters for normalized thrombin peak height and normalized thrombin potential models displayed similar parameter estimation, further enhancing the adequate predictive performance of the previously developed models. In the normalized thrombin potential, we estimated an EC50 of 1.93 IU/dl, whereas Bukkems et al. estimated an EC50 of 13.9 IU/dl. In both models, a low level of FVIII was already sufficient to produce a higher normalized thrombin potential. As a result, the normalized thrombin potential displayed a sustained effect after 24 h (Fig. 4C). Moreover, the pharmacodynamic models have a high IIV and residual error. Even after improving the models, there is still considerable inter-patient and residual variability. In this study, we did not opt to use Bayesian approaches because we wanted to investigate the predictive performance of the previous models and re-estimate parameters if no adequate predictions were made. However, Bayesian approaches can be a valuable tool for improving

predictive performance by individualizing models and incorporating prior knowledge.

This study has a number of limitations. First, included patients were from Iran, and samples were handled, frozen, and afterwards collectively shipped to Nijmegen, The Netherlands, on dry ice. Whether this could have influenced the quality of the samples and impacted results is not known. For example, the 3-h sample showed lower than expected results in some patients, possibly because of pre-analytical disturbances. To prevent further deterioration by repeated thaw-freeze cycles, all samples were defrosted only once to measure FVIII activity levels, inhibitor titers, and NHA at one occasion. However, lack of standardization or pre-analytical and analytical procedures is still a major. Second, samples for the pharmacokinetic–pharmacodynamic study were only collected for the first 24 h. Therefore, we were unable to identify the course of thrombin generation after 24 h, which would be interesting, as FVIII activity levels had not reached the baseline level corresponding to the grade of hemophilia severity while thrombin potential was still increased. To overcome this difficulty with the model development, the pre-bolus sample was also used as if it were determined after 72 h, which was equal to the washout period. Third, numerous studies have previously shown a clear association between thrombin generation and bleeding phenotype as determined with the annual bleeding rate (ABR). Here, the ABR was not systematically determined when patients were included, and it was not possible to determine the ABR retrospectively. Because this is associated with reporting and recall bias, we were not able to include an analysis between ABR and as such the bleeding phenotype and pharmacokinetic–pharmacodynamic parameters. Lastly, VWF was only measured before pdVWF/FVIII concentrate administration. VWF is known to act as a protector and chaperone of FVIII. In the previously developed population pharmacokinetic model, VWF had an effect on the clearance of FVIII. Unfortunately, the effect of VWF could not be tested in the external dataset because samples after administration did not include measurements of VWF because of insufficient sample volumes.

Despite the development of non-factor replacement therapies for HA (like emicizumab), FVIII concentrate will remain an important part of the treatment. FVIII concentrates are still used for bleeding episodes and during the peri-operative period during prophylactic therapy with non-factor concentrates. Therefore, it remains important to measure and improve dosing of FVIII concentrates. Furthermore, expensive non-factor concentrates will remain out of reach for large parts of the world while the decreasing price of FVIII concentrates means they will remain an important cornerstone in the treatment of HA. This study adds to the knowledge on optimal dosing FVIII concentrates based on pharmacokinetic–pharmacodynamic measurements, which can become available to larger parts of the world because of point-of-care measurement techniques and digitally powered devices.

5 Conclusion

The previously developed population pharmacodynamic models of normalized thrombin peak height and thrombin potential were able to adequately predict the observations in our external dataset. These thrombin generation models can be used to guide the application of pharmacokinetic–pharmacodynamic-guided dosing of FVIII concentrates in patients with hemophilia A. A prospective study in which this thrombin generation pharmacodynamic-based dosing model is used and combined with bleeding phenotype data to individualize prophylactic therapy with FVIII concentrate will answer the question of whether the current prophylactic HA management can be further individualized and improved.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13318-024-00876-6.

Acknowledgements *SYMPHONY consortium* The SYMPHONY consortium, which aims to orchestrate personalized treatment in patients with bleeding disorders, is a unique collaboration among patients, health care professionals, and translational and fundamental researchers specialized in inherited bleeding disorders as well as experts from multiple disciplines. It aims to identify best treatment choice for each individual based on bleeding phenotype. To achieve this goal, workpackages have been organized according to three themes: Diagnostics (workpackages 3 and 4), Treatment (workpackages 5–9), and Fundamental Research (workpackages 10–12). This research receives funding from the Netherlands Organisation for Scientific Research (NWO) in the framework of the NWA-ORC Call grant agreement NWA.1160.18.038. Principal investigator: Dr. M.H. Cnossen. Project coordinator: Dr. S.H. Reitsma. Beneficiaries of the SYMPHONY consortium: Erasmus University Medical Center-Sophia Children's Hospital, project leadership and coordination; Sanquin Diagnostics; Sanquin Research; Amsterdam University Medical Centers; University Medical Center Groningen; University Medical Center Utrecht; Leiden University Medical Center; Radboud University Medical Center; Netherlands Society of Hemophilia Patients (NVHP); Netherlands Society for Thrombosis and Hemostasis (NVTH); Bayer B.V., CSL Behring B.V., Swedish Orphan Biovitrum (Belgium) BVBA/SPRL. Additional beneficiaries, not included in the SYMPHONY consortium, currently funding parallel projects are: Novonordisk (OPTI-CLOT TARGET), Roche (Partitura), and Stichting Haemophilia (patient-reported outcomes project).

Author contributions LV analyzed the data, performed the research, and wrote the manuscript. MC analyzed the data and wrote the manuscript. HM recruited the patients and prepared the samples. WB conducted the NHA measurements. MHC, SS, WvH, NB, and RM critically revised the manuscript. RM also supervised the analysis. WvH designed the study and oversaw execution of measurements and analysis of the data. All authors gave final approval for publication.

Funding MC was funded by SYMPHONY (grant no. NWA.1160.18.038).

Data availability The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Code availability NONMEM codes are available from the corresponding author on reasonable request.

Declarations

Conflict of interest MC was funded by a grant from the Netherlands Organisation for Scientific Research (NWO) in the framework of the NWA-ORC Call grant agreement NWA.1160.18.038. MHC has received grants outside the submitted work from governmental research institutes such as NWO: ZonMW and NWO-NWA and the Innovation Fund, and an unrestricted investigator initiated research grants as well as educational and travel funding from the following companies over the years: Pfizer, Baxter/Baxalta/Shire, Bayer Schering Pharma, CSL Behring, Sobi Biogen, Novo Nordisk, Novartis, and Nordic Pharma, and has served as a member on steering boards of Roche, Bayer, and Octapharma. All grants, awards, and fees are always collected by the institution. RM has received grants from governmental and societal research institutes such as NWO, ZonMW, and Innovation fund and unrestricted investigator research grants from Baxter, Baxalta, Shire, Takeda, Bayer, CSL Behring, and Sobi. He has served as advisor for Bayer, CSL Behring, Merck Sharp & Dohme, Baxter, Baxalta, Shire, and Takeda. All grants and fees were paid to the institution. WvH received unrestricted grants from Bayer, Takeda, Novo Nordisk, and CSL Behring. WvH is the co-founder and CSO of Enzyre BV, a Radboudumc spinoff company. LV, MC, WB, HM, NB, and SS have no conflicts of interest to declare.

Ethical approval This project was a sub-study of the IMPALA study (registered at the Dutch Trial Register, no. NL2808) and was approved by the Medical Ethics Committee of the Radboud University Medical Center on 21 July 2011 and by the regional ethics committee of Mashhad University of Medical Sciences on 9 November 2016 (no. 89-88215).

Consent to participate All patients gave written informed consent, and the study was conducted in accordance with the Declaration of Helsinki.

Consent to publish Not applicable

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