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A Novel, Enriched Population Pharmacokinetic Model for Recombinant Factor VIII-Fc Fusion Protein Concentrate in Hemophilia A Patients

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Abstract

Background The currently published population pharmacokinetic (PK) models used for PK-guided dosing in hemophilia patients are based on clinical trial data and usually not externally validated in clinical practice. The aim of this study was to validate a published model for recombinant factor VIII-Fc fusion protein (rFVIII-Fc) concentrate and to develop an enriched model using independently collected clinical data if required.

Methods Clinical data from hemophilia A patients treated with rFVIII-Fc concentrate (Elocta) participating in the United Kingdom Extended Half-Life Outcomes Registry were collected. The predictive performance of the published model was assessed using mean percentage error (bias) and mean absolute percentage error (inaccuracy). An extended population PK model was developed using nonlinear mixed-effects modeling (NONMEM).

Results A total of 43 hemophilia A patients (FVIII $\leq$ 2 IU/dL), aged 5 to 70 years, were included. The prior model was able to predict the collected 244 rFVIII-Fc levels without

Keywords

- recombinant factor VIII-Fc
- population
- pharmacokinetics
- child
- validation

* Shared first and last authorship.

significant bias (−1.0%, 95% CI: −9.4 to 7.3%) and with acceptable accuracy (12.9%). However, clearance and central distribution volume were under predicted in patients <12 years, which was expected as this age group was not represented in the previous model population. An enriched population PK model was constructed, which was able to successfully characterize PK profiles of younger children.

Conclusion We concluded that the existing rFVIII-Fc population PK model is valid for patients ≥ 12 years. However, it is not reliable in younger patients. Our alternative model, constructed from real world patient data including children, allows for better description of patients ≥ 5 years.

Introduction

Hemophilia A is a severe bleeding disorder caused by a deficiency of coagulation factor VIII (FVIII). Treatment consists of replacement therapy with plasma-derived or recombinant FVIII concentrates, administered prophylactically to prevent bleeding, or on demand to treat bleeding episodes and to prevent bleeding in the perioperative setting. Traditionally, prophylactic therapy aims to maintain FVIII trough levels >1 IU/dL, thereby altering patients with severe hemophilia A with a baseline FVIII activity <1 IU/dL into patients with a moderate hemophilia A with FVIII activity between 1 and 5 IU/dL.^{1,2}

In most hemophilia A patients, standard half-life (SHL) FVIII concentrates are infused intravenously every 24 to 48 hours. Due to the invasiveness of therapy, efforts have been made to prolong the FVIII elimination half-life by improving the pharmacokinetic (PK) properties of these concentrates. Recombinant FVIII coupled to the Fc portion of human immunoglobulin G1 (rFVIII-Fc) is one of these so-called extended half-life (EHL) concentrates, demonstrating a 1.5- to 1.7-fold increase in elimination half-life when compared with SHL rFVIII concentrates.^{3,4}

Traditionally, the dose and interval of prophylactic replacement therapy are based on the patients' body weight and are increased in frequency and dose when (spontaneous) bleeding or bleeding after minor trauma occurs. However, this approach results in large differences in achieved FVIII activity levels after infusion due to large interindividual variability in PK.⁵ This may therefore result in unnecessarily high levels in some patients and insufficient levels in others. A more optimal approach would be administration of a dose which is directly able to safeguard targeted trough levels at initiation of therapy, resulting in less frequent monitoring. This is possible by application of PK-guided dosing using Bayesian estimation, leading to tailoring of dose and dosing interval based on a patients' individual PK.⁶ During this procedure, individual PK parameters are estimated by combining observed FVIII activity levels from an individual patient with information from the population at large on the basis of a population PK model. With these individual PK parameters, the required dose to maintain a desired target level can be calculated. This approach is however not feasible if reliable and validated population PK models are lacking.

Currently, available population PK models for factor concentrates are often based on drug trial data and seldomly externally validated in clinical practice. This means that the predictive performance of the population PK models are usually not assessed with independent data. Likewise, the published population PK model for the EHL concentrate rFVIII-Fc is not externally validated yet.⁷ Moreover, this population PK model is only based on data from severe hemophilia A patients ≥ 12 years while rFVIII-Fc is registered for use in all age groups. It is therefore favorable to describe the rFVIII-Fc PK in a population PK model for this broader application. Currently, to enable description of rFVIII-Fc PK in younger patients, extrapolation of the available population PK model must be applied which may lead to errors due to significant differences in FVIII PK in children versus adults.⁸ Therefore, the aim of our study was to validate the previously published rFVIII-Fc concentrate population PK model in hemophilia A patients on prophylaxis using clinical data from all age groups and to subsequently construct an enriched model if deemed necessary.

Methods

Data Collection

Clinical data from hemophilia A patients (baseline FVIII activity: ≤ 2 IU/dL) treated prophylactically with rFVIII-Fc concentrate participating in the United Kingdom Extended Half-Life (UK-EHL) Outcomes Registry were collected. The UK-EHL registry is registered at ClinicalTrials.gov under identifier NCT02938156. The included patients were treated in eight different centers in the United Kingdom and children as well as adults were included. Informed consent was obtained from all patients before inclusion in the UK-EHL Outcomes Registry. The EHL FVIII concentrate used in this study was a novel single B-domain-deleted recombinant FVIII protein fused with a Fc domain of the human immunoglobulin G1 (Elocta).

The UK-EHL Outcomes Registry contains patient characteristics and treatment information such as the administered rFVIII-Fc dosages and measured FVIII levels during PK-profiling. During PK-profiling, FVIII levels were measured at approximately 15 minutes, 3, 6, 24, 48, 72, and 96 hours post-rFVIII-Fc concentrate dose by one stage assay (OSA) according to United Kingdom Hemophilia Centre Doctors' Organization guidelines for PK profiling when switching to an EHL concentrate.⁹ As suggested in this guideline, fewer

blood samples were obtained in children and 24 or 96 hour blood sampling was omitted. Exact timing of rFVIII-Fc infusion and blood sampling was documented; however, timing could differ due to circumstances. Sparse additional data were obtained during subsequent prophylactic and breakthrough bleeding treatment. In a subset of cases, blood samples were also measured by chromogenic substrate assay (CSA). The lower limit of quantification (LLOQ) was 1 IU/dL for the OSA method. For the CSA method, LLOQ differed between 1 and 6 IU/dL, depending on the treatment center. None of the patients were tested positive for inhibitors during study inclusion. Other patient characteristics collected were: height, weight, sex, age, blood group, hematocrit levels, hemoglobin levels, von Willebrand Factor Antigen (VWF:Ag), baseline FVIII, F8 DNA mutation, number of joint bleeds in the year before switching to EHL concentrate, total number of bleeds before switching, and the presence of target joints, defined as three joint bleeds in specific joint during last 6 months. The data also included presence of comorbidities such as liver function anomalies, hepatitis C, HIV, hypertension, diabetes, and ischemic heart disease and history of inhibitors.

Population Pharmacokinetic Model

A published rFVIII-Fc concentrate population PK model was validated with clinical data using Bayesian estimation in NONMEM (v7.4.1, Icon Development Solutions, Gaithersburg, Maryland, United States).^{7,10} Data visualization, model management, and evaluation were accomplished with R 3.5.1, Pirana 2.9.9., and PsN 4.8.1. The validated population PK model is a two-compartment model and includes the following PK parameters: central volume of distribution (V1, scaled to body weight), peripheral volume of distribution (V2), clearance (CL), and intercompartmental clearance (Q). The model is based on FVIII samples measured with OSA and incorporates a negative association between VWF:Ag level and clearance plus an association between hematocrit level and volume of distribution.⁷ The PK parameter estimates of the final population PK model can be found in ►Table 1. Detailed information on the population PK model can be found in reference¹¹.

Clusters of observations, separated from previous observations by at least 1 week, were defined as a single occasion. This variable was added to incorporate interoccasion variability (IOV) and was handled in the same way as done in the previously published population PK report.⁷ Observed FVIII levels before the administration of rFVIII-Fc concentrate could represent the baseline of the patient (endogenous FVIII; lowest FVIII ever measured) as well as the residual activity after a previous dose of the SHL product before switch (exogenous FVIII). Therefore, FVIII levels were corrected for FVIII baseline by subtraction, and residual levels by assuming first-order elimination of the observed residual levels.^{7,11–13}

The VWF:Ag and hematocrit levels were assumed to be constant until the next measured value, using the last observation carried forward method. In case hematocrit levels were not available for a patient, they were denoted as missing and set to the median value described in the published population PK model. Missing VWF:Ag levels were

imputed on basis of patient age.¹⁴ A linear regression line, characterizing the relationship between age and VWF:Ag, was created based on the available VWF:Ag levels in the dataset. Missing VWF:Ag values were calculated using this regression line.

Validation of Published Recombinant Factor VIII-Fc Concentrate Population Pharmacokinetic Model

Individual PK parameters were estimated and predicted FVIII levels were calculated using Bayesian estimation. Predictive performance of the population PK model was evaluated by comparing observed FVIII levels with predicted FVIII levels. Therefore, mean percentage error (MPE, Eq. 1) and mean absolute percentage error (MAPE, Eq. 2) were calculated by representing bias and inaccuracy, respectively. Observed FVIII levels measured by OSA were used for validation, as samples were measured with this assay in the published model. The validation analysis was also performed with CSA measured levels for comparison of the results using different assays.

$$MPE = \frac{1}{n} \sum_{j=1}^n \left(\frac{C_{pred} - C_{obs}}{C_{obs}} \right) \times 100\% \quad (1)$$

$$MAPE = \frac{1}{n} \sum_{j=1}^n \left| \frac{C_{pred} - C_{obs}}{C_{obs}} \right| \times 100\% \quad (2)$$

Cpred represents the population predicted level and Cipred the individually predicted level of measurement *j*. The total number of measurements is represented by *n*. Bias was regarded as nonsignificant when zero was included in the confidence interval (CI). Inaccuracy less than the an arbitrarily chosen 25% was accepted. Additionally, predictive performance was visualized in goodness-of-fit plots. In these plots, the relationship between population or individual prediction and observed FVIII plasma levels were presented. The goodness-of-fit plots also include plots depicting difference between observed and predicted FVIII level, specified as the conditional weighted residuals, to the time after dose and population prediction.

Evaluation of predictive performance of the model in more detail was done by plotting patient characteristics such as age and body weight against the interindividual variability (ETA, η) in the PK parameters CL and V1. Interindividual variability displays the variation in a PK parameter between patients due to varying physiology.

Development of Alternative Population Pharmacokinetic Model

An alternative population PK model was constructed when validation results were insufficient or to describe the expanded population with children. The PK parameters were reestimated and number of compartments, inclusion of IIV and IOV were evaluated. Initial evaluation of the models was done by examination of the PK parameters estimates, their residual standard errors, goodness-of-fit plots, and objective function value (OFV), which expresses the ability of a model to describe observations by minimizing likelihood. Application of the M3 method was evaluated when the number of samples below quantification limit was >10%.^{15,16}

Table 1 Pharmacokinetic parameter estimates of the previously published and novel population pharmacokinetic FVIII-Fc models

	Published model ⁷	Final enriched model	
Parameter	Estimate	Estimate (RSE %) [Shr.]	Bootstrap: estimate (95% CI)
V1 (dL)	36.8	35.5 (5.7)	35.5 (31.0–39.9)
Body weight exponent on V1	0.423	0.89 (12.6)	0.90 (0.60–1.18)
Hematocrit exponent on V1	–0.412		
V2 (dL)	4.09	5.32 (20.9)	5.37 (3.60–9.27)
CL (dL/h)	1.73	1.74 (7)	1.70 (1.46–1.92)
VWF exponent on CL	–0.391	–0.395 (45.3)	–0.41 (–0.86 to –0.03)
Weight exponent on CL		0.635 (13.3)	0.63 (0.25–0.90)
Q (dL/h)	0.279	0.665 (12.3)	0.71 (0.37–4.87)
Exponent CSA		1.06 (1.2)	1.06 (1.03–1.09)
Interindividual variability			
IIV on V1 (CV%)	13.6	27.0 (17.9) [10.6]	25.6 (14.8–37.1)
IIV CL (CV%)	25.1	36.5 (16.8) [13.2]	34.8 (22.8–47.1)
Correlation IIV CL and V1	0.563	0.599	0.56 (0.091–0.88)
Interoccasion variability			
IOV V1 (CV%)	9.27		
IOV CL (CV%)	22	20.9 (12.5) [41.9]	20.6 (15.3–25.8)
Correlation IOV CL and V1	0.526		
Residual variability			
Proportional error OSA (%)	15.4	17.8 (20.7)	0.17 (0.10–0.24)
Additive error OSA (IU/dL)	0.240	1.01 (12.4)	1.0 (0.66–1.27)
Proportional error CSA (%)	–	24.6 (12.4)	0.23 (0.16–0.29)

Abbreviations: CL, clearance; CSA, chromogenic assay; CV, coefficient of variation calculated as $\sqrt{(\exp(\omega^2)-1)}$.

*100; OSA, one stage assay; Q, intercompartment clearance; RSE, relative standard error; Shr, shrinkage; V1, central volume of distribution; V2, peripheral volume of distribution.

Published model:

$$CL = \theta_{CL} * \left(\frac{VWF:Ag_{ij}}{118} \right)^{-0.391} * e^{\eta_{CL}};$$

$$V1 = \theta_{V1} * \left(\frac{Weight_i}{73} \right)^{0.423} * \left(\frac{Hematocrit_{ij}}{45} \right)^{-0.412} * e^{\eta_{V1}};$$

$$Q = \theta_Q;$$

$$V2 = \theta_{V2}$$

New expanded model:

$$CL = \theta_{CL} * \left(\frac{Weight_i}{73} \right)^{0.635} * \left(\frac{VWF:Ag_{ij}}{118} \right)^{-0.395} * e^{\eta_{CL}};$$

$$V1 = \theta_{V1} * \left(\frac{Weight_i}{73} \right)^{0.89} * e^{\eta_{V1}};$$

$$Q = \theta_Q;$$

$$V2 = \theta_{V2}$$

$$CSA\ activity = OSA\ activity^{1.06}.$$

Covariate analysis was performed by testing whether the collected patient characteristics described under data collection reduced IIV of PK parameters. A forward/backward selection approach was applied: first, univariate covariates were screened for relevance. When one covariate was added to the model an OFV drop of 3.84 was regarded as a significant improvement of the model ($p < 0.05$, Chi-squared distribution). Thereafter, all significant covariates were added to the model at once, and backward elimination of a covariate should result in an OFV raise of >6.64 ($p < 0.01$) for the parameter to be considered as significantly improving the model. Missing covariates were handled as described before.

The alternative population PK model was first developed using only OSA data. Thereafter, CSA data were added to the final OSA PK model and analyzed simultaneously with the OSA data. In this model, a function was incorporated to describe relationships between OSA and CSA results. Different residual proportional and/or additive errors were installed for the OSA and CSA measured samples. The newly developed population PK model was internally validated with visual predictive checks and a bootstrap.

Clinical Case

A clinical case from the Pediatric Hematology Department of the Amsterdam University Medical Centers was used to demonstrate the difference between the published and the novel population PK model. Following national and international guidelines for concentrate switching, a PK profile was constructed for a 2-year-old patient with severe hemophilia A to safely switch from a SHL to an EHL concentrate.^{9,17} The boy received a dose of 500 IU rFVIII-Fc concentrate and serial blood samples were collected at approximately 15 minutes, 4, 24, 48 and 72 hours after administration. The FVIII levels were measured by chromogenic assay (LLOQ: 0.4 IU/dL) on the Siemens CS-2500, using the Siemens factor FVIII chromogenic kit. PK profiles were composed based on the previously published model and our novel expanded population PK model. Individual PK parameters were assessed by Bayesian analysis, and dosing schemes were calculated to maintain trough levels >1 IU/dL while administering rFVIII-Fc concentrate twice a week using multiplications of 250 IU rFVIII-Fc, which corresponds to the available industry vials. Informed consent from this patients' parents was not needed according to the Dutch law (WMO, article 1) as only treatment data obtained during routine clinical care—following the Dutch SHL/EHL switching protocol—was used.

Results

Patient Characteristics and Pharmacokinetic Profiling

Data from 43 patients, including eight children aged 5 to 11.5 years and 35 adults aged 12 to 70 years, were analyzed and used for validation of the previously published population PK model. Data included 244 FVIII levels measured by OSA. From 28 patients, a total of 111 FVIII levels measured using CSA were also available. Patients received rFVIII-Fc bolus infusions with a median dose of 35 IU/kg ranging from 10 to 132 IU/kg, resulting in a median *in vivo* recovery of 2.07 IU/dL per IU/kg

(range: 1.53–3.75). In adult patients, a median of six samples (range: 1–7) was measured during the first occasion. In children (age <12 years), this median number of samples was 4.5 (range: 1–6). Of the total number of samples, 9 (4%) OSA and 17 (15%) CSA FVIII levels were below the limit of quantification. Baseline FVIII of 41 patients was <1 IU/dL. Two patients had baseline levels ≥ 1 and ≤ 2 IU/dL; baseline correction was performed for these patients. VWF:Ag levels at the start of inclusion were reported for 25 patients. The obtained linear regression line, based on the available VWF:Ag levels and age, was used to estimate the VWF:Ag levels when missing. The equation of this regression line was $y = 82.009 + 1.1609x$ (► **Supplementary Fig. S1** [available in the online version]). Hematocrit levels were measured in 18 patients, in these patients the number of observed levels ranged from 1 to 7 with a median of 2. In ► **Table 2**, the demographics of all patients are summarized.

Validation of the Published Recombinant FactorVIII-Fc Concentrate Population Pharmacokinetic Model

The predictive performance of the published population PK model for rFVIII-Fc was evaluated by comparing the observed levels with the predicted levels. The model showed a non-significant bias with a MPE value of -1.0% (95% CI: -9.4 to 7.3%). MAPE was 12.9% (95% CI: 10.7 – 15.1%), indicating adequate accuracy of the model. The goodness-of-fit plots of the validation with the OSA samples can be found in ► **Supplementary Fig. S2** (available in the online version) and showed no structural bias, which is demonstrated by the approximation of the trend lines to the line of identity. In both the population as well as the individual prediction versus observed FVIII levels plots, acceptable accuracy was visualized by the closeness of the data points to the line of identity. For comparison, the goodness-of-fit plots of validation with CSA measured FVIII levels are added to ► **Supplementary Fig. S2** (available in the online version). The trend lines of the samples measured with CSA deviate further from line of identity indicating a structural bias. This was expected as FVIII levels measured by CSA were generally higher than levels measured by OSA in severe hemophilia patients (► **Supplementary Fig. S3** [available in the online version]). The validated population PK model was built with OSA data and thus includes “OSA PK parameters” of rFVIII-Fc.

When focusing on the data from the eight patients <12 years of age, a bias was seen in the interindividual variability of clearance and central distribution volume which was caused by the 8 patients <12 year weighing ≤ 38 kg (► **Fig. 1A, B**). In principle, no trend should be visible in these plots, as interindividual variability in CL and V1 should be divided randomly over all age and weight groups without bias, meaning that a typical patient in the model is described well over the complete age and weight range.

Development of an Alternative Population Pharmacokinetic Model

Using the prospectively collected data from this study, a novel and expanded population PK model was developed to enable accurate description of children younger than 12 years. During

Table 2 Baseline study patient characteristics

Demographics	Median (minimum–maximum) or number (frequency)	Number of patients available data
Number of patients	43	–
Male sex	43 (100.0%)	43
Age (y)	28 (5–70)	43
Bodyweight (kg)	72 (20–113)	43
Height (cm)	172 (113–190)	42
Baseline FVIII (IU/dL) ^a	<1 (<1–2)	43
von Willebrandfactor:Ag (IU/dL)	110 (53–237)	25
Hematocrit (%)	43.5 (25.1–48.9)	37
Blood group O	17 (39.5%)	36
Ethnicity		
White	34 (79.1%)	–
Asian	4 (9.3%)	–
African	1 (2.3%)	–
Other	4 (9.3%)	–
Treatment		
Dose (IU/kg)	35 (10–132)	43
Samples per occasion	4 (1–9)	43
Joint status		
Presence of target joints	3 (7.0%)	42
Total number of bleed before switch	1 (0–19)	30
Number of joint bleeds before switch	0 (0–10)	30
Comorbidities		
Liver function disorder	3 (7.0)	39
Hepatitis C cleared/sustained remission	16 (39)	41
Active hepatitis C	1 (0.02)	41
HIV positive	6 (14.0)	43
Hypertension	7 (16.3)	43
Diabetes	2 (4.7)	43
Ischemic heart disease	1 (2.3)	43

^aMeasured by one-stage assay.

development of a population PK model, a whole population can be investigated at once even though different PKs are expected between children and adults as is the case in hemophilia patients.⁸ In these integrated models, influence of body size on the PK parameters is accounted for by scaling the parameters by body weight or by inclusion of an association between the parameter and age.

The PK of rFVIII-Fc concentrate was best described by a two-compartment model. IIV could be estimated for both CL and V1; IOV was estimated for CL only. The current data did not support the estimation of IOV for V1. The M3 method was applied as the number of samples BQL in the CSA data was >10%. Incorporation of a residual error per center or combination of centers did not significantly improve the model; therefore, all centers were described by the same residual error. PK parameters were normalized to bodyweight as both

data of children and adults were available. Univariate analysis of the covariates showed statistically significant effects of age, blood group O, VWF:Ag, and presence of target joints on CL. After forward inclusion and backward deletion, only the effect of VWF:Ag on CL was retained in the final model. Since 15 different DNA mutations were present in the collected patient data, we were not able to demonstrate the influence of F8 gene mutations on the PK parameters.

The correlation between the OSA and CSA measured FVIII levels was explored by plotting the levels of the samples that were measured with both assays against each other (► **Supplementary Fig. S3** [available in the online version]). Linear, exponential, and polynomial functions were tested to describe relation between the two assays. The model containing an exponential function showed the lowest OFV, best goodness-of-fit plots and more precise PK parameter

Fig. 1 Interindividual variability of (A) clearance (CL) or (B) central volume of distribution (V1) versus body weight using the earlier published model. Corresponding plots C and D present the interindividual variability for the developed population pharmacokinetic rFVIII-Fc model. The individual data (black circles) are visualized as a trend line (blue solid line) that approximates the line of identity (black solid line). With the introduction of weight as covariate on clearance in the new model a less biased estimation is obtained at lower weight.

estimates, and therefore proved to describe the relation between the two assays best. The relation between the two assays was modeled as in Eq. 3, meaning that a FVIII level of 100 IU/dL measured with OSA relates to a FVIII level of 132 IU/dL measured with CSA.

$$\text{Predicted CSA activity} = \text{predicted OSA activity}^{1.06} \quad (3)$$

The final alternative real world model did not display biased values of IIV of CL and V1 when plotted versus body weight (►Fig. 1C, D). In ►Table 1, the parameter estimates of the final real world rFVIII-Fc PK model can be found and compared with those from the previously published model. ►Fig. 2 presents the internal validity of the final model using a visual predictive check for both OSA and CSA; bootstrap results are added to ►Table 1. Goodness-of-fit plots can be found in ►Supplementary Fig. S4 [available in the online version].

Clinical Case

A PK profile was constructed for a boy aged 2 years with severe hemophilia A (FVIII baseline activity <1 IU/dL) to compose PK-guided prophylactic dosing advice. The VWF:Ag and hematocrit level were not available for this patient. The missing VWF:Ag level was imputed with the obtained linear regression line. One level was BQL and therefore, the M3 method was used to derive the individual PK parameters. PK profiles were constructed using the previously published

and the alternative real world PK model. In the latter model, calculations were also made with uncorrected activity levels (e.g., OSA levels). In ►Fig. 3, the PK profiles are shown. The PK profile defined using the new model with adequate correction for CSA samples could best describe both peak as the trough levels, and this dosing scheme is therefore regarded as most trustworthy. The PK profile made with the new PK model without correction for the OSA samples was able to describe FVIII peak levels well; however, FVIII trough levels could not be described adequately.

►Fig. 3 also displays the PK profiles and the calculated dosing schemes to maintain trough levels >1 IU/dL when dosing twice weekly. Considerable difference existed between the applied population models: calculated twice weekly doses were 2,750 IU for the published model, 1,750 IU for the new model using correction for CSA levels, and 1,000 IU for the new model without this correction (e.g., OSA levels).

Discussion and Conclusion

The aim of this study was to validate a previously published rFVIII-Fc population PK model with clinical data and to construct an expanded model if necessary. The published population PK model showed adequate predictive performance in the collected clinical data of patients ≥ 12 years, expressing adequate performance of the model in external populations ≥ 12 years. Since the published population PK

Fig. 2 Visual predictive check of (left) real world enriched rFVIII-Fc model for one stage assay samples and (right) chromogenic assay samples are shown in the upper panel. The median (red line) and 95% confidence interval (blue lines) of the observed data (black dots) are plotted against the simulated data ($n = 1,000$) indicated as highlighted areas: the red box being the median and the blue box the 95% prediction interval. A model predicts the concentrations adequately when the blue and red lines run through the corresponding boxes. The line in the lower panel shows the proportion of the observed samples below the limit of quantification (LOQ). The blue shaded area indicates the 90% confidence interval of the proportion of the 1,000 simulated samples <LOQ. The different LOQ values of 1, 3, and 6 IU/dL were taken into account. The dataset also contained several samples with an FVIII level of 1 IU/dL, LOQ, limit of quantification.

model for rFVIII-Fc was developed with data from patients ≥ 12 years, it is not surprising that the population parameter estimates for clearance and central distribution volume in children < 12 years weighing ≤ 38 kg were underestimated and that the model is not suitable for these patients. With the newly collected data, containing eight children < 12 years with a median age of 9.5 years (range: 5–11.5 years), an expanded population PK model could be developed.

The expected differences between children and adults in FVIII PK could be explained by the introduction of total body weight on clearance, enabling the model to give patients with a lower body weight a reduced clearance. Other published FVIII population PK models based on children and adult data also contain the relationship between weight (total body weight or lean body mass) and clearance.^{5,18–20} The wider range in body weight in the new dataset (20–113 kg) compared with the data

Fig. 3 PK profile of the clinical case using the (A) previously published, (C) real world rFVIII-Fc PK model and (E) without correction for CSA method. Based on the PK profile of the (B) previously published, (D) real world rFVIII-Fc PK model with correction for CSA samples, and (F) without correction for CSA method, the required dosing schemes to maintain FVIII levels >1 IU/dL (dashed line) with two times a week dosing are drafted for this patient. The dark blue line displays the individually predicted concentration. The red dots are the measured FVIII levels; one level (orange dot) was below quantification limit (<0.4 IU/dL). CSA, chromogenic assay; PK, pharmacokinetic.

of the previously published model (42 and 127 kg) enabled statistically significant description of the effect of body weight on FVIII clearance.^{3,4} However, since only eight children were included in this analysis, validation with a larger pediatric population is recommended.

Unfortunately, data on VWF:Ag and hematocrit as covariates were limited and missing in almost half of the patients. Therefore, the data were insufficient to prove the effect of hematocrit on CL that was described in the published population PK model.⁷ When this relation was excluded, the OFV increased by only 0.94 and was thus excluded. However, implementation of this covariate in clinical practice is also less feasible, as hematocrit levels are not regularly monitored in hemophilia A patients when on prophylaxis. Of 18 patients, including six of the eight children <12 years, VWF:Ag levels were not available. Since several studies have shown that VWF:Ag levels increase with age with approximately 15 to 17 IU/dL per 10 years, VWF:Ag levels were estimated based on the age of the patients using linear regression.^{14,21,22} The obtained linear regression line showed an VWF:Ag increase of 11.6 IU/dL per 10 years, which is in the same order of magnitude as found in literature. Estimation of VWF:Ag levels gives a better representation of true VWF:Ag levels than inserting the median

VWF:Ag of the population. Nonetheless, missing VWF:Ag values represent an important limitation of this study.

In many studies, discrepancies between FVIII levels measured with OSA or CSA are observed.^{23–25} For rFVIII-Fc concentrate, CSA seems to overestimate the FVIII *in vivo* recovery with 24% on average, resulting in higher FVIII peak level measurements.^{25–27} Higher FVIII levels as determined by CSA were also found in the present study. Consequently, when the previously published PK model based on OSA samples is used to predict the CSA levels, higher distribution volumes will be found. Therefore, the collected CSA samples were added to the population PK model and a correction factor was applied to correlate the levels of the different assays; a technique that has also been used in other population PK models.²⁸ An exponential function with a parameter of 1.06 could describe the relation between the different assay methods best, which indicates that a FVIII activity measured on an OSA of 100 IU/dL on average corresponds to a FVIII activity level of 132 IU/dL measured by CSA. This is about the same discrepancy as found in the published population PK model of BAX 855 in the higher FVIII level ranges.²⁸ The visual predictive check (–Fig. 2) shows that the population PK model describes the data well for both assays.

The presented clinical case underlines that PK guided dosing can only be performed when the model building population corresponds to the characteristics of the individual patient and validation is of importance. This young patient would have received a less optimal dosing scheme if the published population PK model was used for PK-guided dosing. Furthermore, it also demonstrates that the developed PK model with correction for CSA levels describes the achieved FVIII levels more optimally than the model without correction; that is, assuming that CSA levels are similar to OSA levels. This underlines the general assumption that the assay used to measure FVIII samples must be taken into account when performing PK-guided dosing. Naturally, this single case is not sufficient to significantly prove the validity of the alternative population PK model. However, it visualizes the clinical implications the use of a model not built for the used population can have.

In conclusion, the previously published rFVIII-Fc model was developed for patients ≥ 12 years and regarded as valid in this population. However, this population PK model will give erroneous results in patients <12 years as the relationship between body weight and clearance was not adequately described yet. This underlines that PK-guided dosing can only be performed when the model building population corresponds to the characteristics of the individual patient and that model validation is of importance. The novel expanded population PK rFVIII-Fc concentrate model, consisting of a population with a greater age range, is able to describe the clinical data of younger patients more adequately and is therefore more suitable for PK-guided treatment in severe to moderate hemophilia A patients receiving prophylaxis with this specific concentrate. However, external validation of this model including younger patients is recommended. Use of this population PK rFVIII-Fc model can aid in defining individual PK profiles with this concentrate and thereby calculating the most adequate dosing scheme.

What is known on this topic?

- Reliable population pharmacokinetic (PK) models are necessary to perform PK-guided dosing and individualize dosing regimens with factor concentrates.
- Population PK models are mostly based on drug trial data and are generally not externally validated before use in clinical practice.

What does this paper add?

- An existing rFVIII-Fc population PK model, based on patients ≥ 12 years, was validated with clinical data from the UK-EHL Outcomes Registry.
- The population PK model was regarded as valid in patient ≥ 12 years but under predicted PK parameters in patients.
- An alternative model was younger developed, which describes the rFVIII-Fc PK of younger patients more accurately and seems preferable for PK-guided treatment in younger patients.

Authors' Contributions

L.H.B. and R.A.A.M. analyzed the data and developed the population pharmacokinetic model. J.M.H. collected and checked all clinical data from the EHL registry. R.A.A.M., M. H.C. and P.C. designed and supervised the study, while M.M., P.W.C., C.R.M.H., F.W.G.L., K.M., and K.F. gave critical guidance. Patient inclusion in the UK EHL registry was monitored by M.M., P.W.C., C.R.M.H., R.C.T., S.M., B.M., G.E., B.B., N. C., J.P., and S.A. The clinical case was treated by K.F. All authors contributed substantially to the writing and critical revision of the manuscript and approved the final draft.

Note

This study is a collaboration between the international multicenter OPTI-CLOT consortium (Patient tailored-Pharmacokinetic-guided dosing of CLOTting factor concentrate and desmopressin in bleeding disorders) and the United Kingdom – Extended Half-Life (UK-EHL) Outcome registry. OPTI-CLOT aims to implement PK-guided dosing of clotting factor concentrates by initiating studies that emphasize the impact of PK-guided dosing, by constructing prophylactic and on-demand population PK models, and by evaluating the cost-effectiveness of a PK-guided approach. The UK-EHL Outcome registry aims to evaluate the impact of EHLs on real world outcomes for patients with hemophilia and develop an evidence base for the introduction of new clinical strategies for improved patient outcomes. A list of the members of the “OPTI-CLOT” and UK-EHL outcome registry programs are available in **Supplementary Appendix A** (available in the online version).

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Conflict of Interest

J.M.H. has received a grant from CSL Behring outside the submitted work. M.M. has received consultancy fees from Takeda, Bayer, Roche Novo Nordisk, Sobi and Freeline and gave advisory board input to Takeda, Travel support from Takeda, CSL, Novo Nordisk and Roche. P.W.C. has received research support from CSL Behring and consultancy fees from Sobi, Roche and Bayer. R.C. Tait has received consultancy fees from Bayer Healthcare, Pfizer, Shire, NovoNordisk, Sanofi, Sobi and Daiichi-Sankyo, and travel grants from Bayer Healthcare, NovoNordisk and CSL Behring. S. M. has received consultancies from Novonordisk, Roche, Pfizer and Takeda, and funding for travel/conference registration from Octapharma and Sobi. B.M. has received financial support to attend meetings by SOBI and Amgen and gave lectures for Pfizer over the last year. F.W.G.L. has received unrestricted research grants from CSL Behring and Shire outside the submitted work, and is consultant for Shire, uniQure and Novo Nordisk, for which the fees go to the institution. He is a DSMB member for a study supported by Roche. The institution of K.F. has received

unrestricted research grants from CSL Behring, Bayer and Novo Nordisk and her institution received consultancy fees from Shire, Roche, Novo Nordisk and B.K.M. has received research support from Bayer, Sanquin and Pfizer; speaker fees from Bayer, Sanquin, BoehringerIngelheim, BMS and Aspen; and consulting fees from uniQure. P.C. has received grants and/or personal fees from Bayer, Baxalta, Biogen, Bioverativ, CSL Behring, Chugai, Freeline, Novo Nordisk, Pfizer, Roche, Shire, Spark and Sobi. M.H.C. has received grants from governmental research institutes such as Dutch Research Institute (NWO), ZonMW, Innovation fund, and 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, SobiBiogen, Novo Nordisk, Novartis and Nordic Pharma, and has served as a member on steering boards of Roche and Bayer. All grants, awards and fees go to the institution. R.M. has received travel grants from Shire and Bayer. The UK Enhanced Half-Life registry was funded by a grant from Sobi. The remaining authors declare no competing financial interests.

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