Population pharmacokinetics of factor IX in hemophilia B patients undergoing surgery

Running head: Perioperative pharmacokinetics of factor IX

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Key Points

- Factor IX (FIX) dosing using body weight frequently results in under and overdosing during surgery
- This study aims to establish a population pharmacokinetic (PK) model describing the perioperative FIX levels
- Population PK parameter values for CL and V1 were 284 $mLh^{-1}70kg^{-1}$ and 5450 $mL70kg^{-1}$, respectively
- Perioperative PK parameter estimates are significantly different from the non-surgical prophylactic treatment

Abstract

Background

Hemophilia B is a bleeding disorder characterized by a deficiency of coagulation factor IX (FIX). In the perioperative setting, patients receive FIX concentrates to ensure hemostasis. Although FIX is usually dosed according to body weight, under- and overdosing occurs frequently during surgery.

Aim

The objective was to quantify and explain the inter-patient variability of perioperatively administered plasma-derived (pd) and recombinant (r) FIX concentrates.

Methods

Data were collected from 118 patients (median age: 40 years (range: 0.2-90), weight: 79 kg (range: 5.3-132)) with moderate (28%) or severe hemophilia B (72%), undergoing 255 surgical procedures. Population pharmacokinetic (PK) parameters were estimated using nonlinear mixed-effect modeling in NONMEM.

Results

Measured perioperative FIX level versus time profiles were adequately described using a three-compartment PK model. For a typical 34-year-old patient receiving rFIX, clearance (CL), inter-compartmental clearance (Q2, Q3), distribution volume of the central compartment (V1) and peripheral compartments (V2, V3) plus inter-patient variability (%CV) were: CL: 284 $mLh^{-1}70kg^{-1}$ (18%), V1: 5450 $mL70kg^{-1}$ (19%), Q2: 110 $mLh^{-1}70kg^{-1}$, V2: 4800 $mL70kg^{-1}$, Q3: 1610 $mLh^{-1}70kg^{-1}$ and V3: 2040 $mL70kg^{-1}$. From 0.2 years, CL and V1 decreased 0.89% and 1.15% per year, respectively, until the age of 34 years. Patients receiving pdFIX exhibited a lower CL (11%) and V1 (17%) than patients receiving rFIX. Inter-patient variability was successfully quantified and explained.

Conclusions

The estimated perioperative PK parameters of both pdFIX and rFIX are different from those reported for prophylactic treatment. The developed model may be used to apply PK-guided dosing of FIX concentrates during surgery.

Keywords: Hemophilia B, Coagulation Factor IX, Surgery, Pharmacokinetics, Coagulation Factor Concentrates.

Introduction

Hemophilia B is a bleeding disorder characterized by a deficiency of coagulation factor IX (FIX). Severe and moderate patients have endogenous FIX levels less than 0.01 $IUmL^{-1}$ and between 0.01 and 0.05 $IUmL^{-1}$, respectively [1,2]. In this category of patients, plasma-derived FIX (pdFIX) or recombinant FIX (rFIX) standard half-life concentrates are usually administered prophylactically to prevent spontaneous joint and muscle bleedings [3,4] and 'on-demand' when bleeding occurs in the surgical setting. In the prophylactic setting, FIX trough levels above 0.01 $IUmL^{-1}$ are usually aimed for, as moderate patients have significantly less spontaneous bleedings [5]. In the perioperative setting, higher doses of FIX concentrates are administered to normalize FIX levels for 7-10 consecutive days post-surgery with target trough levels from 1.00 to 0.30 $IUmL^{-1}$ ensuring adequate hemostasis [6].

Currently, prophylactic, "on-demand" and perioperative dosing of FIX concentrates is performed according to body weight with frequent monitoring to ensure sufficient FIX levels. Despite weight-based dosing, considerable under- and overdosing in the surgical setting has been reported by Hazendonk et al [7]. It was shown that 60% of the hemophilia B patients have FIX levels below the target level range during the first 24 hours directly after surgery. This lack of adequate FIX plasma levels confers a considerable potential risk of bleeding and should be avoided. Therefore, more optimal dosing strategies are warranted.

In the prophylactic setting, FIX doses can be tailored to an individual's need by pharmacokinetic (PK)-guided dosing using Bayesian analysis [8]. In this approach, observed individual FIX levels are combined with PK information assessed in the population in order to obtain estimates for individual PK parameters [9]. These individual parameter estimates can be used to calculate doses necessary to achieve and maintain desired target levels by PK-guided dosing, potentially preventing over and under-dosing. This approach can be applied iteratively, as with every new blood sample the calculated dose can be adapted to alterations in the individual PK parameter estimates [10]. This technique may also be applied in the perioperative setting.

A prerequisite for applying Bayesian analysis to perioperative dosing of FIX is the availability of a population model that describes the PK of FIX in hemophilia B patients undergoing surgery. The population PK of pdFIX and rFIX is well documented [5,11–13]. However, these models have all been constructed using data during non-surgical dosing of FIX concentrates. In the perioperative setting, the

PK of FIX may however be altered. In order to apply Bayesian dosing in the perioperative setting, a dedicated population model should be available.

This study was performed to describe the population PK of pdFIX and rFIX concentrates in hemophilia B patients during surgery and the days thereafter. It was investigated whether specific patient and surgical characteristics explain inter-patient variability (IIV) in FIX exposure and whether the perioperative PK of FIX is comparable to the prophylactic situation.

Methods

Patients and clinical data

An international multi-center observational cohort study was performed in which data were collected from 118 severe and moderate hemophilia B patients from five Hemophilia Treatment Centers in the Netherlands and five in the United Kingdom. Patients of all ages, who had undergone a minor or major elective surgical procedure between January 1st 2000 and December 1st 2015, were included [14]. Details of the study data have been reported previously [7].

In summary, severe and moderate hemophilia B patients received replacement therapy during surgery with FIX concentrates according to national and/or hospital guidelines, while aiming for target FIX levels as prescribed. To ensure hemostasis during the surgical procedure, a pdFIX product [AlphaNine® SD (Grifols Biologicals Inc., Los Angeles, USA), Replenine® (Bio Products Laboratory, Hertfordshire, UK), Haemonine® (Biotest Pharma GmbH, Dreierich, Germany), Mononine® (CSL Behring GmbH, Marbourg, Germany) and Nonafact® (Sanquin, Amsterdam, the Netherlands)] or rFIX product [BeneFix® (Pfizer Wyeth Pharmaceuticals Inc., Kent, UK) and IXinity® (Aptevo BioTherapeutics LLC, Berwyn, US)] was administered with a bolus infusion of approximately 100 $IUkg^{-1}$, followed by either multiple intermittent bolus infusions or continuous infusions. FIX levels were obtained in the participating centers using a one-stage assay, according to local protocol.

Pharmacokinetic modeling

In population PK modeling, the PK is assessed in a cohort of patients rather than in an individual patient [15]. In population PK modeling, not only the average or median value of a PK parameter is of interest but also its inter- and intra-patient variability. Population PK parameters can be obtained by the standard two-stage method, in which individual PK parameters are calculated and, subsequently, summarized. A drawback of this method is that for each individual 10 or more serial samples should be available (rich sampling). In the clinical situation, this is often impossible or inconvenient to perform, especially in populations such as children or the elderly. An alternative is the population approach [16], which allows the estimation of population PK parameters by analyzing data from all the patients simultaneously. The simultaneous analysis allows the use of sparsely and heterogeneously sampled

data, which is frequently encountered in the clinical situation. In this study, sparsely and heterogeneously sampled data were used to construct the population PK model.

Using the population-based approach, a structural PK model is established first. This model consists of a number of PK compartments with PK parameters described in terms of clearance and volume of distribution. The structural model provides values for the typical (average) parameter and, importantly, several levels of variability. Differences in PK parameters between patients are quantified in terms of inter-patient variability (IIV). Variability of a PK parameter within a patient may be quantified by estimation of inter-occasion variability (IOV). Furthermore, a population PK model contains residual unexplained variability (RUV), which is the variability of the differences between the predicted and the measured plasma levels. By combining observed individual FIX levels and population PK parameters empirical Bayesian estimates (EBEs) of the individual PK parameters can be obtained. These EBEs can be used in the covariate analysis (below).

In this study, nonlinear mixed-effects modeling was used to estimate the population PK parameters [17]. A detailed description of the methods used to construct the population PK model can be found in the Supplemental data. For each patient, the (historically lowest) endogenous baseline level was subtracted from each observed FIX level. Furthermore, in some subjects, a preoperative FIX level was present that was higher than the measured endogenous baseline level and for which no prior dose information was known. In the modeling procedure, the pre-operative level was accounted for by an arbitrary virtual dose of 8250 IU administered 5 days prior to the pre-dose FIX measurement. To account for inter- and intra-individual variability in the observed preoperative FIX levels, the typical bioavailability of this dose was estimated in combination with its IIV and IOV. For estimation of the IOV, an occasion was defined as a single surgical procedure.

After the structural model was established, it was evaluated whether patient and surgical characteristics (covariates) explained the variability (IIV, IOV, and RUV) in a covariate model. Since FIX levels were available for both children and adults, estimated PK parameters were normalized for a body weight of 70 kg using allometric scaling with the ¾ rule [18]. Body weight was, however, missing in 38 surgical procedures (14.9%) from 18 patients (15.3%). Therefore, a piecewise linear model was developed to impute the missing values for body weight using age as a predictor. Covariate relationships were evaluated using graphical evaluation of plots of the EBEs versus the covariate value. Subsequently, covariates were implemented in the population model and their ability to explain

the IIV, IOV or RUV was tested by univariate analysis. The following covariates were evaluated: severity of hemophilia (severe versus moderate), age, the use of tranexamic acid or heparin during surgery, the type of FIX concentrate (plasma-derived or recombinant), the brand of product, treatment center, country of treatment, presence of hepatitis C, the use of prophylaxis before the surgical procedure, a history of neutralizing inhibitors, having a minor or major surgical procedure, blood group and the presence of an infection or a decrease in hemoglobin concentration during the surgical procedure. The final model, containing multiple covariates, was constructed by multivariate analysis using forward inclusion and backward deletion.

Model evaluation

The objective function value (OFV), which represents the ability of the model to describe the observed FIX levels, was used to discriminate between different models. When comparing nested models, the difference of the corresponding OFVs (dOFV) is known to be described by a χ^2 distribution, in which the difference in the number of parameters between the evaluated models determines the degrees of freedom. Hereby, a dOFV bigger than 3.84, 5.99 or 7.81 indicates a significant difference of $P < .05$ with 1, 2, or 3 degrees of freedom, respectively. In the covariate analysis, covariates were selected in the forward inclusion and backward elimination procedure if a dOFV bigger than 3.84 ($P < .05$, df=1) and 6.63 ($P < .01$, df=1), respectively, were obtained.

To evaluate whether the measured FIX levels were adequately described by the developed population PK model, several criteria were used. The adequacy of the model was evaluated by inspection of precision of the estimated model parameters, creation of goodness-of-fit plots, evaluation of shrinkage of the IIV, IOV, and RUV, the condition number of the model and the creation of visual predictive checks (VPC) [19,20]. In the latter procedure, FIX levels were generated by Monte Carlo simulation (n=1000) using the established population PK model and are, subsequently, compared to the actual measured FIX levels [21]. For the goodness-of-fit plots, the measured FIX levels were compared to the population predicted FIX levels (PRED) using the typical values for the PK parameters and the individual predicted FIX levels (IPRED) predicted on basis of the EBE. Moreover, several plots were evaluated depicting conditional weighted residuals (CWRES). CWRES are the weighted difference between the model predicted and measured FIX levels [22].

The stability and robustness of the final model were tested by a bootstrap analysis [23]. In this analysis, 1000 new datasets were created by randomly sampling from the data from all patients in the original dataset. Subsequently, the final model was re-estimated using the bootstrapped datasets. The median and 95% confidence interval of the obtained bootstrap parameters was compared to the estimated PK parameters of the final model.

Comparison to non-surgical FIX models

The final population model describing the PK of FIX in hemophilia B patients during surgery was compared to published population PK models derived from data of patients on prophylaxis [11–13,24].To evaluate whether the published prophylactic models were able to describe the perioperative FIX levels from this study, predictions of the perioperative FIX levels were calculated using the prophylactic population PK parameters. For each model, the difference between the population predictions and the measured FIX levels was summarized using the relative mean prediction error (rMPE). The latter was calculated using the following equation:

$$rMPE = \frac{1}{n}\sum_{i=1}^{n}\left(\frac{C_{PRED}-C_{FIX:C}}{C_{FIX:C}}\right) * 100\% \quad \text{(Eq. 1)}$$

in which, C_{pred} are the population predictions and $C_{FIX:C}$ the measured FIX level for a total of *n* measurements. Furthermore, the terminal elimination half-life was calculated using the values from all population PK parameters.

Results

Patients

In total, 118 severe and moderate hemophilia B patients were included undergoing 262 surgical procedures. Four occasions were excluded, as FIX levels were not measured. Due to the withdrawal of approval for IXinity® by the European Medicine Agency during data collection in June 2013, another three surgical procedures were also excluded from analysis [25]. As a result, data from 255 surgical procedures were used for PK analysis. Table 1 shows the general patient characteristics.

Body weight was not recorded in 14.9% of all surgical procedures. Therefore, a piecewise linear model was developed, from which the missing values for body weight could be imputed using age (Supplemental methods). Table S1 shows the parameter estimates for the piecewise linear model. The relationship between age and body weight is shown in Figure 1; the blue line depicts the predictions from the model for all ages, which was used to impute values for the missing body weights.

Pharmacokinetic modeling

For constructing the structural model, a three-compartment model more adequately fitted the FIX levels than a two-compartment model (dOFV = 58.1, p<0.001). Table 2 summarizes the parameter estimates of the structural model. For all estimated PK parameters, the imprecision of the estimated value was lower than 20%. A proportional residual error model was most appropriate to fit the data, as compared to an additive or combined residual error model. In the structural model, IIV could be estimated for both CL and V1, as well as a correlation for the IIV between the two parameters. Moreover, shrinkage values for the IIV of CL and V1 were lower than 20%, indicating that there was sufficient information available for each patient to estimate the individual parameters reliably [26]. Although IIV should also be present for the other PK parameters (e.g. Q2, Q3, V2, V3), the available data did not support the estimation of these values. Pre-administration FIX levels (greater than endogenous baseline values) were present in 138 of the 255 evaluated surgical procedures and ranged from 0.01 to 0.67 $IUmL^{-1}$. Administration of a virtual dose of 8250 IU, 120 hours before the start of the surgery, adequately approximated the pre-administration FIX levels and significantly improved the fit of the model; dOFV was -495.5 ($P < .001$). The typical value for the estimated bioavailability of the virtual dose was 99.8% and IIV and IOV values were 91% and 93%, respectively. These values

indicate that virtual doses vary largely between patients and surgical procedures with values ranging from 744.2 IU to 196,824.2 IU. Estimation of IOV for differences in CL and V1 between surgical procedures was not successful. By implementing IOV for CL, the fit of the model improved (dOFV: -141.9, $P < .01$). However, parameter estimates became unstable and IOV was therefore not included.

Covariate analysis

To prevent the covariates influencing the estimation of the virtual dose, the IIV and IOV from the virtual dose were fixed to the values obtained for the structural model. Table 3 shows the dOFV for the selected covariate relations from the forward inclusion and backward elimination procedure. Age of the patient was included for CL and V1 as a piecewise linear model, which is a linear model with two slopes. The best fit was obtained when the first slope was estimated and the second was set to zero from an age of 34 years, which was the median, and higher. As a result, the body weight normalized CL and V1 decreased 0.89% and 1.15% per year, respectively, until the age of 34 years. Moreover, IIV was reduced from 20.8% to 18.5% (10.1%) and from 24.6% to 18.7% (14.6%) for CL and V1 due to the introduction of age. In Figure 2A-B, age versus individual values for CL and V1, as obtained by Bayesian analysis using the final model, are shown. In these figures, the combined effect of body weight and age is observed, as CL and V1 increase with body weight and decrease with age up to 34 years.

Patients receiving pdFIX concentrates exhibited a lower CL and V1 as compared to patients receiving rFIX concentrates; respective values were 11% and 17% lower for pdFIX. Moreover, V1 was 10% lower in patients with moderate hemophilia in comparison to patients with severe hemophilia. The parameters of the final model are summarized in Table 2. All other covariate relations did not result in a significant dOFV.

Evaluation of the final model

The fit of the final model was evaluated by inspection of goodness-of-fit plots, as shown in Figure 3A-D. Figure 3A shows the prediction of FIX levels, based on the population PK parameter values, adjusted for the covariate values. Both under- and overprediction is present since IIV is not taken into account for calculating the population predictions. Nevertheless, the population predictions are distributed randomly around the y=x axis, demonstrating the appropriateness of the model. Figure 3B

is obtained after Bayesian analysis, in which individual PK parameter estimates (EBEs) are obtained by simultaneous analysis of the individual observations and the population model. The individual FIX levels are predicted using the derived EBEs. Again, these predictions are distributed randomly around the y=x axis. Figure 3C-D show plots of the conditional weighted residuals (CWRES) versus predicted FIX level and time, respectively. CWRES values are distributed randomly around the line y=0. Most of the values are within the -2 and +2 SD range, which confirms the goodness-of-fit of the final model.

To evaluate the stability of the final model, a bootstrap analysis was performed. In this analysis, 1000 model estimations were performed from which 98.3% were successful. Table 2 shows that the medians for the parameter estimates from the bootstrap analysis were similar to those from the final model, except for Q3. This deviation for Q3 is caused by the high imprecision of its estimation, as shown by the 95% CI for Q3 from the bootstrap analysis (667.2 - 5131.9 $mLh^{-1}70kg^{-1}$). For all other parameters of the final model, the CIs were small and corresponded to the RSEs from the parameter estimates of the final model.

The evaluation of the final model comprised of 1000 Monte Carlo simulations for each patient to construct a VPC, as shown in Figure 4. The (grey) lines, depicting the 2.5^{th}, 50^{th} and 97.5^{th} percentiles of the measured FIX levels, are predominantly within their corresponding 95% prediction intervals, as presented by blue and red areas. As a result, the simulated data were similar to the measured data, confirming the adequacy of the final model.

Comparison with non-surgical models

Supplementary Table S2 summarizes population PK parameters of four models that have been published previously and were constructed using data obtained after non-surgical dosing. Higher values for the population PK parameter CL were found for the rFIX models as compared to the pdFIX models. Supplementary Figure S2 shows the predicted FIX levels as obtained using the population PK parameter values analogous to Figure 3A (without IIV). Supplementary Figure S2A-B were constructed using solely the pdFIX data from this study; concentrations were predicted using the population parameters of pdFIX model 1 and 2. Likewise, Supplementary Figure S2C-D were constructed using solely the rFIX data from this study in combination with population parameters from rFIX model 1 and 2. In each case, the non-surgical models underpredicted the observed levels, as shown by the blue lines being above the black line y=x. The rMPE values, calculated for pdFIX model

1 and 2 and rFIX model 1 and 2, were -7.2%, -15.7%, -40.7% and -40.3%, respectively. Furthermore, the half-lives calculated for pdFIX and rFIX using the population parameter values for the final model from the present study were 51 and 49 hours, respectively, whereas the terminal elimination half-lives for pdFIX model 1 and 2 and rFIX model 1 and 2 were: 28, 23, 20 and 20.3 hours, respectively.

Discussion

In this study, the PK of pdFIX and rFIX were characterized in children and adults with severe and moderate hemophilia B undergoing a surgical procedure. Considerable inter-patient variability was identified for clearance and central volume of distribution, which was partially explained by the patient's age, type of FIX product and the severity of hemophilia. Importantly, the perioperative PK of FIX was different from that in the non-surgical situation.

In population PK analysis, the variability within and between patients is quantified and, subsequently, explained using covariates such as age or body weight. When these variabilities are assessed adequately, a population PK model may be used for PK-guided dosing using Bayesian analysis. In contrast to dosing based on body weight, PK-guided dosing allows for individualization of doses while taking the individual's PK into account. To apply Bayesian analysis clinically, an appropriate population PK model is essential. Moreover, Bayesian analysis using a population PK model, which does not describe the PK of FIX adequately, may result in biased individual PK parameters and, hence, biased estimated doses. For factor VIII, a dedicated population PK model for hemophilia A patients undergoing a surgical procedure was constructed in a similar fashion [27]. Therefore, a dedicated population PK model was constructed to describe the perioperative FIX levels.

In this study, the observed pre-surgical FIX level was higher than the endogenous baseline value in 138 of 255 surgical procedures. These elevated pre-surgical FIX levels were taken into account by a virtual dose that was estimated using a typical value and both IIV and IOV. Hereby, each patient having a pre-surgical FIX level can have a different virtual dose for each surgical procedure. Inclusion of these pre-surgical FIX levels greatly improved the fit of the model. Therefore, exclusion of such pre-surgical FIX levels may lead to biased population PK parameter estimates.

In the present study, age partially explained the inter-individual variability from CL and V1. In the final model, the best fit was obtained using a piecewise linear relation using two slopes with -0.89% and -1.15% for ages below 34 years for body weight normalized CL and V1, respectively. Allometric scaling of CL using an exponential factor of 0.75 partly explains the increased clearance when a lower body weight is present. Nevertheless, additional variability was explained by taking age into account.

Björkman et al. reported a similarly piecewise linear relationship between age and CL of rFIX when administered in a non-surgical situation [28,29]. It was shown that clearance and (steady-state) volume of distribution decreased when age increased from 2 to 20 years. Above an age of 20 years, there was virtually no change in clearance or volume of distribution. Suzuki et al explored a similar piecewise relationship of age with CL for the population PK of rFIX as well [13]. However, a relationship between age and CL could not be identified when body weight was included in the model as well. Furthermore, in the covariate analysis, severity of hemophilia B was associated with V1. For a moderate hemophilia B patient, V1 was 10.5% lower as compared to a severely affected patient, which is in agreement with the findings from Ewenstein et al.[30]

In previous studies, differences have been reported between PK parameters from pdFIX and rFIX products in the non-surgical situation [5,30,31]. The *in vivo* recovery for rFIX products was found to be on average 53% that of pdFIX products [5]. As *in vivo* recovery is inversely related to volume of distribution, V1 is lower for pdFIX products. Moreover, the clearance of rFIX products was found to be approximately twice as high as compared to pdFIX products [32]. In the presents study, CL and V1 of pdFIX were 11.2% and 17.3% lower than their respective values for rFIX. These higher values for CL and V1 from rFIX are in accordance with results from previous studies. However, the difference between the types of products is smaller in the surgical situation than in the non-surgical situation.

In Figure S2, each published non-surgical population PK model showed that the observed perioperative FIX levels were underpredicted. These differences were also demonstrated by simulations of the typical FIX level versus time profiles for a patient receiving 100 $IUkg^{-1}$ of pdFIX or rFIX using the available population PK models (Figure S5). The calculated rMPEs and half-lives clearly demonstrate that the PK of FIX in the non-surgical setting is different from the surgical setting. The extent of underprediction was higher for rFIX model 1 and 2 (-40.7% and -40.3%) compared to pdFIX model 1 and 2 (-7.2% and -15.7%). This may be explained by the fact that CL in the non-surgical situation was almost twice as high as the value in the present study: 560 $mLh^{-1}70kg^{-1}$ and 551 $mLh^{-1}70kg^{-1}$ versus 284 $mLh^{-1}70kg^{-1}$, respectively. For the pdFIX models, there was less underprediction. Values for CL in the non-surgical situation were slightly higher than the values from the present study: 290 $mLh^{-1}70kg^{-1}$ and 319.8 $mLh^{-1}70kg^{-1}$ versus 284 $mLh^{-1}70kg^{-1}$. An explanation for

this difference is unknown. Nevertheless, the currently published population PK models for prophylactic treatment with rFIX and pdFIX underpredict the perioperative FIX levels. Consequently, use of these models in the perioperative situation result in overdosing.

Conclusion

In the present study, a population PK model was established that adequately described the perioperative FIX levels as obtained from hemophilia B patients undergoing a surgical procedure. As differences in the population PK were found between the surgical and non-surgical setting, the dedicated population PK model constructed in this study may be applied for patient-tailored dosing in the perioperative period. However, application of a population PK model for clinical use should always be validated.

Acknowledgments

This study is part of the research program of the international multicenter consortium “OPTI-CLOT” (Patient tailOred PharmacokineTIc-guided dosing of CLOTting factor concentrate and DDAVP in bleeding disorders)”, which aims to implement PK-guided dosing of clotting factor replacement therapy by initiating studies which 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. A complete list of the members of the “OPTI-CLOT” research program is available in the Appendix.

Authorship Contributions

TP and RM performed the population PK analysis. TP, RM and MC wrote and revised the manuscript. MC and RM wrote the protocol and supervised the study in the Netherlands. MC, HH, sPWC and RL wrote the protocol for the United Kingdom and organized the clinical study and data collection in the United Kingdom. HH performed data collection in all centers in both countries and analyzed the clinical data. Study protocol was implemented and patient inclusions were organized in the Netherlands by: HH, BL, FM, KM, KF, FL and MC, and in the United Kingdom by HH, PWC, RL, PC, DH and DK. MD gave critical input at initiation of and during the clinical study. MC, RM, FL and KF gave critical guidance during the study and for the population PK analysis. All authors contributed substantially to the writing and critically revised the manuscript, with approval of the final draft.

Disclosure of Conflicts of Interest

BLG has received unrestricted educational grants from Baxter and CSL Behring. FM received grants from Bayer, CSL Behring, Novo Nordisk, Octapharma, Pfizer, Sanquin, and Sobi for the development of a registry of Hemophilia patients in The Netherlands (HemoNED). KM has received research support from Bayer, Sanquin and Pfizer; speaker fees from Bayer, Sanquin, Boehringer Ingelheim, BMS and Aspen; consulting fees from uniQure. The institution of KF has received unrestricted research grants from CSL Behring, Bayer and Novo Nordisk and her institution received consultancy fees from Shire, Roche, Novo Nordisk and Bayer. FL has received unrestricted research grants of CSL Behring and Shire, outside the submitted work and is consultant for Shire, uniQure and Novo Nordisk (DSMB), of which the fees go to the institution. PWC has received funding for research from CSL

Behring, paid consultancy from Shire, Novo Nordisk, CSL and Roche. MC has received unrestricted research grants for investigator-initiated studies and educational as well as travel grants from Pfizer, Baxalta/Shire, Bayer, CSL Behring, Novo Nordisk, Novartis, Nordic Pharma, and for the advisory board activities from Bayer and Roche. RM has received travel grants from Shire and Bayer. The remaining authors declare no competing financial interests.

Appendix: study group members

List of the members of the "OPTI-CLOT" research program

The investigators and institutions participating in the OPTI-CLOT research program in the Netherlands are as follows. Steering committee – M.H. Cnossen (principal investigator and chair), Erasmus University Medical Centre – Sophia Children's Hospital, Rotterdam; F.W.G. Leebeek, Erasmus University Medical Centre, Rotterdam; K. Fijnvandraat, R.A.A. Mathôt, Academic Medical Centre, Amsterdam. Principal Investigators and Local Collaborators in the Netherlands – M.J.A.H. Kruip, S. Polinder, J. Lock, H.C.A.M. Hazendonk, I. van Moort, J.M. Heijdra, A. Nederlof, , Erasmus University Medical Centre, Rotterdam; R.A.A. Mathôt, K. Fijnvandraat, T. Preijers, N. de Jager, M. Coppens, M. Peters, Academic Medical Centre, Amsterdam; K. Meijer, R.Y.J. Tamminga, University Medical Centre Groningen; P. Brons, B.A.P. Laros-van Gorkom, Radboud University Medical Centre, Nijmegen; F.J.M. van der Meer, H.C.J. Eikenboom, Leids University Medical Centre, Leiden; R.E.G. Schutgens, K. Fischer University Medical Centre Utrecht; M.H.E. Driessens, Dutch Haemophilia Patient Society; Website, Trialbureau and Databases – C.M. Zwaan, I. van Vliet, Erasmus University Medical Centre, Rotterdam.

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TABLES AND FIGURES

Population pharmacokinetics of factor IX in hemophilia B patients undergoing surgery

Tables

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Table S1. Model for body weight imputation using age

Parameter	Unit	Estimate	RSE (%)
β_1 – y-intercept	kg	6.3	(7)
β_2 – coefficient for all ages	kg year^{-1}	3.6	(5)
β_3 – coefficient for age > β_4	kg year^{-1}	-3.73	(7)
β_4 – Inflection point	year	23.5	(8)
Residual variability			
Proportional error	%CV[†]	19.1	(6)

RSE indicates relative standard error; kg, kilogram. CV, coefficient of variation. By the estimated parameter, the following equation was obtained: WT_{est} (kg) = 6.3 + 3.6*age - 3.73*(age - 23.5)^{DAGE}. In this equation, DAGE is 1 in the case that the age of the patient is 23.5 years or older, in every other case it is zero.

Table S1. Population PK parameter estimates from published models

	pdFIX model 1		pdFIX model 2		rFIX model 1		rFIX model 2	
	Estimate	RSE (%)	Estimate	RSE (%)	Estimate	RSE (%)	Estimate	RSE (%)
Structural model								
Clearance (CL; $mLh^{-1}70kg^{-1}$)	290	3.7	319.8	7.2	560	3.1	551	2.2
Volume of central compartment (V1; $mL70kg^{-1}$)	5710	3.6	5922	7.0	6090	14	9770	4.0
Distribution CL to compartment 2 (Q2; $mLh^{-1}70kg^{-1}$)	1990	35	1049	20.3	22400	28	577	12.6
Volume of compartment 2 (V2; $mL70kg^{-1}$)	810	19	828.9	50.7	4160	17	4620	4.1
Distribution CL to compartment 3 (Q3; $mLh^{-1}70kg^{-1}$)	170	6.7	160.4	8.1	430	15	ND	
Volume of compartment 3 (V3; $mL70kg^{-1}$)	2890	10	2234	73.9	3900	7.7	ND	
Baseline FIX level	ND		0.01588	10.9	ND		ND	
Inter-individual variability (%CV)								
IIV on CL	23	64	36.8	36.8	19	18	25.6	18.9
IIV on V1	19	37	41.2	34.2	46	36	23.2	10.2
IIV on V2	63	30	97.4	72.6	28	44	37.5	13.7
IIV on V3	78	81	133.2	34.5	19	71	ND	
IIV on Q2	ND		ND		ND		69.1	17.6
Inter-occasion variability (%CV)								
IOV CL	15	36	48.8	10.1	ND		24.7	14.3
IOV V1	12	24	47.2	17.8	ND		18.9	8.6
Residual variability								
Additive residual variability (SD; $IUmL^{-1}$)	0.0037	10	0.0067	3.1	0.0064	12	0.00614	8.4
Proportional residual variability (%CV)	9.2	11	6.95	1.5	8.7	4.9	6.8	11.7

RSE indicates relative standard error; mL, milliliter; h, hour; kg, kilogram; CV, coefficient of variation; SD, standard deviation; Allometric coefficients, covariate relations and correlations for IIV and IOV are not shown. pdFIX model 1[12], pdFIX model 2[24], rFIX model 1[11], rFIX model 2[13].

TABLES AND FIGURES

Population pharmacokinetics of factor IX in hemophilia B patients undergoing surgery

Tables

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Table 1. General characteristics of the study population

	Total cohort		Adults		Children*	
	No. (%) or median [range]					
Patient characteristics						
No. of patients	118		82		36	
Age (years)	40	[0.2-90]	46	[18-90]	6	[0.2-18]
Body weight (kg)	79	[5.3-132]	85	[47-132]	19	[5.3-117]
Severe hemophilia B (<0.01 $IUmL^{-1}$)	85	(72)	57	(70)	28	(78)
On prophylaxis	36	(31)	28	(34)	8	(22)
Blood group O†	33	(28)	24	(29)	9	(25)
Neutralizing antibodies (historically)	6	(5)	5	(6)	1	(3)
Chronic hepatitis C	47	(40)	46	(56)	1	(3)
Patient treated in the United Kingdom	93	(79)	63	(77)	30	(83)
Surgical characteristics						
No. of surgical procedures	255		201		54	
Total no. of patients undergoing:						
1	118		82		36	
2	63		49		14	
3	32		28		4	
>3	42		42		0	
Minor surgical procedures	135	(53)	96	(48)	39	(72)
Major surgical procedures	120	(47)	105	(52)	15	(28)
Replacement therapy with FIX concentrate						
Mode of infusion						
Continuous	56	(22)	54	(27)	2	(4)
Bolus	199	(78)	147	(73)	52	(96)
Product type						
Recombinant	201	(79)	150	(75)	51	(91)
Plasma-derived	54	(21)	51	(25)	3	(6)
PK data						
Total number of observations	1555		1324	(85%)	231	(15)
No. of observations per occasion	4	[1-23]	10	[1-23]	5	[1-16]
No. of doses per occasion	7	[1-52]	12	[1-52]	12	[1-39]

No. indicates number; kg, kilogram; and $IUmL^{-1}$, international units per milliliter. *Children were defined as having an age less than 18 years.
†Blood group available in 80 patients. Adapted from Hazendonk et al[7] with permission.

Table 2. Estimated population PK parameters for the structural model, final model, and bootstrap analysis

	Structural model			Final model			Bootstrap analysis	
	Estimate	**RSE (%)**	**Shr. (%)**	**Estimate**	**95% CI**	**Shr. (%)**	**Estimate**	**95% CI**
Structural model								
Clearance (CL; $mLh^{-1}70kg^{-1}$)	296	2.5		284	[266 - 302]		283	[265 - 300]
Volume of central compartment (V1; $mL70kg^{-1}$)	5370	2.7		5450	[5005 - 5895]		5426	[4933 - 5886]
Distribution CL to compartment 2 (Q2; $mLh^{-1}70kg^{-1}$)	112	25		110	[86 - 134]		110	[92 - 140]
Volume of compartment 2 (V2; $mL70kg^{-1}$)	4720	16.7		4800	[3793 - 5807]		4879	[4118 - 6367]
Distribution CL to compartment 3 (Q3; $mLh^{-1}70kg^{-1}$)	2210	19.9		1610	[-32 - 3252]		1943	[667 - 5132]
Volume of compartment 3 (V3; $mL70kg^{-1}$)	2160	17.7		2040	[1344 - 2736]		2079	[1376 - 2753]
Virtual dose	0.998	22.0		ND			ND	
Inter-individual variability (%CV)								
IIV on CL	20.8	9.1	12.3	18.5	[16.4 – 21.3]	10.2	18.5	[15.3 – 21.5]
IIV on V1	24.6	12.2	14.5	18.7	[16.5 – 21.4]	15.0	18.7	[14.0 – 23.5]
Correlation between CL and V1 (%)	91.3	11.2		89.4	[85.0 – 92.5]		89.1	[88.6 – 91.6]
IIV on virtual dose	94.5	18.0		ND			ND	
Inter-occasion variability (%CV)								
IOV on virtual dose	93.4	10.2		ND			ND	
Residual variability								
Proportional residual error (%CV)	23.0	4.2		21.9	[20.2 – 23.6]		21.7	[20.0 – 23.3]
Covariate relations								
CL – (% change with age different from 34 years)	-			-0.89	[-1.4 - -0.4]		-0.89	[-1.4 - -0.4]
V1 – (% change with age different from 34 years)	-			-1.15	[-1.7 - -0.6]		-1.14	[-1.7 - -0.6]
CL – plasma-derived product (%)	-			88.8	[83.8 - 93.8]		88.9	[84.3 - 94.6]
V1 – plasma-derived product (%)	-			82.7	[74.7 - 90.7]		82.3	[72.5 - 90.8]
V1 – if moderate hemophilia patient (%)	-			89.5	[82.8 - 96.2]		89.1	[82.3 - 96.3]
Model characteristics								
Objective function value	-2827.12			-2905.27			ND	
Condition number	68.65			119.65			ND	

RSE indicates relative standard error; CI, confidence interval as obtained using the 2.5th and 97.5th percentiles from the non-parametric distributions; mL, milliliter; h, hour; kg, kilogram; CV, coefficient of variation; Shr., shrinkage. The typical values are obtained for a severe haemophilia B patient weighing 70 kg receiving a recombinant factor IX product.

$$CL\ (mLh^{-1}) = 284\ x\ \left(\frac{BW}{70}\right)^{0.75}\ x\ \left(1 - 0.0089\ x\ (Age - 34)\right)^{AGE<34}\ x\ 0.888^{Plasma-derived\ product}$$

$$V1\ (mL) = 5450\ x\ \left(\frac{BW}{70}\right)^{1.0}\ x\ \left(1 - 0.0115\ x\ (Age - 34)\right)^{AGE<34}\ x\ 0.827^{Plasma-derived\ product}\ x\ 0.895^{moderate\ hemophilia}$$

Table 3. Model building-steps

		OFV	dOFV	No. of parameters
Structural model				
1	Structural model with doses calculated using the virtual dose	-2827.1	ND	9
Covariate relationships – forward inclusion				
2	Structural model and age on CL	-2832.7	-5.6	10
3	Model 2 and age on V1	-2856.4	-23.6	11
4	Model 3 and plasma-derived products on CL	-2876.6	-20.3	12
5	Model 4 and plasma-derived product on V1	-2897.6	-20.9	13
6	Model 5 and moderate patient, as compared to severe patient on V1	-2905.3	-7.7	14
Covariate relationships – backward deletion				
7	Model 6 without moderate patient, as compared to severe patient on V1	-2897.6	7.7	13
8	Model 6 without age on CL	-2881.4	23.9	13
9	Model 6 without age on V1	-2883.2	22.1	13
10	Model 6 without plasma-derived products on CL	-2871.8	33.5	13
11	Model 6 without plasma-derived products on V1	-2880.3	25.0	13

OFV indicates objective function value, as calculated by minus two times the logarithm of the likelihood (-2LL) of the model describing the data; No., number; * For these models, the coefficients for covariate age on both CL and V1 were estimated using a piecewise linear model. However, the slope for the ages above 33.6 years was fixed to 0. Therefore, the number of parameters does only increase by 1.

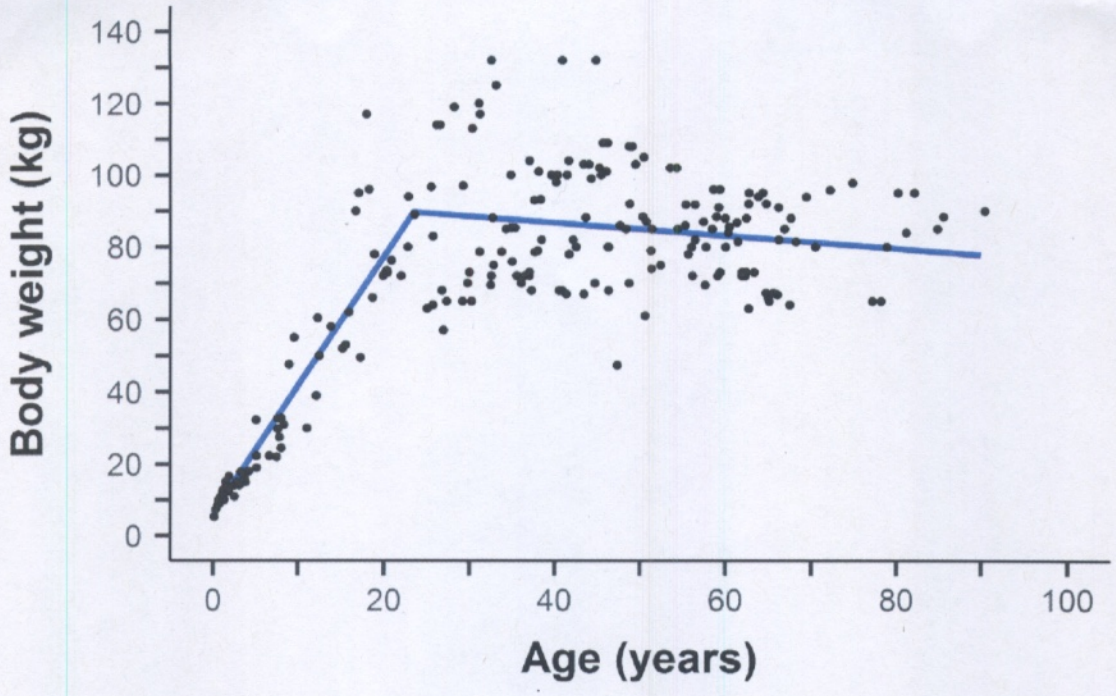

Figure 1. Imputed body weight versus age. Black dots are individual body weights for 99 patients. The blue line depicts the predicted body weight with age as a predictor, as described by the following piecewise linear model: WT_{est} (kg) = 6.3 + 3.6*AGE - 3.73*(AGE - 23.5)^{DAGE}. In this equation, WT_{est} is the estimated body weight and DAGE is 1 in the case that the age of the patient is 23.5 years or older, in every other case it is zero.

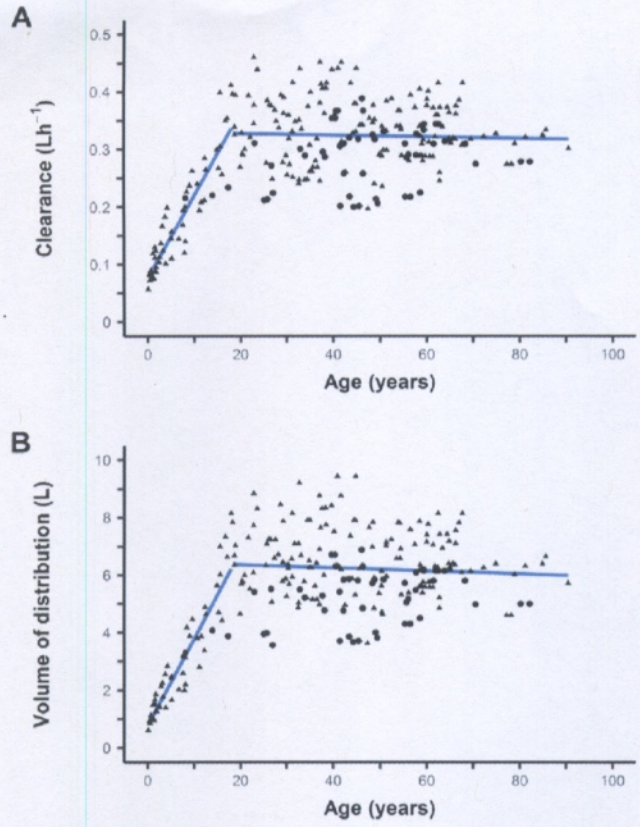

Figure 2. Clearance and volume of distribution versus age. The black dots depict the individual PK parameter estimates obtained by Bayesian analysis for the pdFIX (▲) and rFIX (●) data. (A) clearance. (B) volume of distribution of the central compartment. The blue line represents two (piecewise) linear fits of the individual PK parameters for patients having an age between 0.2 – 18 years and 18 – 90 years, respectively. CL and V1 increase with body weight and decrease with age up to 34 years. In this figure, the combined effect of body weight and age is observed. Both parameters slightly decrease from the age of 18 years due to decreasing weight with increasing age.

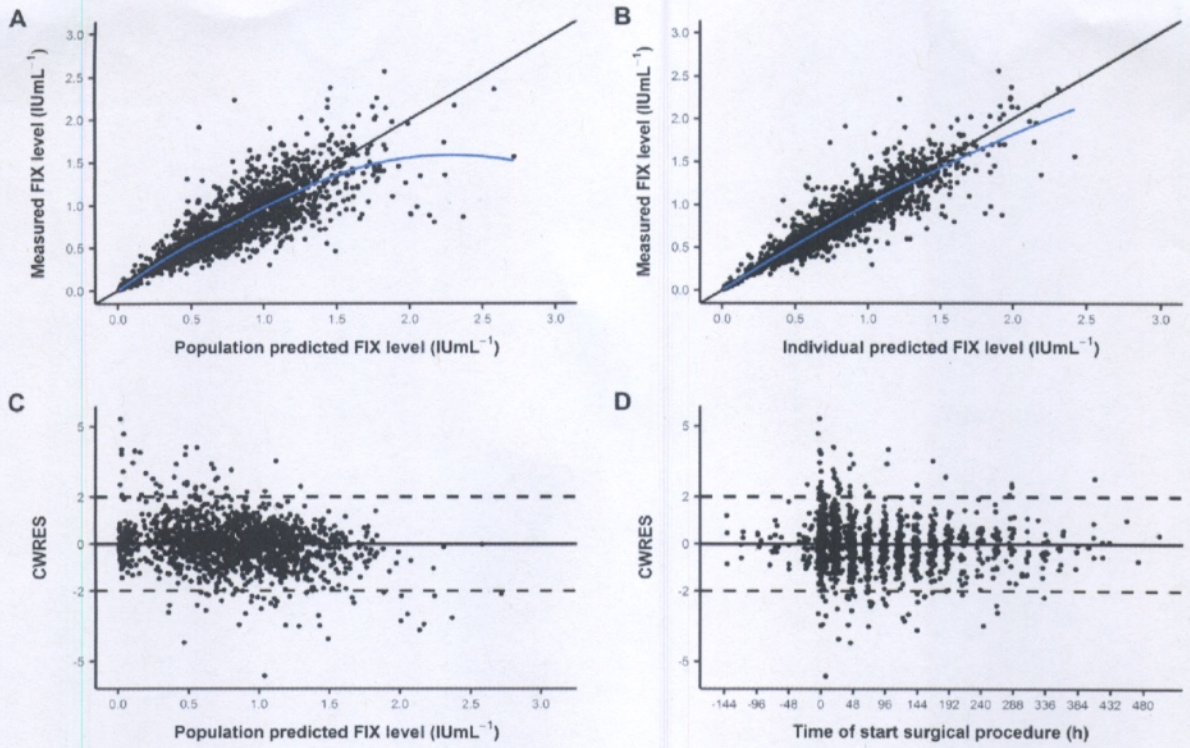

Figure 3. Goodness-of-fit of the plot for the final model. (A) Population predicted versus measured FIX levels. (B). Individual predicted versus measured FIX levels. (C) Conditional weighted residuals (CWRES) versus population predicted FIX levels. (D) CWRES versus time, defined as the time of start of the surgical procedure. Negative times represent samples taken before the start of the surgical procedure.

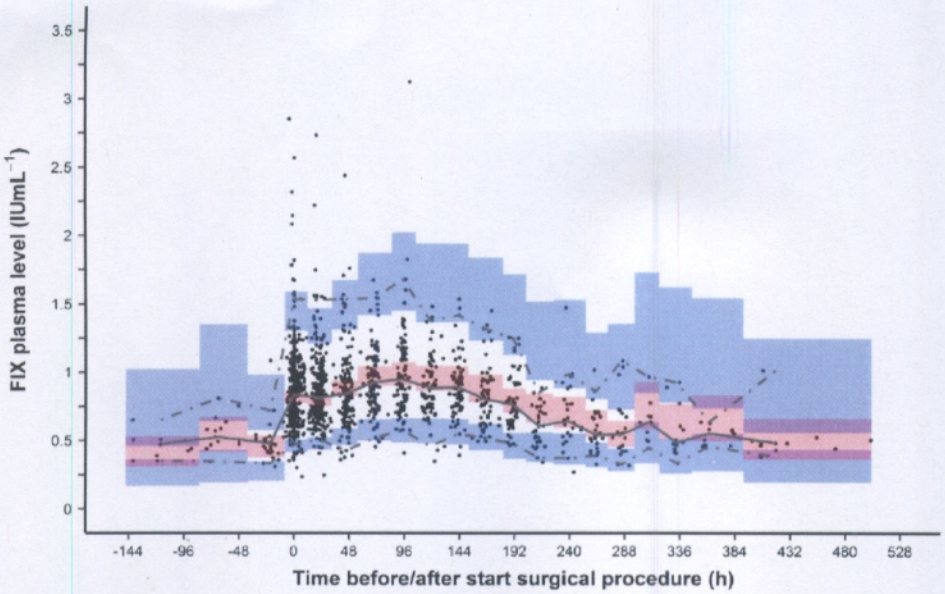

Figure 4. Prediction-corrected Visual Predictive Check of the final model. Time is defined as the time of start of the surgical procedure. Data with negative times represent samples taken before the start of the surgical procedure. Black dots represent the measured FIX levels for all patients. Solid grey line represents the median and the dashed grey lines represent the 2.5th and 97.5th quantiles of the measured FIX levels. Red and blue-shaded areas show the 95% confidence intervals for the predicted individual FIX levels, as obtained by 1000 Monte Carlo simulations using the final model. The binning of the areas for the prediction intervals were created using the auto-bin option in Perl-Speaks-NONMEM.

Supplemental data

Software

Perioperative FIX dosing and coagulation factor IX (FIX) level measurement data were analyzed simultaneously for all patients using NONMEM version 7.4 (ICON Development Solutions, Ellicott City, MD, USA).[1] First-order conditional estimation with interaction was applied to obtain estimates for all model parameters. Perl-speaks-NONMEM version 4.7.0 was used to aid model development and Pirana served as a model management tool.[2] Data preparation and model diagnostics were performed using R version 3.4.1 (R Core Team, 2017) with Xpose version 4.6.0.[1,3,4]

Structural model development

In literature, the population PK of FIX is predominantly described by three-compartment models.[5–8] In our study, however, it was uncertain whether the data would allow estimating all parameters from a three-compartment model with adequate precision, which depends on the timing of sampling. Therefore, development of the model was initiated by fitting a two-compartment model to the data. Subsequently, it was evaluated if the addition of a third compartment would improve the fit. The observed FIX levels and the model predicted FIX levels were both transformed by calculating the natural logarithm.[9]

PK parameters estimates for a two-compartment model were clearance from the central compartment (CL), inter-compartmental clearance (Q2), the volume of the central compartment (V1) and second peripheral compartment (V2). For a three-compartment model, the inter-compartmental clearance between the central and third peripheral compartment (Q3) and the volume of the third peripheral compartment (V3) were added.

Different residual error models were evaluated, including an additive, proportional and combined (additive and proportional) error model[10], as shown by the following equations respectively:

$$(1) \qquad C_{ij} = \hat{C}_{ij} \cdot (1 + \varepsilon_{ij1}) + \varepsilon_{ij2}$$

in which C_{ij} is the measured FIX level for the i^{th} observations from the j^{th} individual, $\hat{C}_{ij}$ is the predicted FIX level, ε_{ij1} is the proportional residual error and ε_{ij2} is the additive residual error. In case a proportional or combined error model was applied to the model, interaction between the residual variability and IIV was allowed.

First, a structural model was developed, in which typical values for the PK parameters were estimated in combination with their inter-patient variability (IIV). Because patients could have undergone more than one surgical procedure, inter-occasion (between-surgery) variability (IOV) of the population PK parameters was also estimated. An exponential model was used to estimate IIV and IOV, as described by the following equation:

(2) $\theta_{ik} = \theta_{TV}\ x\ exp^{\eta_i + \kappa_{ik}}$

in this expression, the subscripts *i* and *k* denote the number of the individual and the occasion, respectively, θ_{TV} is the estimated typical value for a population PK parameter, θ_{ik} is the estimated individual PK parameter, and η and κ are the random-effects accounting for IIV and IOV, respectively.

In some cases, preoperative FIX levels were increased due to a prior FIX dose of which timing was unknown. In these cases, this dose was estimated by a preoperative virtual dose of 8250 IU given 5 days prior to the pre-dose FIX measurement. The bioavailability of the virtual dose was estimated with IIV and IOV, as described by equation 2, allowing the dose to adjust to the dose necessary to describe the preoperative FIX level.

For all other dosing and FIX level measurement data, the bioavailability is set to one. This allows describing the PK from the time of administration of the virtual dose by all PK parameters, instead of using only the terminal elimination half-life to correct for pre-dose FIX measurements.[11]

Since FIX PK data were available for both children and adults, PK parameters were allometrically normalized to a body weight of 70 kg. Scaling was performed *a priori;* i.e. no evaluation of scaling performance was conducted by the following equation:

(3) $\theta_{TV} = \theta_i\ x\ \left(\frac{BW}{70}\right)^{\theta_p}$

in which θ_i is a population PK parameter, θ_{TV} is the typical value for this parameter, BW is body weight and θ_p is the allometric exponent. Allometric exponents were fixed to 1 in case of a volume parameter (V1, V2, V3) and to 0.75 for all clearance parameters (CL, Q2, Q3).[12]

For every patient, age was known at the time of each surgical procedure. However, body weight was missing in 40 surgical procedures (16%). Therefore, a linear model in NONMEM was developed to impute the missing values for body weight using age as a predictor. A piecewise linear model was selected by graphical exploration. The following equation was used to estimate body weight using the values for age:

(4) $\theta_{BW} = \beta_1 + \beta_2 x AGE + \beta_3 x (AGE - \beta_4)\ x\ \beta_5 \begin{cases} AGE \leq \beta_4 : \beta_5 = 0 \\ AGE > \beta_4 : \beta_5 = 1 \end{cases}$

in which θ_{BW} is the imputed body weight, β_1 is the y-intercept, β_2 the slope of the predictor age for all ages, β_3 the slope for the predictor age higher than the value for the inflection point using β_4. Since β_3 only describes body weight higher than this inflection point, β_5 is 0 below this age and 1 in other cases.

Covariate analysis

After the structural model was established, patient demographics and pathophysiological and surgical-characteristics, which can explain the inter-individual variability or the residual variability, were evaluated. First, a univariate analysis was performed to obtain a pre-selection of eligible covariate relationship. Hereby, a significant decrease in OFV (associated with a $P < .05$) determines if the covariate relation is eligible to be included in the covariate model. Subsequently, a multivariate analysis was conducted to test whether the eligible relationships would also significantly decrease the OFV when included simultaneously. Consecutively, a backward elimination procedure was performed, in which the included relations are discarded one by one from the final covariate model. In this procedure, relations are retained in the covariate model if the increment in OFV is associated with a $P > .01$.

All covariate-relationships were evaluated by means of univariate analyses and graphical evaluations to explain the inter-individual variability or the residual variability. The following covariates were evaluated by a dichotomous relationship: the distinction between severe or moderate hemophilia, the use of tranexamic acid or heparin during surgery, administration of recombinant (r) or plasma-derived (pd) FIX, country of treatment, having hepatitis C, the use of prophylaxis before the surgical procedure, a history of neutralizing inhibitors, having a minor or major surgical procedure, having blood group O, having an infection or a decrease in hemoglobin concentration during the surgical procedure, and the brand of product. For all dichotomous covariate relationships, the following equation were used:

(5) $$\theta_i = \theta_{TV} * \theta_{cov}$$

and in case the covariate values was missing:

(6) $$\theta_i = \theta_{TV} * \theta_{cov\_missing}$$

in which θ_{cov} and $\theta_{cov\_missing}$ are the fraction of the typical PK parameter θ_{TV}. For example, an estimate for θ_{cov} was obtained in case a patient used pdFIX during the surgical procedure or if a patient was diagnosed with moderate hemophilia. In other cases, i.e. when a rFIX product was administered or the

patient was diagnosed with severe hemophilia, the value of θ_{cov} was 1, meaning that θ_{TV} was not altered. Thereby, estimated values for θ_{TV} describe the case when θ_{cov} is set to 1. For the dichotomous relationships, the missing values were regarded as being missing at random and, therefore, regarded as a separate group with a fraction value of $\theta_{cov\_missing}$. To describe the relationship of age, the following linear model was applied:

(7) $$\theta_i = \theta_{TV} * \left(1 + \theta_{SL} * (AGE - AGE_{med})\right)$$

in which θ_i describes the individual PK parameter, θ_{TV} is the typical (median) value for a population PK parameter and θ_{SL} is the slope of the relation of age. This model could be used as a piecewise linear model by allowing θ_{SL} to be different, based on a cut-off point for age. For instance, the median age could be used for this cut-off point between the two slopes and, thereby, describing a piecewise linear model.

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