Pharmacokinetic modelling of clinically relevant situations in the treatment of hemophilia A and B

by

Alanna McEneny-King

A thesis

presented to the University of Waterloo

in fulfillment of the

thesis requirement for the degree of

Doctor of Philosophy

in

Pharmacy

Waterloo, Ontario, Canada, 2020

Examining Committee Membership

The following served on the Examining Committee for this thesis. The decision of the Examining Committee is by majority vote.

External Examiner	CATHERINE M. T. SHERWIN Professor and Vice Chair Research
Supervisor	ANDREA N. EDGINTON Associate Professor
Internal Member	DAVID EDWARDS Professor and Hallman Director
	JEFF NAGGE Associate Clinical Professor
Internal-External Member	JOEL DUBIN Associate Professor

Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

Abstract

Hemophilia is a congenital bleeding disorder caused by a deficiency of clotting factor VIII (FVIII; hemophilia A) or IX (FIX; hemophilia B). Severe hemophilia patients have less than 1% (or 1 IU dL^{-1}) of normal factor activity, and often experience spontaneous bleeding episodes. These frequently occur in the joints, causing debilitating arthropathy later in life. The only proven method for preventing joint damage is the prophylactic administration of the appropriate clotting factor from a young age. However, prophylaxis with both FVIII and FIX is complicated by considerable between-subject variability in pharmacokinetic (PK) response. While a traditional PK study can be difficult to implement in routine care, a population pharmacokinetic (PopPK) approach – which reduces sampling burden and allows patients to forego a washout period – represents a more feasible method of dose tailoring for both patients and treatment providers. Nonetheless, PopPK can be challenging to implement due to a significant data requirement and a complicated process for model development and evaluation.

To overcome these barriers, the Web Accessible Population Pharmacokinetic Service for Hemophilia (WAPPS-Hemo) was launched in 2015. This service provides individual estimates of clinically relevant PK parameters (e.g. half-life, time to 1%, factor level at 72 hours post-infusion) from sparse patient samples and demographic data using Bayesian forecasting, with PopPK models serving as prior information. In order to meet its goals, the WAPPS-Hemo project requires PopPK models that are valid for Bayesian estimation for as many of the currently available factor concentrates as possible. In order to standardize the model building process, a data analysis protocol, outlining the steps of model development and evaluation and the criteria for decision-making, was established. This protocol was put into practice in the development of generic models for two classes of factor concentrate (standard half-life FVIII and standard half-

life recombinant FIX). To date, the models described in this dissertation have been used to process over 2,000 PK requests on the WAPPS-Hemo platform.

In addition to developing models for use on the WAPPS-Hemo platform, the clinical factors impacting model performance were investigated. Since Bayesian forecasting relies on only a few samples from the individual patient, the timing of these samples is of considerable importance. Limited sampling analyses were conducted to confirm that accurate estimates of relevant PK parameters can be obtained from a variety of limited sampling schemes. When sampling is extremely limited (e.g. collected during a single clinic visit), estimation can be improved by incorporating knowledge of prior doses, and by conducting the PK study when samples are likely to be within assay quantification limits. Model performance is also dependent on the patient's endogenous (baseline) factor production. In severe hemophilia patients, baseline factor activity may range from truly zero up to 1 IU dL^{-1}; however, baseline is often unknown as 1 IU dL^{-1} is typically the lower limit of quantification for both the one-stage clotting and chromogenic assays, forcing an assumption to be made. The consequences of these assumptions are highly variable; while baseline has little influence on the estimation of half-life, it makes a substantial difference when estimating time to 1% activity.

The models developed for this project have the potential for high impact with respect to the patient, who benefits from an individualized dosing regimen rather than a trial and error approach, resulting in fewer adverse events and, in many cases, more cost-effective use of factor concentrates. The work presented in this dissertation also explores a variety of factors that impact model performance; this knowledge can be used by treatment providers to ensure they receive the most accurate estimates possible when utilizing the service.

Acknowledgements

I would first like to thank my doctoral supervisor, Dr. Andrea Edginton, for her continual support throughout the past six years. Her mentorship and guidance have shaped me into the researcher I am today. I am also grateful to my advisory committee members, Dr. David Edwards and Dr. Jeff Nagge, for their support and valuable discussion over the course of this work.

I wish to thank Dr. Alfonso Iorio and the WAPPS-Hemo tea, for the opportunity to work on such a far-reaching, dynamic, and impactful project during my doctoral studies.

I also owe a debt of gratitude to Dr. Pierre Chelle for being an incredible collaborator over the past several years – your expertise, enthusiasm, and insight are always appreciated.

Finally, I would like to thank Dr. J. Carl Panetta at St. Jude Children's Research Hospital and Dr. Stacy Croteau at Boston Children's Hospital for the illuminating and rewarding experiences at their institutions during my doctoral studies; it was a pleasure to learn and experience the comprehensive nature of hemophilia treatment with both teams.

Dedication

I dedicate this thesis to my husband, Scott, who has been a constant source of reassurance, humour, and love throughout this process, and to my parents, Elgin and Linda, for their boundless encouragement to pursue this, and all of my other aspirations.

Table of Contents

Examining Committee Membership ii
Author's Declaration iii
Abstract iv
Acknowledgements vi
Dedication vii
List of Tables xii
List of Figures xiv
List of Supplemental Material xviii
List of Abbreviations xx
Chapter 1: Background 1
The Clotting Cascade 1
Hemophilia A and B 2
Current Hemophilia Therapy 2
Pharmacokinetics of Clotting Factor Concentrates 3
Role of Pharmacokinetics in Hemophilia Treatment 10
Population Pharmacokinetics 10
The WAPPS-Hemo Project 13
Overarching Thesis Objective 17
Objectives & Hypotheses 17
Chapter 2: The use of pharmacokinetics in dose individualization of factor VIII in the treatment of hemophilia A 18
Introduction 18
Pharmacokinetics of FVIII 21
Covariates Affecting Pharmacokinetic Parameters 27
A Pharmacokinetic Comparison of Recombinant and Plasma-Derived Products 29
Development of Extended Half-Life Products 30
Conclusions 32
Addendum 33
Chapter 3: Data analysis protocol for the development and evaluation of population pharmacokinetic models for incorporation into the Web Accessible Population Pharmacokinetic Service – Hemophilia (WAPPS-Hemo) 37
Introduction 37

Methods 39
Data Sources 39
Software and Handling of BLQs 41
Base Model Development 42
Residual Unexplained Variability 43
Between Subject Variability 44
Covariate Model Selection 46
Model Evaluation 48
Results 56
Discussion 57
Conclusions 59
Chapter 4: Development and evaluation of a generic population pharmacokinetic model for standard half-life factor VIII for use in dose individualization 60
Introduction 60
Methods 65
Patient Data 65
Population Modelling 66
Model Evaluation 69
Results 71
Discussion 80
Conclusions 83
Chapter 5: Development of a population pharmacokinetic model for recombinant factor IX and its use in evaluating limited sampling strategies for pediatric patients 84
Introduction 84
Methods 86
Patient data 86
Population modelling 87
Model evaluation 89
Limited sampling analysis for pediatric clinical trial 90
Results 91
Model development and evaluation 91
Limited sampling analysis for pediatric clinical trial 95

Noncompartmental analysis 100
Discussion 102
Conclusions 106
Chapter 6: Limited sampling strategies for accurate determination of extended half-life factor VIII pharmacokinetics in severe hemophilia A patients 107
Introduction 107
Methods 109
Software 109
Population pharmacokinetic model 109
Simulated populations and activity profiles 110
Design of limited sampling strategies 112
Evaluation of limited sampling strategies 115
Results 116
Adult Population 116
Pediatric and Adolescent Populations 121
Discussion 126
Conclusions 128
Chapter 7: Clinical application of Web Accessible Population Pharmacokinetic Service – Hemophilia (WAPPS-Hemo): Patterns of blood sampling and patient characteristics among clinician users 129
Introduction 129
Methods 131
Results 133
Discussion 141
Conclusions 143
Chapter 8: The effect of unmeasurable endogenous plasma factor activity levels on factor VIII dosing in patients with severe hemophilia A 144
Introduction 144
Methods 147
Population pharmacokinetic modeling 147
Development of simulated populations 148
Simulations 148
Real patient data 150

Results 150
Discussion 157
Conclusions 163
Chapter 9: Modeling of body weight metrics for effective and cost-efficient conventional factor VIII dosing in hemophilia A prophylaxis 164
Introduction 164
Methods 167
Population Generation 167
Definitions of Weight Metrics 168
Population Pharmacokinetic Model 169
Simulation and Assessment of Treatment Regimens 170
Results 171
Discussion 176
Conclusions 180
Chapter 10: Discussion, Conclusions, and Future Directions 181
Discussion 181
Conclusions 187
Future Directions 188
References 190
Appendices 210
Letters of Copyright Permission 210
Chapter 2 210
Chapter 4 211
Chapter 7 212
Chapter 8 213
Chapter 9 214
Supplemental Information 215
List of WAPPS-Hemo Centres 230

List of Tables

Table 1. Studies on the pharmacokinetics of plasma-derived and recombinant FIX. PK parameters are presented as mean ± SD (%CV) unless otherwise noted; n. g. denotes values that were not reported in the studies. 4

Table 2. Studies on the pharmacokinetics of plasma-derived and recombinant FVIII. PK parameters are presented as mean ± SD (%CV); n. g. denotes values that were not reported in the studies. 24

Table 3. Studies on the pharmacokinetics of plasma-derived and recombinant FVIII identified in the 2019 update. n. g. denotes values that were not reported in the studies. 34

Table 4. Description of the components for a typical NONMEM dataset 40

Table 5. NONMEM subroutines used to implement kinetic equations for linear models following intravenous administration 41

Table 6. Simulation details for limited sampling analysis by product type 53

Table 7. Published PopPK models for standard half-life FVIII products 62

Table 8. Demographics of the patient population used to develop the generic SHL FVIIII model 66

Table 9. Modelling of brand as a covariate 68

Table 10. Parameter estimates for the final SHL FVIII model 74

Table 11. Summary of parameter estimates from published PopPK models for SHL FVIII 80

Table 12. Demographics of the patient population used to develop the generic SHL rFIX model. Data are presented as median (range) where appropriate. 87

Table 13. Parameter estimates for the final SHL rFIX model 92

Table 14. Error (presented as mean (range) in percent) and absolute error (presented as median [Q95] in percent) relative to the 10-sample design using Bayesian estimation 97

Table 15. Error (presented as mean (range) in percent) and absolute error (presented as median [Q95] in percent) relative to the 10-sample design using noncompartmental analysis 101

Table 16. Details of the base rFVIIIFc model developed by Nestorov et al [181] 110

Table 17. Demographic data of rFVIIIFc studies used for development of Nesterov model and of simulated populations. 112

Table 18. Details and nomenclature of tested limited sampling strategies. All doses were 50 IU kg^{-1} (rounded to the nearest 250 IU vial) unless otherwise indicated..... 114

Table 19. Pharmacokinetic outcomes for simulated populations from the rich sampling design. Results are presented as mean (standard deviation). Age is presented as median (range), except in the adult case where the median for the PK subgroup was unknown. Simulated values of PK parameters are presented as mean (SD); reported values of half-life are presented as mean (95% CI). 116

Table 20. Comparison of predose methods A and B for 2- and 3-sample designs following Thursday sampling in adults. Absolute errors are presented as mean [95^{th} percentile]..... 118

Table 21. Categorical covariate summary for final WAPPS-Hemo dataset for Period 1 and 2 134

Table 22. Mean and median number of blood samples per patient per infusion for each group of factor products during Period 1 and 2..... 137

Table 23. Details of the model developed by Garmann et al [182] 148

Table 24. The effect of baseline assumption on dose adjustments using a single observed level 154

Table 25. The effect of baseline assumption on estimated PK outcomes using a Bayesian approach..... 155

Table 26. Details of the model developed by Garmann et al [182] 169

Table 27. Comparison of the typical 20 IU kg^{-1} total body weight (TBW) dose and the lowest dose meeting the safety threshold (i.e., 14 IU kg^{-1} TBW) in overweight and obese patients. Results are presented as median (90% confidence interval)..... 171

Table 28. Summary of safety and economic evaluations of different weight metrics used in a Q48 h regimen across BMI subgroups, assuming a baseline factor level of 0.5 IU dL^{-1}. Dose is the dose required to have 95% of patients with a steady state C_{min} over 1 IU dL^{-1}. Optimal regimens for each subgroup and the overall population are bolded. 174

Table 29. Summary of safety and economic evaluations of different weight metrics used in a Monday-Wednesday-Friday regimen across BMI subgroups, assuming a baseline factor level of 0.5 IU dL^{-1}. Dose is the Friday dose required to have 90% of patients with a weekly $C_{min} \geq 1$ IU dL^{-1}. Optimal regimens for each subgroup and the overall population are bolded..... 174

List of Figures

Figure 1. Partitioning of variability in pharmacokinetic response 12

Figure 2. Schematic of the WAPPS-Hemo service, including PK estimation, clinical calculator module, and patient app 15

Figure 3. The global WAPPS-Hemo network, where the colour of each country indicates the number of registered centres 16

Figure 4. η-distribution of a structural model parameter (e.g. CL) with low (left) and high (right) shrinkage. The red line indicates the expected distribution from the model assumptions, while the blue line shows the actual distribution of individual η-values. 45

Figure 5. Illustration of different functional forms (left to right: power, linear, piecewise linear, and categorical) for covariate relationships, with varying effect sizes 48

Figure 6. Example of prediction-corrected visual predictive check (pcVPC) for a SHL FVIII model shown on linear (top) and log (bottom) scales. Dashed red lines denote median, 5^{th} and 95^{th} percentiles of observed data. Solid lines denoted the same percentiles in the simulated data, with shaded regions representing the 90% prediction regions for the simulated percentiles. 51

Figure 7. Sample results from cross-validation, presented as histograms of relative errors on key PK parameters 52

Figure 8. Example of results from limited sampling analysis for a SHL FVIII model, depicting the error on estimates of half-life and time to 2% factor activity for two-sample designs 54

Figure 9. Process of external validation using WAPPS-Hemo data. (A) Comparison of covariate spaces of modelling (grey) and WAPPS-Hemo (blue) data (B) Goodness-of-fit plots (log scale) (C) Comparison of individual PK estimates for patients from modelling and WAPPS-Hemo datasets (D) Predicted individual activity-time profiles with 95% confidence intervals (shaded region) and observed data overlaid (×). 56

Figure 10. Sources and structures of the brands of SHL FVIII included in the modelling dataset 64

Figure 11. Boxplots of η-values for clearance (left) and central volume (right) across brands before (top) and after (bottom) inclusion of brand as a covariate. 72

Figure 12. Individual predicted values from the final SHL FVIIII model versus observed values by brand group on linear (left) and log (right) scale. Samples that were BLQ are not depicted.. 75

Figure 13. Prediction-corrected visual predictive check (pcVPC) for the final SHL FVIII model shown on linear (top) and log (bottom) scales. Shaded regions are the 90% confidence intervals for the simulated percentiles. 76

Figure 14. Errors on estimates of half-life (top) and time to 2% activity (TAT2%, bottom) from limited sampling strategies consisting of 2 post-infusion samples 77

Figure 15. Comparison of PK parameter estimates generated from the SHL FVIII model and brand specific models for Kovaltry (blue, n = 213), ReFacto AF/Xyntha (purple, n = 132), and Fanhdi/Alphanate (orange, n = 49). 79

Figure 16. Goodness-of-fit plots on linear (left) and log (right) scales. 93

Figure 17. Population prediction-corrected visual predictive check (pcVPC) for the final SHL rFIX model. Shaded regions are the 90% confidence regions for the simulated percentiles. 94

Figure 18. Histograms of prediction errors on half-life, TAT2%, CL, and V_1 estimates 95

Figure 19. Half-life error relative to the rich 10-sample design for 4- (top), 3- (middle), and 2- (bottom) sample designs. 99

Figure 20. Impact of inclusion of a late sampling tie (72 or 96 h) on PK parameter estimates compared to EMA-based sampling strategy when using NCA 102

Figure 21. Comparison of errors on estimates of half-life (blue), clearance (yellow), V_{ss} (orange), and AUC (purple) from Bayesian estimation (left) and NCA (right) 105

Figure 22. Boxplot of relative error on half-life estimates from limited sampling strategies using (top) Method A and (bottom) Method B for predose handling in a simulated adult population. Error is calculated relative to design AT1 and BT1, respectively. 120

Figure 23. Effect of predose handling on estimates of PK parameters and IDAR for adults, adolescents, and children for the predose-peak sampling strategy 122

Figure 24. Influence of study day on PK parameters and IDAR for different simulated age groups. Predose is handled using Method B 125

Figure 25. Dosages (IU/kg) of FVIII (A: Period 1; B: Period 2) and FIX (C: Period 1; D: Period 2) administered for infusions submitted for PK analysis 135

Figure 26. Age (A: Period 1; B: Period 2) and body weight (C: Period 1; D: Period 2) distributions of patients submitted for PK analysis 135

Figure 27. Number of samples collected per infusion for each of the product types (A. SHL FVIII; B: EHL FVIII; C: SHL FIX; D: EHL FIX) during Period 1 (left) and Period 2 (right).. 136

Figure 28. Post-infusion timing of factor activity levels for each of the product types (A. SHL FVIII, B. EHL FVIII, C. SHL FIX, and D. EHL FIX) collected during Period 1 (left) and Period 2 (right). Shaded regions represent sampling windows recommended by ISTH subcommittee for the specific product type: SHL FVIII: 4 – 8 h, 16 – 28 h, and 40 – 60 h, EHL FVIII add 60 – 84 h, SHL FIX: 24 – 36 h, and 48 – 60 h, EHL FIX: add 5 – 14 days. 138

Figure 29. Factor activity levels for infusions containing a single sample, excluding confirmatory samples. SHL FVIII – blue circles, SHL FIX – red squares, EHL FVIII – green triangles, EHL FIX – yellow diamonds. 139

Figure 30. The Q48h dose required to keep 95% of individuals above the trough as a function of baseline value. The shaded region is the 95% confidence interval of the mean of 500 populations each containing 500 individuals..... 151

Figure 31. Effect of baseline assumption on the infusion frequency needed to keep 95% of individuals above a desired trough with a dose of 40 IU kg^{-1} when frequency is considered as a continuous variable. The shaded region is the 95% confidence interval of the mean of 500 populations each containing 500 individuals..... 152

Figure 32. Estimated FVIII activity profiles following a dose of ~60 IU kg^{-1}, assuming different baseline levels (legend, IU mL^{-1}). White circles denote observed data..... 156

Figure 33. Correlation of body weight metrics with body mass index (BMI) for each BMI subgroup (blue = normal weight, red = overweight and obese). TBW: total body weight; HT: height; LBW: lean body weight; IBW: ideal body weight; ABW: adjusted body weight. 172

Figure 34. Percentage of patients with $C_{min} \geq 1$ IU dL^{-1} (safety) at increasing doses per kg of various weight metrics, stratified by BMI subgroup, administered at 48 h intervals. 175

Figure 35. (a) Median and 90% confidence intervals for C_{min} and (b) 95th quantile for time spent below 1 IU dL^{-1} (hours per week) for TBW-based dosing regimen administered at different intervals for the combined group (normal + overweight/obese) for both Q48 h (blue) and Monday-Wednesday-Friday (red) dosing schedules. For the Q48 h regimen, all doses are increasing along the *X*-axis; for the Monday-Wednesday-Friday schedule, only the Friday dose is changing (Monday and Wednesday doses are fixed at 20 IU per kg TBW) 175

Figure 36. Comparison of safety profiles for patients simulated with baseline (BL) 0.5 IU dL^{-1} and 0 IU dL^{-1} for a Q48 h regimen. Safety (%) is the percentage of patients with $C_{min} \geq 1$ IU dL^{-1} at various doses per kg of IBW. 176

List of Supplemental Tables

Table S1. Categorical covariate summary among high-use centres (HUCs, top 25th percentile) and other centres (non-HUCs) for Period 1 and Period 2 215

Table S2. Summary of number of samples per patient per infusion for each product type for Period 1 and Period 2 for high-use centres (HUCs) and other centres (non-HUCs). 216

Table S3. Model building log 217

Table S4. Results of 5-fold cross-validation of final SHL FVIII model 218

Table S5. Details of sampling schemes explored and results of limited sampling analysis 219

Table S6. Median [95th percentile] absolute error on PK outcomes for limited sampling designs for the 2-year-old population. 220

Table S7. Absolute error (%, as median [95th percentile]) on half-life estimates, for all permutations of study day and predose handling for each age group. To assist with interpretation, a gradient has been applied, with absolute error increasing from green (lowest) to red (highest). 221

Table S8. Absolute error (%, as median [95th percentile]) on C_{72} estimates, for all permutations of study day and predose handling for each age group. To assist with interpretation, a gradient has been applied, with absolute error increasing from green (lowest) to red (highest). 222

Table S9. Absolute error (%, as median [95th percentile]) on C_{96} estimates, for all permutations of study day and predose handling for each age group. To assist with interpretation, a gradient has been applied, with absolute error from green (lowest) to red (highest). 223

Table S10. $IDAR_{72}$ and $IDAR_{96}$ for all permutations of study day and predose handling for each age group. To assist with interpretation, a gradient has been applied, with IDAR increasing from green (lowest) to red (highest). 224

List of Supplemental Figures

Figure S1. Scatter plots of η-values versus age following the inclusion of fat-free mass on CL, V_1 and V_2. 225

Figure S2. Histograms of parameters estimates for fixed effects from bootstrap analysis..... 226

Figure S3. Histograms of parameter estimates of variability from bootstrap analysis 227

Figure S4. Histograms of prediction errors on half-life, time to 2% activity, clearance, and central volume from internal cross-validation 227

Figure S5. Correlation between FFM and age 228

Figure S6. Correlation plots for all body size metrics used in simulations for the normal BMI subgroup. Diagonal elements contain histograms. 229

Figure S7. Correlation plots for all body size metrics used in simulations for the overweight/obese subgroup. Diagonal elements contain histograms. 229

List of Abbreviations

ABW_x	adjusted body weight with correction factor x%
AE	absolute error
BDD	B-domain deleted
BLQ	below limit of quantification
BMI	body mass index
BSV	between subject variability
BW	body weight
CFC	clotting factor concentrate
CI	confidence interval
CL	clearance
CRM	cross-reactive material
CV	coefficient of variation
dL	decilitre
EHL	extended half-life
FFM	fat-free mass
FIX	factor IX
FVIII	factor VIII
h	hour
HUC	high use centre
IDAR	inappropriate dose adjustment rate
IBW	ideal body weight
IOV	inter-occasion variability
ISTH	International Society on Thrombosis and Haemostasis

IU	international unit
L	litre
LBW	lean body weight
LLOQ	lower limit of quantification
LSS	limited sampling strategy
mL	millilitre
NCA	noncompartmental analysis
NHANES	National Health and Nutrition Examination Survey
pcVPC	prediction-corrected visual predictive check
pd	plasma-derived
PK	pharmacokinetics
PopPK	population pharmacokinetics
Q	intercompartmental clearance
r	recombinant
RUV	residual unexplained variability
SD	standard deviation
SHL	standard half-life
TBW	total body weight
V_1	central compartment volume
V_2	peripheral compartment volume
vWF	von Willebrand factor
WAPPS-Hemo	Web Accessible Population Pharmacokinetic Service – Hemophilia
WSV	within-subject variability

Chapter 1: Background

The Clotting Cascade

When the body suffers an injury to the vascular system, the physiological response is to minimize blood loss at the site of damage and restore hemostasis. The coagulation cascade is one of the mechanisms triggered by vascular trauma, and the end product of this pathway is a cross-linked fibrin scaffolding for aggregated platelets to form a clot. The components of the cascade are inactive enzyme precursors, or zymogens, known as coagulation factors. The coagulation cascade consists of two distinct pathways, each of which is prompted by a different event and plays a unique role in achieving hemostasis.

The extrinsic pathway is initiated when damaged endothelial cells release tissue factor, which binds and activates coagulation factor VII [1]. The resulting complex activates factor X, which joins with factor V to form the prothrombinase complex. Prothrombinase activates prothrombin to thrombin, which not only converts inactive fibrinogen into clot-forming fibrin, but also activates several components of the intrinsic pathway of the coagulation cascade including factors XIII, V and VIII [2]. The main function of the extrinsic pathway is to generate an initial burst of thrombin; it is quickly inhibited by tissue factor pathway inhibitor, at which point the more kinetically efficient intrinsic pathway takes responsibility for the growth and maintenance of the fibrin clot [3]. The intrinsic pathway begins by activation of clotting factor XII upon binding to collagen. Factor XII proteolytically cleaves factor XI, which then activates factor IX (FIX). Finally, FIX joins with co-factor VIII (FVIII) to form a complex that also activates factor X. The rate of factor X activation by this intrinsic tenase complex is estimated to be at least 50-fold higher than the extrinsic counterpart; as a result, the vast majority (95%) of

thrombin is produced by this pathway [4,5]. Without sufficient production of the intrinsic tenase complex, an inadequate amount of activated Factor X is generated and the propagation phase of vascular repair is ineffective.

Hemophilia A and B

Hemophilia is an inherited bleeding disorder caused by a deficiency in one of the clotting factors that forms the intrinsic tenase complex (hemophilia A: FVIII; hemophilia B: FIX). This results in bleeding episodes, often in the joints, and eventual arthropathy. In the most severe hemophilia patients (those with less than 1 IU dL^{-1} [i.e. 1% of normal] of the respective clotting factor activity), these bleeds may occur spontaneously. A recent study estimates the prevalence of hemophilia A and B to be roughly 17 and 4 cases per 100,000 males, respectively, of which roughly 30% are the severe form. This amounts to an expected 1,125,000 hemophilia patients worldwide [6].

Current Hemophilia Therapy

Although strides are being made in the development of alternative therapies (e.g. monoclonal antibodies, gene therapy), the mainstay of hemophilia treatment is prophylactic intravenous administration of the deficient clotting factor. Prophylactic treatment aims to convert severe hemophilia patients to a moderate phenotype by regularly administering clotting factor concentrates, and the strategy is based on several clinical observations. The first is a 1965 study, which found that the joint scores of moderate hemophilia A patients (i.e. those with FVIII levels between 1 and 3 IU dL^{-1}) were markedly improved compared to severe patients [7]. The second, described by Nilsson and colleagues, is the observation that patients on prophylaxis who spent more time per week with FVIII levels above 1 IU dL^{-1} had improved joint function [8]. A 2007

study by Manco-Johnson *et al* demonstrated reduced incidence of joint bleeds, life-threatening bleeds, and lowered risk of joint damage following prophylaxis in young hemophilia A patients [9]. Finally, Collins *et al* observed an association between time spent below 1 IU dL^{-1} per week and occurrence of bleeding events [10].

Despite global consensus regarding the initiation of prophylaxis at a young age [11–13], the implementation of this approach is highly variable [14]. While part of this variation may be attributed to the cost or availability of factor concentrates [15], the considerable between-subject variability in the pharmacokinetic (PK) handling of both FVIII and FIX is the major obstacle to the determination of an effective 'one-size-fits-all' dosing regimen.

Pharmacokinetics of Clotting Factor Concentrates

The pharmacokinetics of FVIII are well characterized, and are discussed in detail in Chapter 2, which contains a comprehensive review of FVIII PK studies conducted in recent years with a focus on variability and the sources thereof.

The pharmacokinetics of FIX, on the other hand, are not as well understood. A comprehensive review, analogous to the one presented in Chapter 2 for FVIII, was performed to identify original PK studies for factor IX products in hemophilia B patients; the details and results from the 34 eligible studies are presented in Table 1.

Table 1. Studies on the pharmacokinetics of plasma-derived and recombinant FIX. PK parameters are presented as mean ± SD (%CV) unless otherwise noted; n. g. denotes values that were not reported in the studies.

Study	Product	*n*	Patient Age (years)	Sampling Time	Number of Samples	Terminal Half-Life (h)	Clearance (mL h^{-1} kg^{-1})	Volume of Distribution (mL kg^{-1})	Analysis Method
[16]	Nanotiv® or Immunine®	8	19-65	74 h	11	29.7 ± 4.3 (14%)	4.25 ± 0.64 (15%)	n. g.	n. g.
[17]	pdFIX-SD	11	12-60	72 h	12	34.2 ± 3.5 (10%)	7.4 ± 0.8 (11%)	162.9 ± 47.8 (29%)	n. g.
	pdFIX-SD-15					33.3 ± 3.8 (11%)	6.9 ± 1.2 (17%)	155.4 ± 46.4 (30%)	
[18]	BeneFIX®	11	4-9	72 h	12	20 ± 4.3 (21%)	10.4 ± 2.2 (22%)	270 ± 7 (3%)	n. g.
		10	10-19			20 ± 4.1 (21%)	8.3 ± 2.3 (28%)	210 ± 7 (3%)	
		12	20-29			19 ± 4.9 (26%)	8.5 ± 1.2 (14%)	220 ± 6 (3%)	
		12	30-39			20 ± 6.5 (33%)	7.2 ± 1.4 (19%)	190 ± 4 (2%)	
		7	40-49			19 ± 4.2 (22%)	7.6 ± 1.7 (22%)	200 ± 5 (3%)	
		3	50-56			17 ± 7.1 (42%)	7.5 ± 0.3 (3%)	180 ± 8 (4%)	
[19]	rFIX	19	<15	72 h	12	20.2 h ± 4.0 (20%)	n.g.	n.g.	compartmental and non-compartmental methods
		28	15-40			19.4 h ± 5.5 (28%)			
		9	>40			17.2 h ± 4.8 (28%)			
[20][a]	Mononine / BeneFIX®	38	7-75	48 h	7	14.9 h (7.2-33.7) / 16.8 (10.8-26.1)	n.g.	n.g.	NCA

Study	Product	n	Patient Age (years)	Sampling Time	Number of Samples	Terminal Half-Life (h)	Clearance (mL h^{-1} kg^{-1})	Volume of Distribution (mL kg^{-1})	Analysis Method
[21]	Mononine	12	7-85	76 h	10	16.7 (8.7-36.6)	4.5 (2.4-9.6)	119.9 (77-233.2)	n. g.
[22]	Mononine BeneFIX®	15	≥12	48 h	7	12.9 13.7	4.2 7.1	61 118	Nonlinear regression
[23][a]	unspecified	13	13-70	retrospective analysis; details not provided		32 (26-49)	3.9 (2.9-4.5)	140 (80-200)	compartmental modelling (NONMEM)
[24]	Aimafix DI	12 5	12-36	50 h	12	23.5 h ± 12.3 (52%) 17.6 h ± 3.5 (20%)	6.5 ± 1.4 (22%) 5.3 ± 1.6 (30%)	197.5 ± 72.5 (37%) 131.1 ± 25.6 (20%)	NCA
[25]	BeneFIX®	10	24.7 (8.6)	72 h	11	24.4 ± 6.4 (26%)	4.84 ± 1.03 (21%)	144.3 ± 41.8 (29%)	model-independent method
[26]	BeneFIX®	24	12-61	72 h	12	22.4 ± 6.4 (26%)	n.g.	n.g.	n. g.
[27]	Octanine F	24	0.5-5	n.g.	sparse	n.g.	n.g.	n.g.	n.g.
[28]	Factor IX Grifols® Control (Immunine® or Octanine®)	25	23.1 (8.83)	74 h	11	26.7 ± 3.8 (14%) 26.8 ± 3.7 (14%)	3.8 ± 0.9 (24%) 4.1 ± 1.2 (29%)	n. g.	model-independent method
[29]	BeneFIX®	20	0.6-4	24h	4	10.9 ± 2.3 (21%)	13.6 ± 3.4 (25%)	n.g.	WinNonLin and NLME
[30]	AlphaNine®	25	25.8 (8.68)	74 h	10 to 12	34.5 ± 6.2 (18%)	n.g.	n.g.	model-independent method

Study	Product	n	Patient Age (years)	Sampling Time	Number of Samples	Terminal Half-Life (h)	Clearance (mL h^{-1} kg^{-1})	Volume of Distribution (mL kg^{-1})	Analysis Method
[31]	Nonafact®	13	19-58	48 h	10	18.7 ± 2.0 (11%)	n. g.	n. g.	model-independent method
[32]	N9-GP	5	21-55	168 h	13	82.94 ± 18.15 (22%)	0.76 ± 0.08 (10%)	90.13 ± 13.24 (15%)	NCA
		5				96.25 ± 41.85 (43%)	0.74 ± 0.21 (28%)	99.50 ± 47.42 (48%)	
		5				110.45 ± 17.48 (16%)	0.65 ± 0.13 (20%)	101.96 ± 12.00 (12%)	
	rFIX	7		48 h	9	19.34	6.99	194.98	
	pdFIX	8				17.79	5.48	140.58	
[33]	IB1001	32	15-64	72 h	12	29.7 h ± 18.2 (61%)	5.0 ± 2.0 (40%)	160 ± 40 (25%)	compartmental and non-compartmental methods
	nonacog alfa					33.4 h ± 21.2 (63%)	5.0 ± 1.0 (20%)	180 ± 70 (39%)	
[34]	rIX-FP (albumin)	13	15-58	336 h	11	91.57 ± 20.74 (23%)	0.75 ± 0.19 (25%)	95.0 ± 20.3 (21%)	NCA using WinNonLin
	pdFIX	4		32-48 h	6	14.59 ± 1.73 (12%)	4.76 ± 1.08 (23%)	98.7 ± 14.9 (15%)	
	rFIX	8				17.23 ± 2.28 (13%)	5.24 ± 0.85 (16%)	130.6 ± 29.9 (23%)	
[35]	Haemonine®	13	13-45	72 h	12	27.6 ± 4.5 (16%)	205.4	n. g.	PCModfit
[36]	rFIXFc	11	18-76	240 h	14	56.7 ± 10.4 (18%)	3.18 ± 0.745 (23%)	227 ± 57.1 (25%)	WinNonLin (2comp)
[37]	AlphaNine®	25	27.0 (9.7)	74 h	11	32.7 ± 7.4 (23%)	4.2 ± 1.0 (24%)	134 ± 42 (31%)	model-independent method
	BeneFIX®	22				36.0 ± 12.8 (36%)	4.6 ± 1.0 (22%)	175 ± 52 (30%)	
[38]	rFIXFc	22	12-71	240 h	n. g.	82.1	n. g.	n. g.	n. g.
	BeneFIX®			96 h		33.8			

Study	Product	n	Patient Age (years)	Sampling Time	Number of Samples	Terminal Half-Life (h)	Clearance (mL h^{-1} kg^{-1})	Volume of Distribution (mL kg^{-1})	Analysis Method
[39][b]	AlphaNine®	9	15-73	48 h	10	16.6 (13.2-20.9)	n. g.	n. g.	n. g.
	BeneFIX®					17.5 (14.8-25.6)			
[40]	BAX326	25	12-65	72 h	12	25.4 ± 6.9 (27%)	6.0 ± 1.5 (24%)	179 ± 45 (25%)	
[41]	rIX-FP (albumin)	15	13-36	336 h	10	94.8 h	n. g.	n. g.	NCA (using Phoenix WinNonLin)
[42]	BeneFIX®	7	n.g.	48-72 h	5-9	36 ± 8.3 (23%)	3.8 ± 0.4 (10%)	n .g.	Non-compartmental
[43]	N9-GP	12	1-6	168 h	6	69.6	0.758	76.1	non-compartmental method
		13	7-12			76.3	0.65	71.5	
[44]	rIX-FP (albumin)	12	<6	n.g.	n.g.	89.6 (12%)	1.184 (28%)	142.5 (24%)	NCA (using Phoenix WinNonLin)
		15	6-11			92.8 (20%)	1.059 (28%)	131.6 (20%)	
	FIX (21 rFIX, 6 pdFIX)	12	<6			19.9 (40.3%)	7.158 (39.0%)	167.5 (24.8%)	
		15	6-11			17.7 (25.6%)	5.812 (23.7%)	143.1 (20.5%)	
[45]	BeneFIX®	17	12-59	72 h	12	23.7 ± 5.6 (24%)	7.5 ± 1.8 (24%)	216 ± 66 (30%)	SAS
[46]	rIX-FP (albumin)	n. g.	12-61	n. g.	n. g.	101.7	0.769	n. g.	NCA using Phoenix WinNonLin

Study	Product	n	Patient Age (years)	Sampling Time	Number of Samples	Terminal Half-Life (h)	Clearance (mL h^{-1} kg^{-1})	Volume of Distribution (mL kg^{-1})	Analysis Method
[47][c]	rFIXFc	11	<6	168 h	8	66.5 (55.9-79.1)	4.4 (3.9-4.9)	365.1 (316.2-421.6)	NCA
		13	6-11			70.3 (61.0-81.2)	3.5 (3.0-4.1)	289.0 (236.7-352.9)	
	rFIX	11	<6	48 h	6	18.2 (15.5-21.3)	n. g.	n. g.	
		9	6-11			19.2 (17.6-20.9)	n. g.	n. g.	
[48]	N9-GP (nonacog beta pegol)	25	1-12	168 h	7	73.0 (22%)	0.7 (20%)	70.2 (19%)	NCA
		9	18-54			85.1 (22%)	0.4 (20%)	50.6 (17%)	
		9	18-54			110.8 (12%)	0.4 (12%)	64.0 (19%)	
[49]	trenonacog alfa	32	14.8-64.5	72	12	24.2 ± 6.9 (28%)	5.1 ± 1.3 (25%)	175 ± 57 (32%)	n. g.
	nonacog alfa					26.4 ± 13.6 (52%)	5.0 ± 1.2 (24%)	181 ± 57 (31%)	

[a]Results presented as median (range)
[b]Results presented as mean (range)
[c]Results presented as median (IQR)

Relative to FVIII, standard half-life FIX has a larger volume of distribution, higher clearance, and longer half-life; typical values for these parameters are roughly 150 mL kg^{-1}, 4.0 mL h^{-1} kg^{-1}, and 30 h, respectively [50], compared to 48 mL kg^{-1}, 3.0 mL h^{-1} kg^{-1}, and 14 h for FVIII [51]. The increased volume of distribution of FIX is attributed to its smaller size (57 kDa, compared to 300 kDa for FVIII) which allows for extensive distribution into the extravascular space. The inter-individual variation in FIX PK parameters is high, with a three to fourfold range between the most extreme values, though the PK within an individual is quite stable over time [52]. Few covariates have been found to explain this variability. Clearance and volume of distribution are correlated to body weight, but increasing age (beyond adolescence, when body weight is no longer changing appreciably) has no noticeable effect on PK parameters. Considerable differences have been observed between plasma-derived and recombinant FIX, although the terminal half-life was found to be similar in comparison studies [20,53,54].

In recent years, considerable success has been found in the extension of FIX half-life through a variety of techniques. Three new concentrates are currently available; one pegylated product and two rFIX fusion proteins (albumin, and the Fc fragment of IgG1). All three products achieve significant extensions of half-life (80–100 h), allowing for less frequent infusions, reduced weekly FIX consumption, and lower bleed rates [55].

Role of Pharmacokinetics in Hemophilia Treatment

Hemophilia patients are typically dosed by total body weight, but evidence suggests that this approach is suboptimal, resulting in either overdosing (and excessive use of expensive clotting factor concentrate) or underdosing (and ineffective or unsafe treatment). To improve both patient outcomes and cost-effectiveness, PK-based individualization of dosing regimens has been proposed [51,56]. However, a classic PK study for FVIII or FIX requires rigorous sampling (ten samples over the course of two to three days, as recommended by the International Society on Thrombosis and Haemostasis). Furthermore, this type of study requires the patient to undergo a potentially hazardous washout period. For these reasons, the classical PK approach can be difficult to apply in clinical settings.

Population Pharmacokinetics

The population PK (PopPK) approach can be used to address some of these hurdles. The primary advantage of PopPK is a significantly reduced sampling burden; accurate PK parameter estimates can be obtained from as few as two to three well-timed samples [57]. This is achieved by leveraging information from a large patient population in the form of a PopPK model, which is in turn comprised of three sub-models: a structural model (defining the shape of the factor activity vs. time profile), a covariate model (describing the relationships between PK parameters and patient characteristics), and a statistical model (describing the variability). The total variability around a given parameter may be partitioned into predictable variability, which can be attributed to demographic, environmental, or genetic covariates, and unpredictable variability. The unpredictable component of the variability may be further divided into between-subject variability (BSV, η) and within-subject variability (WSV, ρ; also referred to as inter-occasion variability [IOV]). Finally, the remaining discrepancy between predicted and observed factor activities is deemed residual unexplained variability (RUV, ε). A simple example, consisting of a

1-compartment model (parameterized by clearance [CL] and volume [V]), with BSV on CL, a body weight effect on V, and an additive error model, is shown below:

$$\hat{C}_j(t) = \frac{Dose}{V_j} \cdot e^{-\frac{CL_j}{V_j} \cdot t}$$

$$CL_j = \theta_{CL} \cdot e^{\eta_j}$$

$$V_j = \theta_V \cdot \left(\frac{BW_j}{BW_{med}}\right)^{\theta_{BW-V}}$$

$$C_{ij} = \hat{C}_{ij} + \varepsilon_{ij}$$

The parameters denoted with θ are known as fixed effects, which are constant across individuals; these include the typical values of PK parameters within the population as well as the covariate effects on those parameters. Random effects (η, ε, and, if applicable, ρ) describe the unpredictable variability, and are specific to the individual. Further details regarding PopPK model development and evaluation can be found in Chapter 3.

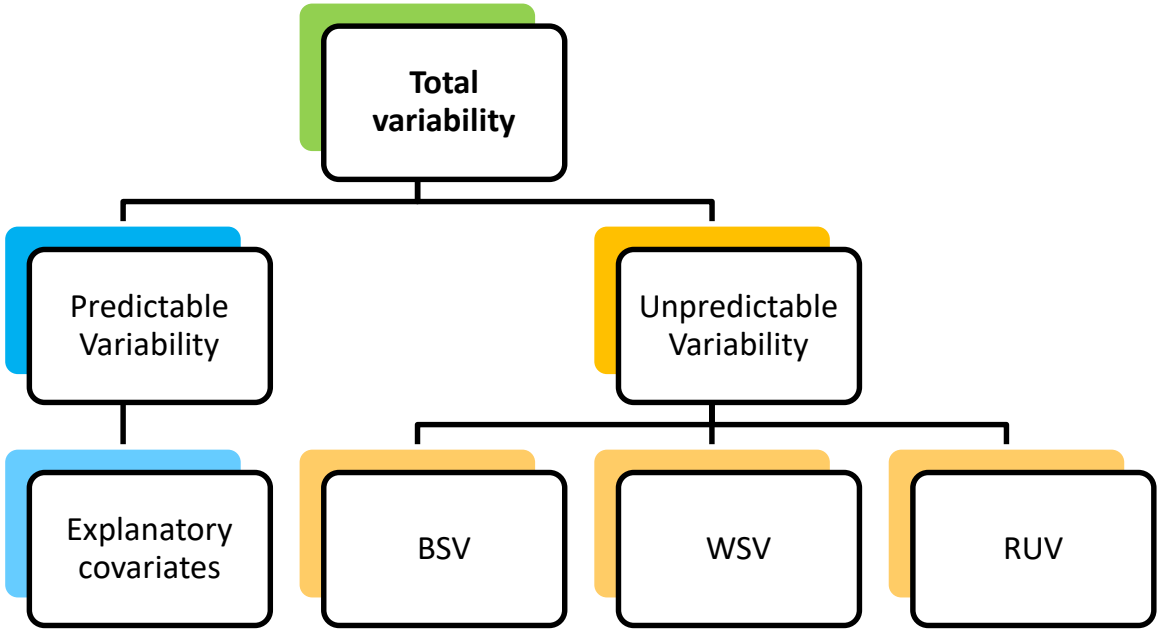


Figure 1. Partitioning of variability in pharmacokinetic response

The magnitude of these different variabilities plays a role in determining the appropriate dosing strategy for a therapy [58]. If a drug has low BSV and low WSV, along with a relatively wide therapeutic window, a generic population dose will maintain safe and effective concentrations in most patients. Conversely, when a drug that displays WSV that is wider than the therapeutic window, it becomes impossible to identify a safe and effective dose for a patient. In the case of clotting factor concentrates, BSV is quite high, but WSV is relatively low; that is, patients vary considerably from one another, but each patient's individual PK is reasonably stable over time. In this scenario, an individualized approach to dosing, which takes the patient's unique PK profile into account, should be implemented.

The WAPPS-Hemo Project

Despite its many advantages, PopPK is not without its own challenges. The development of a PopPK model requires a significant amount of data (a considerable obstacle in a rare disease setting), as well as proficiency in model building and evaluation. In the hemophilia community, a lack of confidence in model performance, as well as skepticism surrounding specific therapeutic targets (especially in the case of FIX), have also traditionally hampered the uptake of the PopPK approach. To facilitate its incorporation into routine care, a number of tools have been developed. Björkman evaluated the free Bayesian computer program TCIWorks for estimation of individual FVIII PK [59] but found that its efficient use required a level of expertise beyond that of the typical target user. myPKFiT, an industry-sponsored, brand-specific PopPK calculator recently received class II medical device approval from the US Food and Drug Administration. However, its application is currently limited in the United States to the on-label use of a single FVIII product (Advate), leaving a substantial portion of the hemophilia population unserved [60].

The Web Accessible Population Pharmacokinetic Service – Hemophilia (WAPPS-Hemo, Figure 2) program is an online service developed at McMaster University that is designed to help clinicians overcome the barriers typically associated with PK-tailoring of clotting factor regimens. Hemophilia treatment providers submit patient demographic information (e.g. age, weight, height) and 2–4 blood samples on the WAPPS-Hemo website (www.wapps-hemo.org). Using Bayesian forecasting, estimates of individual PK parameters are obtained and validated before being returned to the clinician. The patient report includes a graphical representation of the predicted concentration-time profile (with observed factor levels overlaid), as well as estimates of terminal half-life, times to critical factor activity levels (e.g. 5, 2, and 1 IU dL^{-1}), and

expected factor activity at key post-infusion times (e.g. 24, 48, and 72 hours) along with confidence intervals.

After the PK request has been completed, clinicians can enter the clinical calculator module, which allows for the exploration of alternative dosing regimens. There are three foundational components to a hemophilia regimen: dose, frequency of administration, and the target trough factor activity level. The user defines two of these three variables and the third is calculated using the patient's individual PK. Finally, the clinician can activate myWAPPS, a patient-facing app that allows patients to record their infusions and see predicted current or future factor activity levels.

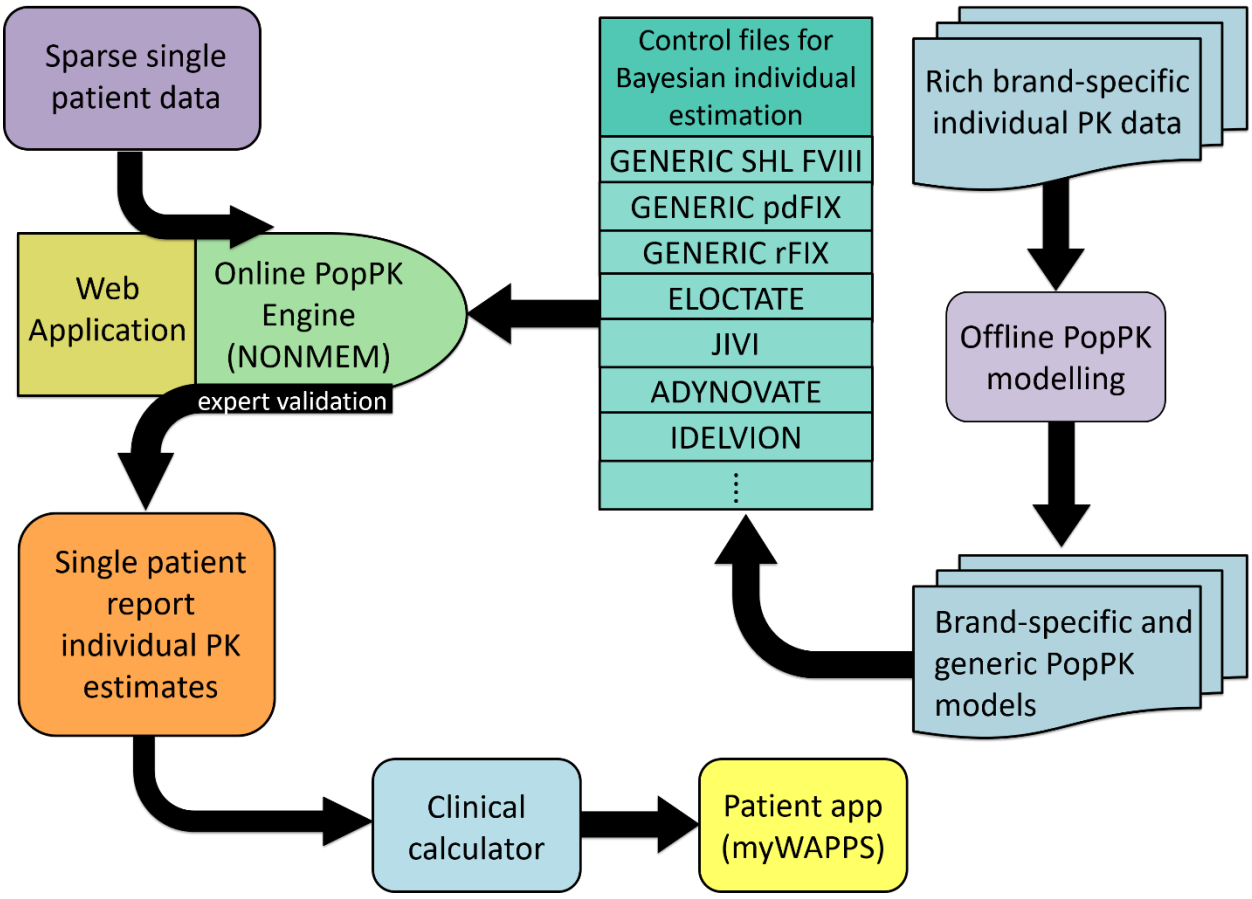


Figure 2. Schematic of the WAPPS-Hemo service, including PK estimation, clinical calculator module, and patient app

First launched in 2015, the WAPPS-Hemo network has been enthusiastically welcomed by the community as a useful tool in support of individualized dosing, and has grown rapidly in the last several years, as detailed in Chapter 7. At the time of writing, the network currently boasts over 400 centres in more than 50 countries, representing a truly global effort to provide hemophilia patients with truly individualized care.

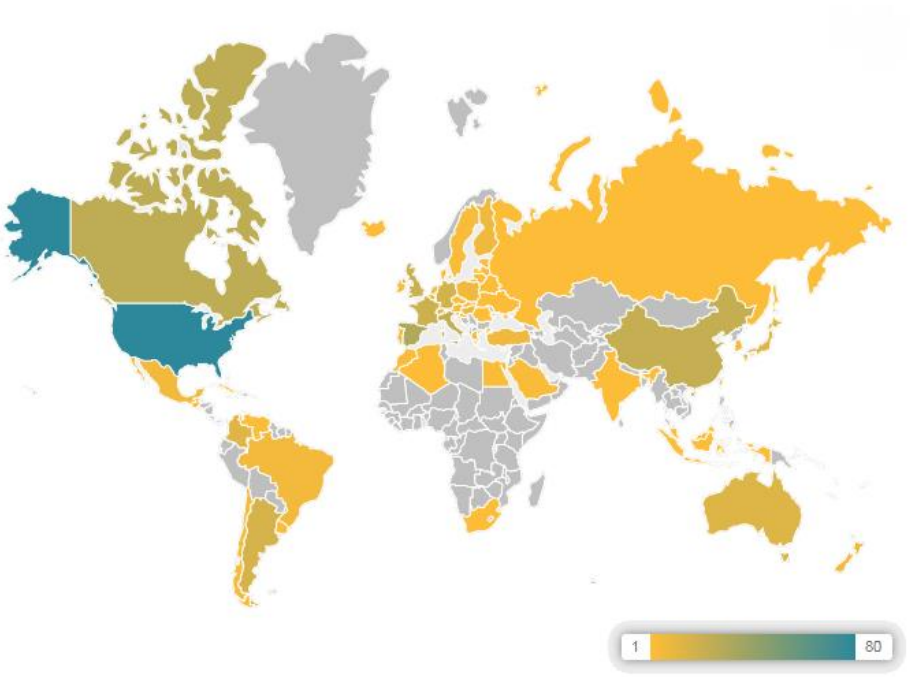


Figure 3. The global WAPPS-Hemo network, where the colour of each country indicates the number of registered centres (obtained from www.wapps-hemo.org, December 1, 2019).

Overarching Thesis Objective

To develop clotting factor PopPK models that are fit for the purpose of Bayesian forecasting for use in dosing regimen design in hemophilia A and B

Objectives & Hypotheses

1. Propose a data analysis protocol for the development and evaluation of PopPK models for all brands of clotting factors VIII and IX for use on the WAPPS-Hemo platform

   Hypothesis: A systematic approach to model development and evaluation will result in robust models that are fit for Bayesian forecasting

2. Apply the data analysis protocol to develop and evaluate generic models for brands of factor concentrate lacking brand-specific models on WAPPS-Hemo service

   Hypothesis: Factor VIII products are pharmacokinetically similar enough to that data from multiple brands can be combined to build a generic PopPK model to handle PK requests on WAPPS-Hemo for brands lacking a dedicated model; the same hypothesis applies to recombinant factor IX products

3. Develop an understanding of clinical factors that influence model performance and identify conditions that improve PK parameter estimation

   Hypothesis: Clinical factors such as timing of samples, information relating to prior doses, and knowledge of endogenous factor production level affect estimates of relevant PK parameters obtained through Bayesian forecasting

Chapter 2: The use of pharmacokinetics in dose individualization of factor VIII in the treatment of hemophilia A

Portions of this chapter are reflective of an original manuscript published by the Ph.D. candidate (Alanna McEneny-King) in *Expert Opinion on Drug Metabolism & Toxicology*. All pertinent dialogue in this chapter was written by the Ph.D. candidate.

McEneny-King A, Iorio A, Foster G, Edginton AN. The use of pharmacokinetics in dose individualization of factor VIII in the treatment of hemophilia A. Expert Opin Drug Metab Toxicol. 2016; 12(11):1313-1321. DOI: 10.1080/17425255.2016.1214711.

Introduction

Hemophilia A is an inherited bleeding disorder caused by a deficiency in clotting factor VIII (FVIII) and resulting in spontaneous, often recurring, joint bleeds and eventual arthropathy. As an X-linked condition, hemophilia A affects approximately 1 in 5,000 males [61] but reported prevalence varies considerably between countries, with many cases going undiagnosed in lower income countries [62]. Although evidence of hemophilia can be found in ancient Egyptian and Hebrew texts, modern replacement therapy did not begin until the 1960s [63,64]. With the introduction of plasma-derived clotting factor concentrates, hemophilia became a manageable

disorder rather than a catastrophic diagnosis. While a gene therapy cure remains elusive, considerable progress in the treatment of hemophilia patients has been made. When it was discovered that viruses such as hepatitis B, hepatitis C and HIV could be transmitted through these products, more rigorous safety measures were introduced, including stricter donor screening and the implementation of viral inactivation processes [65]. Advances in DNA technologies and lingering concerns about the safety of plasma-derived concentrates propelled the development of recombinant coagulation factors in the early 1990s [66,67].

Two main treatment strategies exist for the management of hemophilia A: on-demand, or episodic, and prophylactic. The concept of prophylaxis, initiated by Nilsson and colleagues in the 1970s [7,68], is derived from the clinical observation that patients with moderate hemophilia (those whose factor levels are >1% of normal) are less prone to the arthropathy and spontaneous bleeds seen in those with severe hemophilia [69]. Today, there is global unanimity that prophylaxis should be initiated in young children before joint disease is apparent [11–13], but the implementation of this approach varies widely between countries [14]. The cost and availability of factor concentrates are major barriers to its widespread adoption, as is the challenge of patient compliance [15]. Prophylaxis is the only known method for preventing joint damage in hemophilia patients, as episodic treatment has been shown to be ineffective for the prevention of arthropathy [9,12]. However, an optimal dosing strategy has yet to be determined; instead, evidence suggests that treatment with FVIII should be individualized for best results, both from a therapeutic and economic perspective [51,56]. Typically, patients are dosed by weight but a tailored treatment plan must take into account individual variation in pharmacokinetic (PK) parameters, beyond what can be predicted by age and weight [70,71]. [17] However, a classical PK study for FVIII requires 11 samples – four in the distribution phase (0–1 h) and seven in the

elimination phase (3–48 h) – as outlined by recommendations from the International Society on Thrombosis and Haemostasis [72], making it a difficult approach to apply in a clinical setting.

For this reason, population pharmacokinetic (PopPK) models are desirable. In addition to the rich sampling schedules, classical PK studies are also typically carried out in a homogeneous group of subjects, usually healthy, young males. PopPK studies, on the other hand, use sparse sampling in a more heterogeneous group of subjects to gain understanding of variability. Since fewer samples are required, populations that are unable to undergo the rigid sampling of classical PK studies (e.g. paediatrics, elderly, critical care patients) can be included [73]. Often, subjects that appear similar exhibit different PK behaviour due to unpredictable variability. For example, Collins et al. examined the variability in time to reach 1 IU dL^{-1} and found significant variation not only between children and adults, but within each group as well, with times ranging from 44 to 78 h in children and 46 to 103 h in adults [74]. Total variability in population parameter values can be split into predictable and unpredictable variability. Predictable variability can be ascribed to covariates that influence PK. These covariates may be demographic (body weight, age), behavioural (smoking status, diet), genetic (phenotypes affecting drug clearance), or physiologic (pregnancy, disease state) in nature, and the identification of meaningful covariates can help recognise subpopulations that are at risk of over- or under-dosing if the standard weight-based strategy is employed [75].

Unpredictable variability may be between patients or within a patient, and a main goal of PopPK is to estimate the magnitude of this unexplained variability. When unexplained variability is high, the chances of reaching drug concentrations outside the target range increase and issues of safety and efficacy can occur [76]. The target concentration window of the drug can be used to define an upper limit on the unpredictable variability, below which the drug is considered to be

safe and effective. By comparing each element of variability to this threshold, a suitable dosing regimen can be chosen. If the therapeutic window is large, variability is not an issue and a generic population dose is acceptable; if the within subject variability is greater than the permissible variability, it is not possible to define a safe and effective dose for an individual. However, if the variability threshold lies between the total unpredictable variability and the unpredictable within subject variability, individualized dosing is beneficial [58]. Since within subject variability is small relative to intersubject variability in the case of FVIII PK parameters [70], individualizing dose by incorporating subject-specific PK behaviour is the appropriate method.

This approach is used in the therapeutic monitoring of several other conditions [77–79], and a 2010 study by Björkman et al. indicated that a limited sampling strategy could be as useful for prediction as a full study [57]. However, adoption of this approach has been hampered recently due to the complexity of the models involved and a relative shortage of PK data due to the rarity of the disease. To contribute overcoming these barriers, we have reviewed the available pharmacokinetic data for FVIII in the treatment of hemophilia patients with the aim of facilitating their uptake and use in the development of PopPK models for dose individualization.

Pharmacokinetics of FVIII

Studies on the PK of plasma-derived factor VIII were reviewed comprehensively by Björkman and Carlsson in 1997 [50]. A similar review in conducted here for studies published in the years 1998 through 2015 for both plasma-derived and recombinant products. Mean values of the PK parameters and associated variabilities were assessed across included studies. In the absence of a specific research question, this review is considered comprehensive as opposed to

systematic. The search was completed using PubMed with the following search criteria: ('hemophilia' [all fields] or 'hemophilia a' [MeSH terms] or 'hemophilia a' [all fields] or 'haemophilia' [all fields]) and ('pharmacokinetics' [subheading] or 'pharmacokinetics' [all fields] or 'pharmacokinetics' [MeSH terms]). Further selection was made for only those original studies presenting new PK data in hemophilia A patients.

An issue faced at the time of the Björkman review was inadequate sampling time (<48 h), resulting in an underestimated half-life and overestimated clearance. Since highlighting the effect of inadequate sampling on estimation of PK parameters, studies have generally extended sampling time to at least 48 h, hopefully eliminating one source of variability in estimated PK. On the other hand, the recent introduction of concentrates with extended half-life (EHL) has made the optimal length of sampling time a new matter of discussion, which in general needs to be reconciled with the general PK recommendation that sampling time should cover at least five half-lives [80]. The study details and calculated PK parameters for each reference is presented in Table 2. Patients included in each of the studies were in stable condition (i.e. not presenting any recent bleed) and in absence of any inhibitors.

The pharmacokinetics of FVIII are generally well characterised, with the exception of EHL products. Typical values for clearance (0.3 dL h^{-1} kg^{-1}), volume of distribution at steady state (V_{ss}, 48 mL kg^{-1}, or slightly larger than plasma volume) and half-life (14 h) are known [51], and the average parameter values for the studies in Table 2 are quite similar (12.94 h, 0.32 dL h^{-1} kg^{-1}, and 56 mL kg^{-1}, respectively). The half-lives for the EHL products ranged from 18.2 to 19.04 h and this extension is due to reduced clearance (0.3 vs. 0.18 dL h^{-1} kg^{-1}) rather than more extensive distribution, since V_{ss} is unchanged. As seen in Table 2, the PK parameters obtained from the 23 studies are quite varied. For example, half-life varies between 9.42 ± 3.80 and 17.37

± 12.94 h in adult patients. Furthermore, the variability within each study is considerable as well; many studies report a coefficient of variation greater than 30% for estimates of half-life, clearance and V_{ss}. Though this may encompass both patient-related variability and experimental error, it reinforces the idea that individual dose adjustments would be of great clinical benefit, to limit both overdosing (associated with wastage of resources) and underdosing (associated with ineffective and unsafe treatment) [30].

Table 2. Studies on the pharmacokinetics of plasma-derived and recombinant FVIII. PK parameters are presented as mean ± SD (%CV); n. g. denotes values that were not reported in the studies.

Study	Product(s)	Assay*	Number of Patients	Patient Age (years)	Sampling Time	Number of Samples	$t_{1/2}$ (h)	CL (mL h^{-1} kg)	V_{ss} (mL kg^{-1})	Analysis Method[c]
[81]	Monoclonate-P® Bioclate®	OS	10	27-41	48 h	14	15.5 ± 2.8 (18%) 14.9 ± 2.9 (19%)	2.76 ± 0.61 (21%) 4.02 ± 0.044 (10%)	77.1 ± 13.9 (18%) 95.0 ± 21.9 (23%)	NCA
[82]	Recombinate®	OS CH	30	≥12	24 h	8	13.1 ± 2.4 (18%) 13.1 ± 1.9 (14%)	1.9 ± 0.3 (16%) 1.52 ± 0.21 (14%)	60.3 ± 5.8 (10%) 49.1 ± 6.9 (14%)	NCA
[83]	Emoclot®	OS	14	18-44	36 h	12	12.51 ± 0.54 (4%)	5.06 ± 3.167 (63%)	n. g.	n. g.
[84]	KOGENATE®	OS	20	12-60	48 h	11	13.8 ± 2.2 (16%) 14.6 ± 3.1 (21%) 12.6 ± 0.1 (8%)	n. g.	n. g.	n. g.
[85]	Koate®-DVI Koate®-HP	OS	18	14-46	48 h	n. g.	16 ± 3 (19%) 16 ± 5 (31%)	n. g.	n. g.	NCA
[86]	BDDrFVIII	CH	113	≥7	24 h	n. g.	10.5 ± 2.6 (25%)	n. g.	n. g.	n. g.
[87]	BDDrFVIII	CH	39	0-5	n. g.	n. g.	7.5 ± 2.7 (36%)	n. g.	n. g.	n. g.
[88]	KOGENATE®	OS	15	12-59	48 h	11	14.4 ± 2.7 (19%)	n. g.	n. g.	n. g.
[89]	ReFacto®	CH	18	n. g.	n. g.	n. g.	14.5 ± 5.3 (37%)	n. g.	n. g.	n. g.
[90]	ReFacto®	OS CH	18	≥12	48 h	9	17.37 ± 12.94 (74%) 11.69 ± 5.21 (44%)	3.80 ± 1.32 (35%) 3.83 ± 1.22 (32%)	78.50 ± 25.60 (33%) 58.13 ± 17.11 (29%)	n. g.
[91]	Advate®	n.s.	53	<6	48 h	5	9.88 ± 1.89 (19%)	4.43 ± 1.40 (32%)	51.4 ± 12.3 (24%)	Two-phase linear regression

Study	Product(s)	Assay*	Number of Patients	Patient Age (years)	Sampling Time	Number of Samples	$t_{1/2}$ (h)	CL (mL h^{-1} kg)	V_{ss} (mL kg^{-1})	Analysis Method[c]
[92]	Recombinate® rAHF-PFM	OS	30	10-65	48 h	11	11.2 ± 2.5 (22%) 12.0 ± 4.3 (36%)	3.0 ± 1.0 (33%) 3.0 ± 1.0 (33%)	46 ± 10 (22%) 47 ± 10 (21%)	Two-phase linear regression
[93]	Haemoctin® SDH	n. g.	13	11-16	30 h	12	11.8 ± 4.2 (36%)	1.52 ± 0.5 (33%)	n. g.	NCA
[94]	BDDrFVIII-A BDDrFVIII-B Hemofil®	CH	18	18-44	48 h	12	15.4 ± 5.4 (35%) 14.8 ± 5.6 (38%) 13.7 ± 3.7 (27%)	n. g.	n. g.	Log-linear regression
[95]	ReFacto® Advate®	CH	17	19-72	48 h	11	13.0 ± 3.1 (24%) 13.6 ± 3.8 (28%)	3.85 ± 1.36 (35%) 3.97 ± 1.40 (35%)	58.6 ± 13.7 (23%) 61.7 ± 18.6 (30%)	NCA
[96]	BAY 79-4980 Recombinate®	OS+CH	24	12-60	168 h	13	11.4 ± 2.49 (22%) 11.6 ± 2.55 (22%)	3.13 ± 0.81 (26%) 3.07 ± 0.72 (23%)	50.0 ± 9.5 (19%) 49.4 ± 9.6 (19%)	NCA
[97]	Moroctocog Alfa	OS	30	12-60	48 h	11	11.2 ± 5.0 (45%)	4.51 ± 2.23 (49%)	n. g.	n. g.
[98]	ReFacto®	OS CH	13	21-69	32 h	9	12.95 ± 4.73 (36%) 7.70 ± 4.26 (55%)	3.5 ± 0.7 (20%) 4.2 ± 1.4 (33%)	46.6 ± 12.1 (26%) 36.2 ± 7.5 (21%)	NCA
[99]	Octivate®	OS	13	12-65	n. g.	n. g.	12.4 ± 2.86 (23%)	3.1 ± 0.79 (25%)	53.4 ± 14.1 (26%)	NCA
[100]	Solvent/detergent-filtered cryoprecipitate	OS	11	n. g.	36 h	7	14.2	2.6	n. g.	Log-linear regression
[101]	N8 Advate®	OS	20	13-54	48 h	9	10.83 ± 4.95 (46%) 11.19 ± 3.51 (31%)	4.11 ± 1.06 (26%) 4.17 ± 1.20 (29%)	59.8 ± 11.7 (20%) 61.3 ± 7.9 (13%)	NCA
[102]	Advate®	n. g.	71	7-59	48 h	10	13.95 ± 5.30 (38%)	3.91 ± 1.18 (30%)	n. g.	n. g.
[103]	NovoEight®	OS	14 14 48	0-5 6-11 ≥12	48 h	14	7.7 ± 1.8 (23%) 8.0 ± 1.9 (24%) 11.2 ± 4.2 (38%)	6.2 ± 3.7 (60%) 5.0 ± 1.7 (34%) 3.5 ± 1.1 (29%)	56.7 ± 26.4 (47%) 46.8 ± 10.6 (23%) 46.7 ± 9.6 (21%)	NCA

Study	Product(s)	Assay*	Number of Patients	Patient Age (years)	Sampling Time	Number of Samples	$t_{1/2}$ (h)	CL (mL h^{-1} kg)	V_{ss} (mL kg^{-1})	Analysis Method[c]
[104]	Nuwiq®	OS	13	2-5	48 h	n. g.	11.91 ± 5.36 (45%)	5.41 ± 2.32 (43%)	68.29 ± 10.42(15%)	NCA
			13	6-12			13.08 ± 2.59 (20%)	4.05 ± 0.92 (23%)	66.07 ± 15.99 (24%)	
		CH	13	2-5			9.49 ± 3.32 (35%)	5.40 ± 2.37 (44%)	55.32 ± 7.09 (13%)	
			13	6-12			9.99 ± 1.88 (19%)	4.33 ± 1.21 (28%)	54.45 ± 14.80 (27%)	
[105]	BAY 81-8973	OS	26	12-61	48 h	10	13.8 ± 3.5 (25%)	3.8 ± 1.4 (37%)	67 ± 16 (24%)	n. g.
		CH					14.3 ± 3.8 (26%)	2.8 ± 1.0 (36%)	54 ± 20 (37%)	
	rFVIII-FS	OS	26				12.6 ± 3.0 (24%)	4.6 ± 1.7 (37%)	71 ± 21 (30%)	NCA
		CH					12.4 ± 3.2 (26%)	3.4 ± 1.3 (38%)	55 ± 23 (42%)	
Extended Half-Life Products										
[106]	Advate®	OS	15	≥12	72 h	n. g.	12.2	2.49	43.9	1-compartment model
	rFVIIIFc				168 h		18.8	1.68	45.4	
[107]	rFVIII-FS	CH	7	18-65	168 h	13	12.9 ± 6.95 (54%)	2.3 ± 2.1 (92%)	42 ± 24 (57%)	NCA
	BAY-949027						18.2 ± 9.72 (53%)	1.6 ± 0.74 (46%)	43 ± 10 (23%)	
[108]	Efraloctocog Alfa	OS	28	≥12	120 h	6	19.0	2.0 ± 0.67 (34%)	n. g.	Compartmental analysis
	Advate®						12.4	3.0 ± 0.94 (31%)		
[109]	N8-GP	CH	26	2-60	168 h	14	19.04 ± 5.53 (29%)	1.79 ± 0.92 (51%)	45.3 ± 17.8 (39%)	NCA
[110]	rFVIIIFc	OS	23	<6	48 h	n. g.	12.67 ± 3.52 (28%)	3.60 ± 1.15 (32%)	58.58 ± 9.02 (15%)	n. g.
			31	6-11	72 h		14.88 ± 8.22 (55%)	2.78 ± 0.98 (35%)	52.13 ± 19.54 (37%)	

*OS = one-stage assay; CH = chromogenic assay.

Covariates Affecting Pharmacokinetic Parameters

The process of model selection for a PopPK model includes both definition of the structural model as well as incorporation of possible covariates. Covariate modelling helps to describe the variability in PK response by establishing relationships between patient characteristics and model parameters. To facilitate the development of PopPK models for FVIII, any covariates that have been already shown to affect FVIII PK were identified.

A 2010 study by Björkman et al [111] gathered PK data from children (aged 1-6 years) and adolescents/adults (aged 10-65 years). Between these two groups, children exhibited lower in vivo recovery (1.84 vs 2.42 IU dL^{-1}/IU kg^{-1}), higher weight-adjusted clearance (4.34 vs. 3.26 mL h^{-1} kg) and a shorter half-life (9.4 vs 11.2 h); weight-adjusted V_{ss} was not different between groups. Within patient variance was also great among children, though still considerably less significant than between patient variance. Within the paediatric group, half-life increased with age. Within the adult group, weight-adjusted clearance and weight-adjusted V_{ss} decreased with both age and weight (expressed as a ratio of actual weight to ideal body weight); no effect was observed for half-life. However, it is important to note that the r^2 values for these predictors are quite low for both the paediatric (<0.31) and adult (<0.13) models, suggesting that the within patient variability cannot be adequately described by these factors. A more recent study conducted by Jiménez-Yuste [103] comparing three age groups (0-5, 6-11 and ≥12 years) found similar trends for half-life, clearance, V_{ss} and IVR (Table 1).

Another characteristic that has been investigated in association with FVIII PK is blood type. Several studies have found that the half-life of FVIII is considerably shorter among patients with O-type blood compared to other blood types [112–115], likely due to the lower plasma levels of von Willebrand factor (vWF) observed in patients with O-type blood. Levels of von

Willebrand factor (vWF) have been shown to be positively correlated with half-life [114,116,117] as binding to vWF serves to stabilize the FVIII molecule and prevent degradation [118]. These levels tend to increase with age [119,120], and may help to explain the shorter FVIII half-life observed in children compared to adults.

As mentioned previously, typical dosing for hemophilia treatment is adjusted according to body weight. However, ideal body weight should be used in place of actual body weight or body mass index when calculating dose for under- or overweight patients. This is because the body proportion of fat does not affect the distribution and elimination of coagulation factors [121]. As mentioned, the typical V_{ss} for FVIII is approximately plasma volume. Since the fraction vascular of fat is low (0.005 to 0.010 [122]), a surplus (or scarcity) of fat does not significantly affect the volume of distribution. In summary, including appropriate weight-based metric and/or other intrinsic or extrinsic covariates may improve dosing in sub-populations.

An additional source of variability inherent in the study of the PK of clotting factors is associated with the measurement of plasma levels themselves. The different ways of measuring FVIII activity can lead to a significant discrepancy in results. Assay results depend not only on the assay method used (one-stage vs. chromogenic), but also on the reagents and calibrants used [123]. The disagreement between assays was thought to be greater among B-domain deleted rFVIII (BDDrFVIII) [124], but more recent and specific studies have shown that the discrepancy is similar to that observed for wild-type recombinant concentrates [123]. Despite the high variability observed with different analytical methods, no definitive recommendations have been made for clinical practice, and attempts to model data obtained from different assays may prove challenging.

A Pharmacokinetic Comparison of Recombinant and Plasma-Derived Products

As mentioned, the transmission of several blood-borne viruses in the 1970s and 1980s emphasized the need for safer treatments and ultimately led to the development of recombinant factor concentrates [65]. The majority of comparison studies for recombinant and plasma-derived FVIII focus on safety, particularly on the risk of developing inhibitors [125–127]. Some early studies comparing the PK of recombinant and plasma-derived products found significant differences in some parameters, but sampling was only performed for 24 hours rather than the suggested 48 hours [67,128]. A more recent study used patients (≥14 years) that were switching from plasma-derived to recombinant FVIII as an opportunity to compare their PK [129]. Differences at early timepoints following infusion precluded proper assessment of bioequivalence, but the half-lives for both groups were similar. There is currently insufficient paediatric data to accurately compare plasma-derived and recombinant products in children [130].

In the study by Deitcher et al. shown in Table 1 [81], changes in FVIII PK characteristics with concomitant use of desmospressin acetate (DDAVP) were investigated for both plasma-derived and recombinant products. DDAVP causes a rapid release of vWF and FVIII from storage sites and is used in the treatment of mild hemophilia; it is assumed to be ineffective for patients with severe hemophilia, since these stores of FVIII do not exist. However, typical replacement theory combined with DDAVP administration was hypothesized to extend half-life of FVIII by fostering the formation of the more stable FVIII-vWF complex. At baseline, clearance was higher for the recombinant group, but no significant differences were observed for other PK parameters. The combined therapy with desmopressin resulted in a significantly larger V_{ss} and mean residence time in the plasma-derived group, but not in half-life, suggesting that neither group would necessarily benefit from this concomitant treatment approach.

Finally, a 2005 study compared the PK of plasma-derived and B-domain deleted recombinant FVIII (BDDrFVIII) [94]. This modification increases the product yield, but is not meant to alter the *in vivo* functionality of the molecule and this study confirmed bioequivalence between two different preparations of BDDrFVIII and a plasma-derived concentrate. In summary, no differences in PK were observed between plasma-derived and recombinant FVIII products and source of factor is not likely an important covariate to include in PopPK modelling.

Development of Extended Half-Life Products

Since the half-life of FVIII is relatively short, prophylactic therapy requires frequent infusion in order to be effective. For this reason, products with longer half-lives are desirable. The first EHL drug for the treatment of hemophilia A to achieve approval from the FDA was efraloctocog alfa, or Eloctate® in June 2014 [131]. Its half-life is approximately 50% longer than traditional recombinant products, allowing for infusion every four days rather than every other day, hopefully improving patient adherence [106]. Several other products are in the pipeline, and the most common strategy involves fusing the FVIII molecule to another large molecule such as polyethylene glycol [132], human immunoglobulin fragments [133,134] and vWF [135]. Another technique involves the manipulation of the FVIII molecular structure. The native structure of FVIII consists of two chains held together by a metal-ion bridge, rendering the molecule rather unstable. By adding a covalent bond between the two chains, a more stable single-chain structure can be formed. The single-chain rFVIII also has greater affinity for vWF. Since the half-life of vWF is approximately 50% longer than that of FVIII, this may translate into a longer-lasting product [136]. A recent study in mice, rabbits and cynomolgus monkeys found a 1.7, 2.1 and 1.6-fold increase in terminal half-life, respectively, for single-chain rFVIII

as compared to its full-length counterpart. The importance of vWF was also confirmed in this study using vWF knockout mice; terminal half-life decreased by a factor of 30 compared to the hemophilia A mice [137].

Since EHL products are relatively new, few PK studies have been conducted in humans. As a result, the covariates affecting their PK parameters remain largely unknown. One study used data from a phase 3 clinical trial to investigate potential covariate effects using PopPK analysis [138]. Their final model included vWF level as a major covariate on clearance and hematocrit as a weak covariate on volume of the central compartment. Thus, the PK parameters of EHL products may be influenced by different covariates than their predecessors and further investigation is required to ensure accurate individualized dosing for these products.

Long-lasting products are not only possibly beneficial from a patient adherence perspective; they could also deliver a major economic advantage, depending of course on costs per unit of factor concentrate. Factor concentrates are expensive (approximately US$1 per unit), and prophylaxis for a 50 kg child can cost up to $300,000 per year [139–141]. As calculated by Björkman [57], an increase in half-life from 8.1 to 10.8 h means that the dose given at each infusion can be reduced by more than half, from 29.7 to 10.7 IU/kg; this translates to a yearly savings of 56,000 IU.

While they may appear to be the first major improvement to hemophilia care since the development of recombinant products, the role of EHL products is still very much unknown and the anticipated advantages may not come to fruition. For example, a simulation exercise performed by Gringeri et al suggests that the longer dosing interval proposed for EHL products results in patients spending a greater amount of time with factor levels below 3%, potentially

increasing the risk of bleeding events [142]. If this is the case, then EHL products may function better by using a similar dosing schedule as current FVIII products and maintaining a higher trough level. Since clinical trials with these products have found no significant change in annualized bleeding rate when adopting a once or twice weekly dosing schedule [143], the role these products will play in the hemophilia community is still largely undefined.

Conclusions

In summary, PK investigations offer valuable information that can subsequently be used in the optimization of hemophilia treatment. From these studies, one can gain a true understanding of the importance of between patient variability in estimation of PK parameters for FVIII. PK studies also afford the opportunity to identify patient characteristics that may help in the parameterization of PopPK models; in the case of FVIII, age, ideal body weight, and blood type should be considered where possible, and levels of von Willebrand factor bear potential but are still in need of confirmation. However, individual PK still varies considerably beyond what is captured by these covariates, and a heterogeneous study population is necessary to capture this variability. Furthermore, intermittent sampling and subsequent model updating may be required to account for variations in individual PK and ensure models remain effective.

Addendum

A substantial number of studies investigating the pharmacokinetics of FVIII products have been conducted in the years since this review was first completed. Using the same search strategy as previously, an additional 19 eligible studies were found for the period following the original review (2016-2019), the details of which can be found in Table 3. Many studies, most of them crossover designs, focused on new EHL products that have entered the market in recent years, and whose potential and role in hemophilia treatment were not well understood at the time of the initial review.

Although the PK profile of these products is somewhat improved, there does appear to be a limit to the degree of half-life extension achievable for FVIII concentrates. Certain EHL FIX products have half-lives of 100 hours or more (a roughly 4-fold increase), allowing for dosing on a weekly or bi-weekly basis; half-lives of EHL FVIII products peak at 20 hours or less (1.5-fold increase), typically extending the dosing interval by just one day [144]. This limited half-life prolongation has been attributed to the complexation of FVIII and vWF. The overwhelming majority of FVIII is present in a complex with vWF, which stabilizes the structure of the FVIII molecule, protects FVIII from proteolytic degradation, and prevents cellular uptake [145]. FVIII is also largely cleared in the FVIII-vWF complex form [146]. As a result, half-life of vWF (approximately 15 h, with considerable BSV [145]) limits the potential for FVIII half-life extension, a hurdle that the currently utilized technologies (protein fusion, PEGylation, and protein sequence modification) have been unable to overcome.

Table 3. Studies on the pharmacokinetics of plasma-derived and recombinant FVIII identified in the 2019 update. n. g. denotes values that were not reported in the studies.

Study	Product(s)	Assay	Number of Patients	Patient Age (years)	Sampling Time	Number of Samples	$t_{1/2}$ (h)	CL (mL h^{-1} kg^{-1})	V_{ss} (mL kg^{-1})	Analysis Method[c]
[147]	rFVIII-SingleChain octocog alfa	CH	27	18-65	72 h	10	14.0 ± 3.4 (24%) 11.6 ± 3.6 (31%)	2.69 ± 0.81 (30%) 3.91 ± 1.38 (35%)	49.6 ± 7.5 (15%) 55.8 ± 11.8 (21%)	NCA
[148]	BAX 855	OS	14 17	<6 6 to <12	48-96 h	4	12.7 13.9	3.07 2.71	n. g.	2-compartment model
		CH	14 17	<6 6 to <12			13.9 13.8	3.00 2.42	n. g.	
	Advate	OS	14 17	<6 6 to <12			8.78 9.44	4.22 5.55	n. g.	
		CH	14 17	<6 6 to <12			9.17 9.52	4.94 5.00	n. g.	
[149]	rFVIII-SingleChain	CH	20 19	<6 6 to <12	48 h	6	10.4 (28.7%) 10.2 (19.4%)	5.07 (29.6%) 4.63 (29.5%)	71.0 (11.8%) 67.1 (22.3%)	NCA
[150]	BAX 855	OS	26 22	12-58	n. g.	n. g.	14.3 (3.84) 16.0 (4.92)	2.76 (2.03) 2.47 (0.82)	n. g.	n. g.
	Advate		26				10.4 (2.24)	4.55 (2.17)		
[151]	Moroctocog alfa	OS	3 10	6 to <12 ≥12	72 h	12	7.2 ± 1.8 (25%) 13.8 ± 3.8 (28%)	6.65 (30%) 2.67 (38%)	67.18 (10%) 50.53 (30%)	NCA
[152]	N8-GP	n. g.	15 12	0-5 6-11	96 h	Up to 7	13.2 14.3	n. g.	n. g.	NLME
	Previous FVIII product		15 12	0-5 6-11	30 h	Up to 5	7.2 7.6			

Study	Product(s)	Assay	Number of Patients	Patient Age (years)	Sampling Time	Number of Samples	$t_{1/2}$ (h)	CL (mL h^{-1} kg)	V_{ss} (mL kg^{-1})	Analysis Method[c]
[10]	Various FVIII products	n. g.	11 7 6	4 to <7 7 to <12 ≥12	48 h	5	8.80 ± 2.45 (28%) 10.15 ± 2.48 (24%) 12.82 ± 2.38 (18%)	n. g.	n. g.	WinNonLin
[153]	Moroctocog alfa Moroctocog alfa AF-CC	CH	25	12-70	48 h	11	10.9 ± 4.5 (41%) 9.9 ± 3.2 (32%)	3.69 ± 1.48 (40%) 3.84 ± 1.69 (44%)	51.1 ± 8.5 (17%) 49.9 ± 9.1 (18%)	NCA
[154]	Moroctocog alfa AF-CC	CH	14	6 to <12	48 h	10	9.12 ± 1.94 (21%)	4.496 (30%)	56.42 (15%)	NCA
[155]	pdFVIII rFVIII	OS	15 21	4.0-16.7	48 h	4	11.16 ± 0.78 (7%) 10.87 ± 0.65 (6%)	3.81 ± 0.36 (9%) 4.69 ± 0.29 (6%)	37.22 ± 2.52 (7%) 42.77 ± 2.04 (5%)	NCA
[156]	BAY 94-9027	CH	14 13 3 19	<6 6 to <12 12 to <18 ≥18	72 h 72 h 96 h 96-168 h	6 6 11 11-13	15.0 ± 4.1 (27%) 16.0 ± 3.5 (22%) 17.9 ± 1.7 (9%) 17.6 ± 4.6 (26%)	3.14 ± 1.41 (45%) 2.14 ± 0.41 (19%) 1.57 ± 0.40 (25%) 1.70 ± 0.58 (34%)	59.5 ± 16.4 (28%) 50.1 ± 9.5 (19%) 39.9 ± 10.7 (27%) 39.6 ± 5.8 (15%)	NCA
[157]	BAY 81-8973 rAHF-PFM	OS CH OS CH	18	18-65	48 h	10	14.5 (25.7%) 13.9 (25.1%) 11.7 (27.3%) 12.0 (23.3%)	2.7 (34.3%) 2.1 (28.5%) 3.6 (32.4%) 3.0 (31.0%)	53 (19.4%) 39 (19.1%) 56 (17.3%) 46 (16.7%)	NCA
[158]	Xyntha (rest) Xyntha (exercise)	OS	21	18-36	24 h	7	10.55 ± 2.87 (27%) 10.45 ± 2.55 (24%)	2.76 ± 0.76 (28%) 2.89 ± 0.71 (24%)	n. g.	NCA
[159]	Advate	OS	21	14-68	24-32 h	2	14.0 ± 2.7 (19%)	3.0 ± 0.7 (23%)	52.1 ± 7.1 (14%)	Bayesian estimation (myPKFiT)
[160]	Unspecified	n. g.	10	22-49	4 h	4	13.4	1.92	38.2	Bayesian estimation

Study	Product(s)	Assay	Number of Patients	Patient Age (years)	Sampling Time	Number of Samples	$t_{1/2}$ (h)	CL (mL h^{-1} kg)	V_{ss} (mL kg^{-1})	Analysis Method[c]
[161]	rFVIII-FS BAY 81-8973	OS	14	10-50	44-52 h	2-3	15.4 [12.0-16.8] 16.9 [15.3-19.1]	n. g.	n. g.	Bayesian estimation (WAPPS-Hemo)
[162]	pdFVIII	CH	4	24-46	48 h	10	14.3 [11.4-16.0]	3.4 ± 0.2	59 ± 6	NCA
[163]	BAY 94-9027 rFVIIIFc	OS	17	22-65	120 h	12	16.3 (34%) 15.2 (33%)	2.0 (38%) 2.5 (32%)	46.2 (15%) 49.7 (22%)	NCA
[164]	N8-GP	CH	13 11 3 42	0-5 6-11 12-17 ≥18	96 h 96 h 96 h 96-168 h	7 7 7 9-14	13.6 (20%) 14.2 (26%) 15.8 (43%) 19.9 (34%)	2.6 (45%) 2.4 (40%) 1.5 (43%) 1.4 (32%)	n. g.	NCA

*OS = one-stage assay; CH = chromogenic assay.

Chapter 3: Data analysis protocol for the development and evaluation of population pharmacokinetic models for incorporation into the Web Accessible Population Pharmacokinetic Service – Hemophilia (WAPPS-Hemo)

This chapter is reflective of an original manuscript prepared by the Ph.D. candidate (Alanna McEneny-King) for submission to *JMIR Research Protocols*. All pertinent dialogue in this chapter was written by the Ph.D. candidate.

Introduction

Hemophilia is a congenital bleeding disorder caused by a deficiency in clotting factor VIII (FVIII, hemophilia A) or IX (FIX, hemophilia B), resulting in bleeding episodes, often in the joints. Hemophilia A is considerably more common than hemophilia B, with reported prevalences of 8.0 and 2.4 per 100,000 males, respectively [62,165]. In more severe hemophilia patients (i.e. those with endogenous factor levels below 10 IU L^{-1}), bleeds may occur spontaneously and can lead to irreversible damage in target joints. Treatment options include replacement with exogenous clotting factor concentrates, which may be administered on-demand when bleeds occur, or prophylactically on a regular schedule.

Prophylactic treatment has been repeatedly shown to improve joint outcomes [9,166], but can be somewhat challenging to implement due to wide interpatient variability in pharmacokinetics (PK) handling of factor concentrates [167,168]. Evidence suggests that an individualized approach is optimal, from both a therapeutic and economic perspective. Previously, PK-based dose tailoring was hampered by the high sampling burden required to perform classical PK estimation. However, the International Society of Thrombosis and Haemostasis (ISTH) Scientific and Standardization Committee recently recommended the use of

the population pharmacokinetic (PopPK) approach to improve the feasibility of PK-tailored dosing in routine care [169]. While this approach does reduce the number of samples needed, the development and evaluation of PopPK models requires specialized software, expertise, and a considerable amount of data. In recent years, web-based PK software such as the Web Accessible Population Pharmacokinetics Service – Hemophilia (WAPPS-Hemo, www.wapps-hemo.org) have emerged to tackle this obstacle and encourage the use of the PopPK approach in hemophilia treatment. Housed at McMaster University in Canada, the service was launched in 2015 and has grown into the largest repository of hemophilia PK data worldwide. Users provide patient demographic data and a minimal number of blood samples, and receive a report containing the patient's predicted PK profile and individual estimates of PK outputs such as half-life, time to critical factor levels (50, 20, and 10 IU L^{-1}), and factor levels at key post-infusion times (24, 48, 72, and 96 hours, as appropriate).

Population pharmacokinetic modelling uses nonlinear mixed effects modelling techniques with the primary goal of partitioning, quantifying, and identifying sources of variability in PK response. Total variability can be divided into predictable and unpredictable variability, where predictable variability is attributed to covariates (e.g. body weight, age, blood type) that are known to influence a patient's PK; the identification of important covariates can also help to detect at-risk subpopulations. Unpredictable variability may occur between separate patients (BSV) or within a single subject on different occasions (BOV). The magnitude of these unpredictable variabilities determines which dosing strategy (e.g. generic population dose vs. individualized dose) is appropriate. PopPK models typically consist of three sub-models: (1) structural model, which defines curve shape (e.g. two-compartment); (2) covariate models, to describe relationships between known patient characteristics and PK parameters (e.g. age effect

on clearance [CL]); and (3) statistical model, which describes residual variance between and within individuals (e.g. 30% BSV on central volume [V_1]). The PopPK model parameters (i.e. typical values and variances) act as informative priors and, combined with sparse blood samples from the patient, are used to estimate individual PK parameters through Bayesian forecasting.

In this update to our previously published protocol [170], we present the model development and evaluation strategy currently being used to produce PopPK models for use in Bayesian forecasting on the WAPPS-Hemo platform.

Methods

Data Sources

To date, PK data from a total of 26 brands of clotting factor concentrate have been used for development of both brand-specific and generic PopPK models on WAPPS-Hemo; these include standard and extended half-life products for both factor VIII and IX. Thus far, most models have been built on data measured by one-stage clotting assay; however, seven products also have models that are valid for chromogenic assay. While the majority of the data used for model development originates from industry-sponsored and investigator-driven studies, some model derivation datasets have also been supplemented with data collected through routine usage of the WAPPS-Hemo service, especially when pediatric patients were not included; in certain cases, models have been built entirely from WAPPS-Hemo data [171,172].

Table 4. Description of the components for a typical NONMEM dataset

Variable	Description	Units
CID	Patient identification number	Positive integer
IID	Infusion identification number	Positive integer
OCC	Dose occasion	Positive integer
TIME	Time of each concentration measurement; TIME = 0 when predose measurement occurs	Hours
TAD	Time of each concentration measurement; TIME = 0 at start of infusion	Hours
AMT	Total Dose	International unit (IU)
RATE	Rate of infusion; AMT/TIME	IU/h
DV	Plasma concentration of valid observation	IU/L DV = 0
AGE	Age	Years
HT	Height	Centimetres
BW	Weight	Kilograms
FFM	Fat-free mass, calculated from AGE, HT and BW	Kilograms
EVID	Event identification variable	0 = valid observation 1 = dose event 3 = reset event 4 = reset and dose event
DOSE	AMT/BW	IU/kg
PREDOSE	Plasma concentration at time of start of bolus	IU/L if measured; -1 if not measured
MDV	Missing dependent variable	0 = valid observation 1 = dose observation
LLOQ	Lower limit of quantification of the assay used	IU/L; assumed 10 IU/L if not provided
BLQ	Below limit of quantification	-1 for non-BLQ measurements; LLOQ for BLQ measurements
BASELINE	Endogenous plasma concentration	IU/L if measured; assumed LLOQ/2 if not provided
Optional covariates:		
VWF	von Willebrand Factor	Percentage
BRAND	Brand of factor concentrate product	Categorical
RACE	Race	Categorical
BTYPE	Blood type	0 = non-O blood type 1 = O blood type -1 = unknown
HCT	Hematocrit	Percentage

Software and Handling of BLQs

Population pharmacokinetic modelling and Bayesian post hoc estimation are both performed in NONMEM using PDx-Pop (v7.3/7.4 and v5.2, respectively; ICON Development Systems, Ellicott City, MD, USA). When available, the modelling dataset will consist of the variables outlined in Table 4. Estimation is performed using the first order conditional estimation with interaction (FOCEI) with the LAPLACIAN option; the ADVAN and TRANS subroutines for each compartmental model are shown in Table 5. Graphical analysis and streamlining of evaluation steps are conducted in MATLAB (R2017b, Mathworks, Natick, MA, USA). As mentioned above, severe hemophilia patients have an endogenous factor activity level below 10 IU L^{-1}. As 10 IU L^{-1} is also the most cited lower limit of quantification (LLOQ) for coagulation activity assays [refs for assay LLOQ], measurements that are below the limit of quantification (BLQ) are common. To handle these measurements, we employ the M3 method proposed by Beal [173]. In brief, this method involves using maximum likelihood estimation to fit the PK model to all observations (where the likelihoods for BLQ observations are the likelihoods that these measurements are truly BLQ). Handling BLQ observations in this manner is less biased than ignoring or imputing the data.

Table 5. NONMEM subroutines used to implement kinetic equations for linear models following intravenous administration

Model	ADVAN Subroutine	TRANS Subroutine
1-compartment	ADVAN1	TRANS2: CL, V
2-compartment	ADVAN3	TRANS4: CL, V1, Q, V2
3-compartment	ADVAN11	TRANS4: CL, V1, Q2, V2, Q3, V3

Base Model Development

The development of the base model consists of defining the structural model (i.e. number of compartments) and the statistical models (i.e. BSV, BOV [if applicable], and residual unexplained variability [RUV]). In the case of hemophilia, where factor activity measurements comprise both endogenous factor and repeatedly administered exogenous factor, measured factor activity is the result of three distinct contributors:

$$C = C_{exogenous} + C_{endogenous}$$

$$C = C_{current\ dose} + C_{residual} + C_{endogenous}$$

For the example of a two-compartment model,

$$C(t) = f(\theta, t) + \left(C_{predose} - C_{endogenous}\right)e^{-\beta t} + C_{endogenous}$$

where $f(\theta, t)$ is function relating factor activity to the individual's parameters (θ), such as PK parameters and covariate effects, and time (t); the precise form of f depends on the number of compartments and the administration route. Residual exogenous FVIII from unaccounted doses $(C_{predose} - C_{endogenous})$ decays according to the terminal rate constant (α for a one-compartment model; γ for a three-compartment model); if no predose level was measured, it is assumed that there was no exogenous FVIII remaining at the time of dose administration. The endogenous FVIII component ($C_{endogenous}$) is assumed to be constant and, when unknown or unmeasurable, is considered to be half of the lower limit of quantification (LLOQ) of the assay.

The selection of the structural model (i.e. 1-, 2-, or 3-compartment) is driven by the objective function value (OFV), a numerical measure of goodness-of-fit, and graphical evaluations. Since the likelihood ratio test is not reliable for this comparison due to boundary

issues, the Akaike Information Criterion (AIC) and Bayesian Information Criterion are also used to determine the most favourable model.

$$AIC = OFV + 2 \cdot n_p$$

$$BIC = OFV + n_p \cdot \ln(N)$$

where n_p is the number of parameters in the model and N is number of observations in the dataset; a decrease in AIC or BIC of at least 2 is considered positive evidence in favour of the model [174]. In addition to these goodness-of-fit measures, the plausibility of the parameter estimates and graphical techniques (described in the Model Evaluation section) are also considered in decision-making. Furthermore, plots of the PK curves generated from estimated population parameters can also be helpful to assess whether meaningful differences exist between structural models.

Residual Unexplained Variability

Residual unexplained variability (RUV) is the unexplained variability after accounting for all other sources of variability, and may arise from assay error, imprecise recording of sample time, model misspecification, or natural variation [174]. Commonly used functions to describe RUV include:

$$C_{ij} = \hat{C}_{ij} + \varepsilon_{ij}$$

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

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

where C_{ij} is the i^{th} observation for the j^{th} individual, $\hat{C}_{ij}$ is the model prediction for the i^{th} observation for the j^{th} individual. In each case, the ε-values are assumed to be independent and follow a normal distribution with a mean of zero and variance σ^2 ($\varepsilon_1 \sim N(0, \sigma_1^2)$, $\varepsilon_2 \sim N(0, \sigma_2^2)$).

Between Subject Variability

Between-subject variability (BSV) is modelled according to the following equation:

$$P_j = TV(P) \cdot e^{\eta_{P-j}}$$

where P_j is the individual value of PK parameter P for the j^{th} individual, $TV(P)$ is the typical value of parameter P. η_{P-j} describes individual j's deviation from the typical parameter value, and $\eta_P \sim N(0, \omega_P^2)$ such that P is log-normally distributed and physiologic PK parameter values remain positive. The decision to include BSV on a given PK parameter is largely driven by η-shrinkage values, defined as:

$$shr_\eta = 1 - \frac{SD(\eta)}{\omega}$$

where ω is the estimate of the standard deviation of η from the population model, and $SD(\eta)$ is the standard deviation of η calculated over the population. Typically, η-shrinkage takes a value between 0% and 100%, with higher values indicating that individual parameter estimates are "shrinking" towards the population value due to a lack of informative data for that particular parameter (Figure 4). This lack of information results in an uncertain estimate of BSV which, when used in Bayesian estimation, leads to uncertainty around the individual estimates and inappropriate outcomes (e.g. implausible half-lives, extremely wide confidence intervals, etc.). In addition to the problems it creates in Bayesian forecasting, elevated η-shrinkage can also

interfere with later steps of model development as many diagnostic plots, particularly those used to assess covariate relationships, become misleading when η-shrinkage is in to 20-30% range [175]. For this reason, BSV is typically removed from parameters with high η-shrinkage (we use 35% as a cut-off value) and the model is re-assessed.

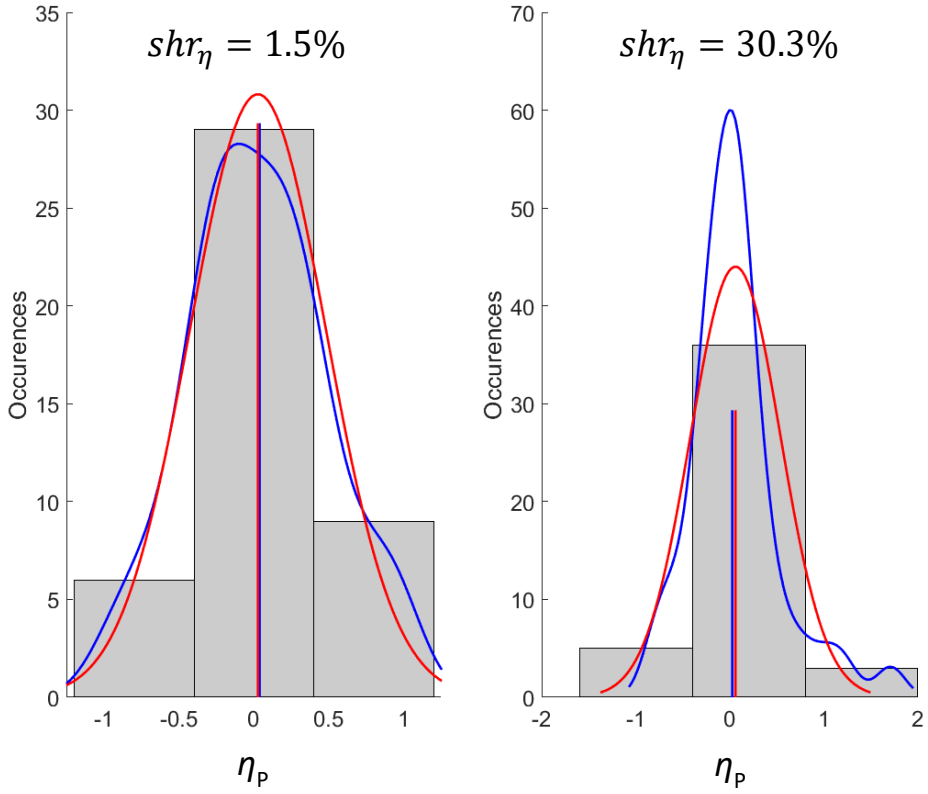


Figure 4. $\boldsymbol{\eta}$-distribution of a structural model parameter (e.g. CL) with low (left) and high (right) shrinkage. The red line indicates the expected distribution from the model assumptions, while the blue line shows the actual distribution of individual $\boldsymbol{\eta}$-values.

Considering the end use of the model, the magnitude of the BSV term was also taken into account, as high BSV may introduce too much flexibility into the model. The inclusion of off-diagonal elements in the omega matrix is only tested after selection of the covariate model, so as

not to obscure covariates effects by prematurely allowing for correlation between η-terms that share a common covariate.

Between-occasion variability (BOV) is modelled similarly, when sufficient data is available:

$$P_{jk} = P_j \cdot e^{\rho_{Pk}}$$

where ρ_{Pk} is normally distributed $\left(\rho_P \sim N(0, \pi_P^2)\right)$ and described the deviation on occasion k for individual j. To diagnose the inclusion of BOV terms, BSV estimates are compared from two different treatments of the modelling dataset. In the first run, each new occasion is treated as a new subject; in the second, occasions are attributed to the original subject, but BOV is not explicitly modelled. Parameters with significant decreases in BSV in the second run are then formally assessed for BOV.

Covariate Model Selection

Possible covariate relationships are first explored by examining plots of η-values against each covariate (either as a scatter plot for continuous covariates or boxplot for categorical covariates). Next, covariates are formally tested in the model using the likelihood ratio test, which assumes that the difference in OFV between two nested models follows a chi-squared distribution with $P_2 - P_1$ degrees of freedom, where P_2 and P_1 are the numbers of parameters in the larger and smaller models, respectively. Thus, if a 5% significance level is employed, the addition of one parameter (i.e. one degree of freedom) must decrease the OFV by at least 3.84 units; when adding two parameters, the decrease must be 6 units or more.

Covariates are added to the model in a stepwise manner, and are kept based on OFV decrease, reduction of unexplained BSV, and physiological/clinical relevance of the estimated covariate effect. If several covariates are highly correlated (e.g. body weight, height, and fat-free mass), only one is included in the final model to avoid collinearity. Typical functional forms used include:

$$TVP_i = TV(P) \cdot \left(\frac{cov_i}{cov_{med}}\right)^{\theta_{cov-P}}$$

$$TVP_i = TV(P) \cdot \left(1 + \theta_{cov-P} \cdot (cov_i - cov_{med})\right)$$

$$TVP_i = TV(P) \cdot \left(1 + \theta_{cov-P} \cdot \max\left(0, \frac{cov_i - cov_{med}}{cov_{med}}\right)\right)$$

$$TVP_i = TV(P) \cdot (1 + \theta_{cov-P} \cdot CAT) \qquad CAT = \{0,1\}$$

where TVP_i is the PK parameter predicted by the model for the i^{th} individual with covariate value cov_i, cov_{med} is the median value of the covariate, and θ_{cov-P} is the effect of the covariate on parameter P. CAT represents a dichotomous categorical covariate (e.g. blood group). Morphometric variables (e.g. body weight, fat-free mass) are modelled using the power function, while age, when significant, tends to follow a linear or piecewise linear relationship depending on the covariate space of the dataset. Graphical representations of these functional forms, with varying values of θ, are presented in Figure 5.

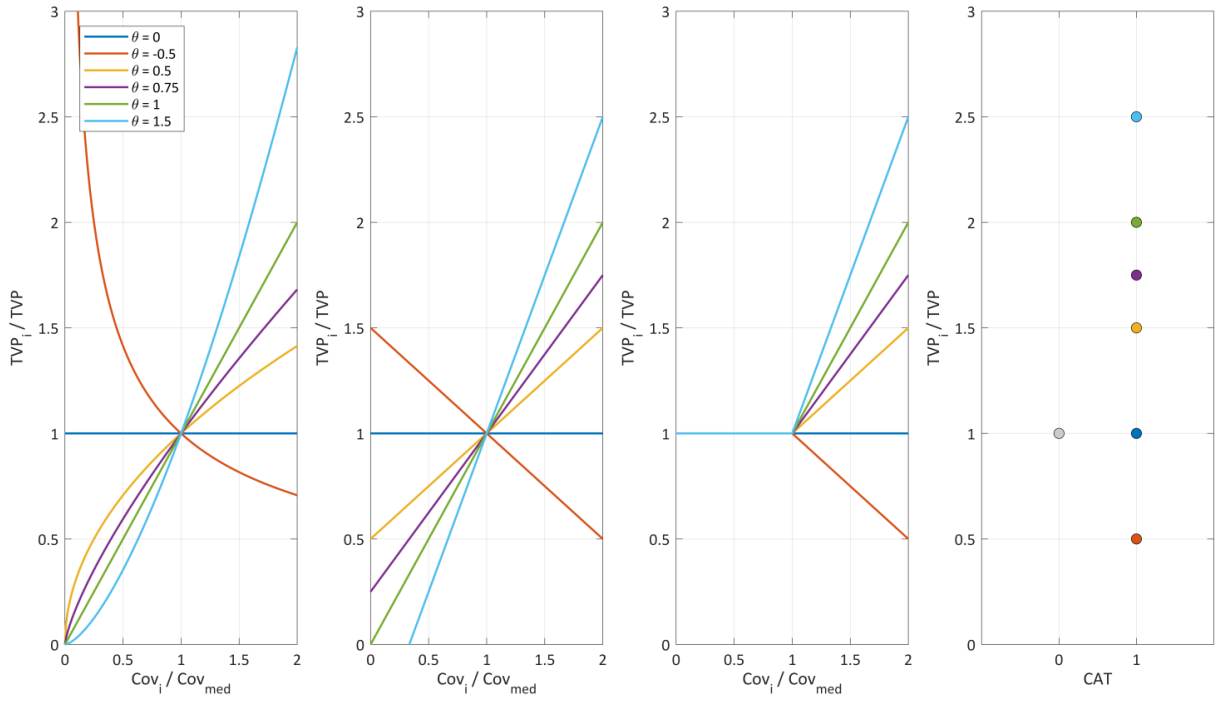


Figure 5. Illustration of different functional forms (left to right: power, linear, piecewise linear, and categorical) for covariate relationships, with varying effect sizes

In light of the final use of these models, multiple covariate models are occasionally required for a single clotting factor concentrate. For example, von Willebrand factor (vWF) level is an excellent predictor of FVIII clearance, as this protein acts as a chaperone for FVIII and protects it from degradation. However, vWF levels are not available for all patients. In these cases, an alternative model containing only basic covariates (e.g. age, body weight) is simultaneously developed.

Model Evaluation

Model evaluation consists of both basic and advanced internal methods that are selected based on the model's intended purpose of Bayesian forecasting. First, models are evaluated using

graphical techniques to assess goodness-of-fit, ensure all underlying assumptions are met, and to identify any model misspecification. Diagnostic plots include:

- Population/individual predicted values vs. observed values
- Conditional weighted residuals (CWRES) vs. predicted values
- CWRES vs. time
- Observed/predicted values vs. time
- Normal QQ plots
- CWRES histograms
- Population covariate plots
- η histograms

In addition to graphical assessment, η-distributions were also numerically evaluated using η-shrinkage.. As previously alluded to, high η-shrinkage increases uncertainty around estimates obtained from Bayesian forecasting and also interferes with commonly used diagnostic plots; preliminary covariate analysis is particularly sensitive to η-shrinkage, as both hidden and induced relationships have been observed at 20-30% η-shrinkage [175]. As a result, we remove BSV from parameters when shrinkage is in excess of 35% and the model is re-assessed When η-shrinkage is above 20%, all covariate relationships are formally explored as the graphical analysis can be misleading.. ε-shrinkage, defined below, is also monitored:

$$shr_{\varepsilon} = 1 - SD(IWRES)$$

where $SD(IWRES)$ is the standard deviation of the individual weighted residuals $\left(IWRES = \frac{observation - IPRED}{\sigma}\right)$.

Thus, high ε-shrinkage is indicative of overfitting. Similar to η-shrinkage, ε-shrinkage can also reduce the power of diagnostic plots (e.g. IPRED vs. observed) to detect model misspecifications [176] and so this value was also considered before consulting graphical diagnostics. While model evaluation is typically thought of as occurring once the final model has been decided upon, these graphical techniques and monitoring of shrinkage are used throughout model development to assist in decision-making.

Next, bootstrap analysis is performed as a non-parametric way of assessing uncertainty in parameter estimates by calculating the standard errors and confidence intervals around model parameters. The modelling dataset is randomly sampled 1000 times with replacement (stratified by covariates as needed) to generate 1000 new datasets of the same size as the original. Parameters are estimated for each prepared dataset, with the median values corresponding to parameter estimates and the 2.5th and 97.5th percentiles forming the 95% confidence interval. Bootstrap standard errors are calculated using the following equation:

$$SE(\hat{\theta}) = \sqrt{\frac{1}{B-1}\sum_{b=1}^{B}\left(\hat{\theta}_b - \bar{\theta}\right)^2}$$

where B is the number of re-sampled datasets, $\hat{\theta}_b$ is the estimate of θ from run b, and $\bar{\theta}$ is the mean estimate of θ from the B datasets.

A prediction-corrected visual predictive check (pcVPC) is then used to assess a model's predictive capacity. Traditional VPCs can be used to assess whether simulations produced by a candidate model are able to recreate both the central tendency and the variability of the observed data. A large number of simulations are performed, and the corresponding percentiles of the simulated and observed data are compared visually. However, model diagnosis by VPC can be

hampered by large variations in independent variables such as dose. To tackle this shortcoming, the observed and simulated factor activity levels in each bin are normalized by the typical population prediction, as shown by Bergstrand et al [177]. The pcVPC is performed with 500 simulations, and an example of a typical pcVPC generated during model evaluation is shown in Figure 6.

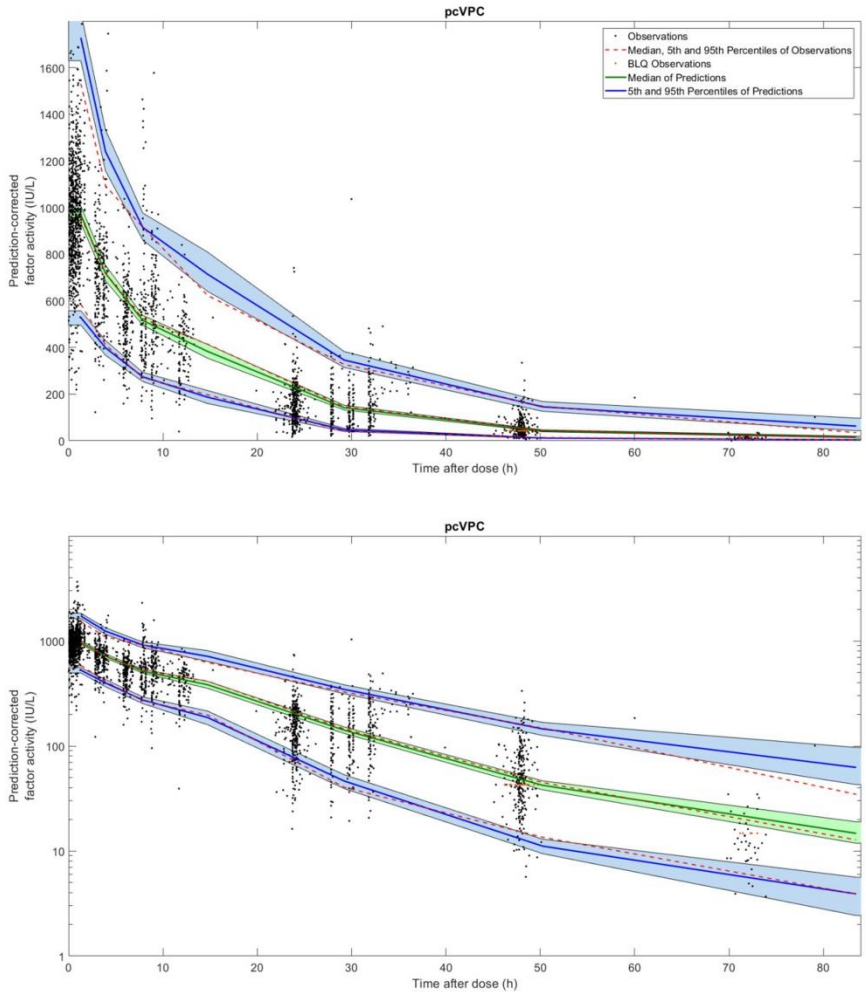


Figure 6. Example of prediction-corrected visual predictive check (pcVPC) for a SHL FVIII model shown on linear (top) and log (bottom) scales. Dashed red lines denote median, 5th and 95th percentiles of observed data. Solid lines denoted the same percentiles in the simulated data, with shaded regions representing the 90% prediction regions for the simulated percentiles.

As the ultimate use of the models is in Bayesian forecasting, internal and external evaluation techniques are used to evaluate them specifically for this purpose. First, cross-validation (typically 10-fold) is performed to assess the model's ability to predict PK parameters for individuals outside of the original modelling dataset. The original dataset is split into a learning subset and a validation subset; in the case of a 10-fold cross-validation, the learning subset comprises 90% of the data while the remaining 10% is used for evaluation. Bayesian forecasting is performed on the validation subset using population estimates obtained from the learning subset. Individual estimates of key PK parameters (e.g. half-life, clearance, central volume, time to 1% factor activity) are then compared to those obtained using the entire modelling dataset by calculating relative error:

$$Relative\ Error = \frac{\hat{\varphi}_i - \varphi_i}{\varphi_i}$$

where $\hat{\varphi}_i$ is the estimate from the cross-validation and φ_i is the estimate when the full dataset is used for model development. This process is repeated 100 times, to prevent the bias that may occur from a single random split of the dataset; typical results are presented in Figure 7.

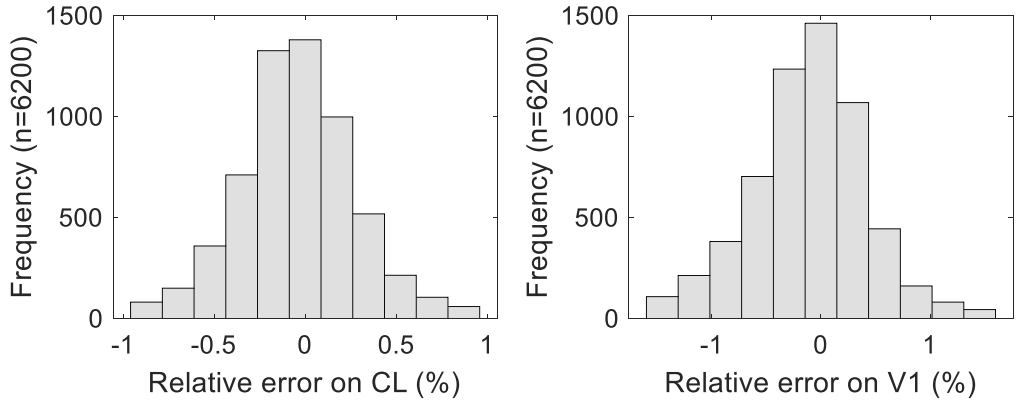


Figure 7. Sample results from cross-validation, presented as histograms of relative errors on key PK parameters

Next, a limited sampling analysis is performed to ensure that the models can estimate individual PK parameters accurately from only a few patient samples. In addition to assessing the performance of the model, this analysis can also identify which sampling times are most informative for estimation of a given parameter using Bayesian forecasting. Based on the method describe by Brekkan et al [178], a population of 1000 individuals is generated using the covariate and PK parameter distributions of the initial dataset. An appropriate treatment strategy is simulated for each subject, depending upon the clotting factor product being modelled (Table 6).

Table 6. Simulation details for limited sampling analysis by product type

Product Type	Dosing Regimen	Steady State Simulation Length	Reference Sampling Design
SHL FVIII	25 IU/kg, 3 times per week	4 weeks	Predose, 1, 3, 6, 12, 24, 48, 72 h
EHL FVIII	40 IU/kg, 2 times per week	4 weeks	Predose, 1, 3, 6, 24, 48, 72, 96 h
SHL FIX	40 IU/kg, 2 times per week	4 weeks	Predose, 1, 3, 6, 24, 48, 72, 96 h
EHL FIX	40 IU/kg, one per week	4 weeks	Predose, 1, 24, 48, 72, 96, 120, 168, 240, 336 h

Limited sampling strategies are created from 2- or 3-sample subsets of the rich design (8+ samples) at clinically convenient times. Individual PK parameters are obtained through Bayesian forecasting from each of the designs, and estimates of relevant PK parameters from the limited sampling strategies are compared to those obtained from the rich sampling strategy using relative error, both numerically and graphically (Figure 8).

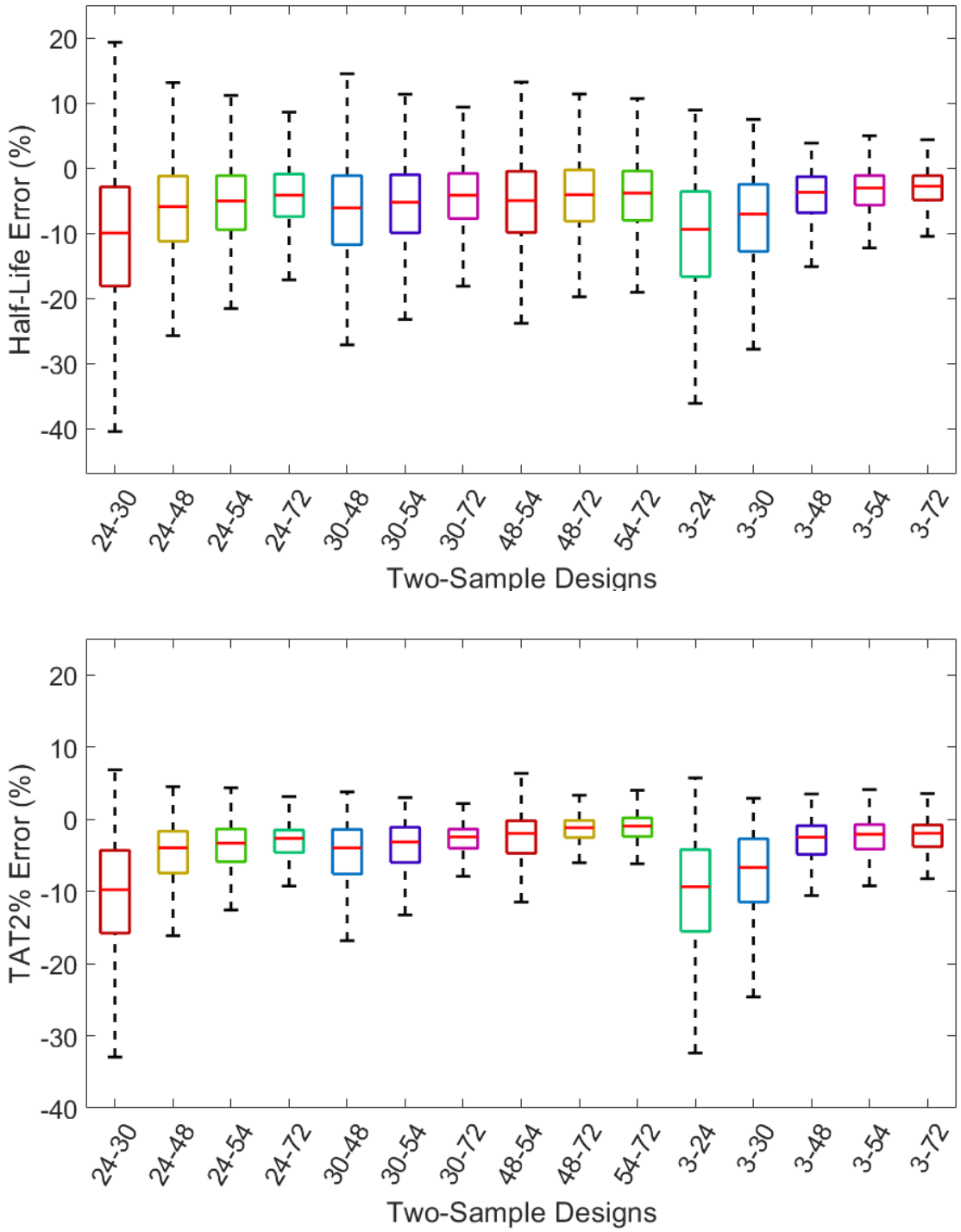


Figure 8. Example of results from limited sampling analysis for a SHL FVIII model, depicting the error on estimates of half-life and time to 2% factor activity for two-sample designs

Finally, data collected through the WAPPS-Hemo network is used to externally evaluate the model, provided the WAPPS data has not already been used to supplement the original modelling dataset. First, histograms of age and body weight for the WAPPS data and the modelling dataset are compared to determine if the WAPPS data is similar to the covariate space on which the model was built (Figure 9A). Next, the model was used to perform Bayesian forecasting, and the results were evaluated using goodness-of-fit plots, histograms of individual estimates of PK parameters (e.g. half-life, clearance, central volume, time to 1% factor activity) and overlaid observed data with the activity-time profile predicted by the model for each individual (Figure 9B-D). Due to the sparse nature of the PK data collected through WAPPS-Hemo, a formal evaluation comparing estimates to some 'true' value is not feasible; rather, this method ensures that the model produces reasonable results in the conditions in which it is intended to perform.

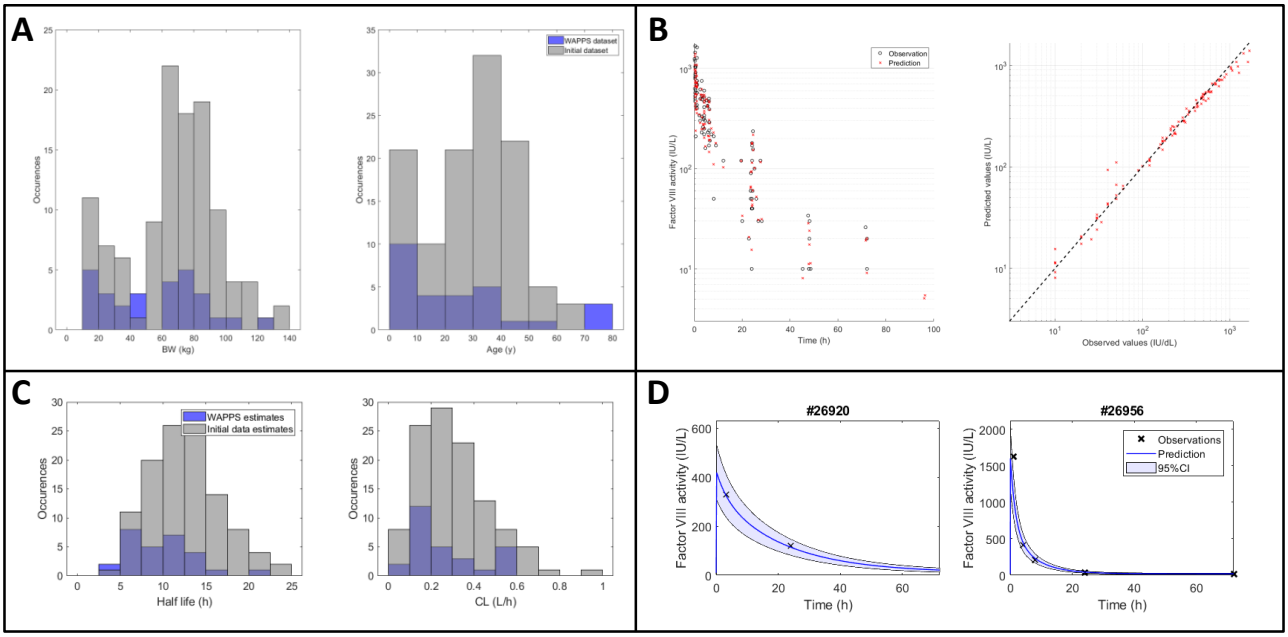


Figure 9. Process of external validation using WAPPS-Hemo data. (A) Comparison of covariate spaces of modelling (grey) and WAPPS-Hemo (blue) data (B) Goodness-of-fit plots (log scale) (C) Comparison of individual PK estimates for patients from modelling and WAPPS-Hemo datasets (D) Predicted individual activity-time profiles with 95% confidence intervals (shaded region) and observed data overlaid (×).

Results

As of this update, 33 models for 26 clotting factor concentrates have been developed according to this protocol. In addition, a generic standard half-life FVIII model was developed to handle requests for products without a brand-specific model. To date, over 9,000 unique PK profiles from nearly 6,000 patients; roughly one third of these infusions correspond to pediatric (<12 years old) patients. The clinical module described in the original WAPPS-Hemo data analysis protocol was implemented in 2017, and has since been used over 1,300 times for dosing regimen design.

With regards to reporting, a recent paper by Hajducek provides a summary of the modelling datasets and evaluation results (pcVPC, cross-validation, and LSA) for all models

currently implemented on the WAPPS-Hemo platform. In brief, the models used on the WAPPS-Hemo platform demonstrate good performance in the context of Bayesian forecasting. Cross-validation reveals low bias and relative error on estimates of relevant PK parameters such as half-life and TAT2%. Results from LSA also showed acceptable bias and error, while also confirming the ability of the models to produce accurate estimates from a number of different sampling schemes; this flexibility in sampling times is an important feature that makes the Bayesian approach more feasible in a real world setting. Furthermore, the development and evaluation of three models (standard half-life FVIII, Fanhdi/Alphanate and Adynovate) are fully detailed in McEneny-King et al [179] and Chelle et al [171,172].

Discussion

We would first like to emphasize that the model development and evaluation strategies described in this report are highly influenced by its end purpose of Bayesian forecasting; models intended for other purposes may employ different decision-making criteria or evaluation techniques. Furthermore, this revised data analysis protocol follows from our collective experience developing over 25 models for clotting factor concentrates for use in Bayesian forecasting; we have observed, through the regular use of the WAPPS-Hemo service, which types of models perform well for this purpose and have incorporated this knowledge into the updated protocol.

Since the publication of the original data analysis protocol for WAPPS-Hemo models in 2016, the uptake of the PopPK approach in the hemophilia community has risen dramatically [180]. The WAPPS-Hemo network, currently with over 400 centres worldwide, boasts the largest global repository of hemophilia PK data, which continues to grow in number, covariate

space, and quality. As we continue to collect data, we aim to standardize our approach to updating models with data submitted to WAPPS-Hemo. This exercise poses an interesting question about which type of model is best suited to Bayesian forecasting: a model using data collected during clinical trials, or model created by merging industry and routine clinical data? The clean, richly sampled data from clinical trials typically enables clearer decisions regarding the structural model and inclusion of BSV or BOV; however, the number of patients may be low and the covariate space may be narrow, and especially lacking in pediatric patients. On the contrary, the WAPPS-Hemo data consists of over 9,000 unique PK profiles (including over 3,000 in children under 12 years of age), but this data is not necessarily clean. To ensure that the inclusion of WAPPS-Hemo data improves rather than pollutes the model of interest, strict data cleaning procedures will be necessary for model updating. Furthermore, it may be prudent to restrict model updating to the re-assessment of covariate effects rather than re-selecting the structural model.

Finally, a limitation – though also a strength of the project – is our constant evolution, necessitating regular updates to our methods, such as this report, so that the hemophilia community is informed of our current practices. For instance, the modelling of BOV is a more recent addition to our model development strategy, as more multi-occasion data became available; its incorporation in some models prompts further research questions regarding the validation of the clinical calculator module.

Conclusions

In summary, the WAPPS-Hemo service is centred on validated PopPK models for clotting factor concentrates. This protocol describes the process of model development and evaluation that has been refined during the last three years, since the original publication of the data analysis plan. The WAPPS-Hemo network continues to grow, contributing to the largest repository of hemophilia PK data.

Chapter 4: Development and evaluation of a generic population pharmacokinetic model for standard half-life factor VIII for use in dose individualization

This chapter is reflective of an original manuscript published by the Ph.D. candidate (Alanna McEneny-King) in *Journal of Pharmacokinetics and Pharmacodynamics*. All pertinent dialogue in this chapter was written by the Ph.D. candidate.

McEneny-King A, Chelle P, Foster G, Keepanasseril A, Iorio A, Edginton AN. Development and evaluation of a generic population pharmacokinetic model for standard half-life factor VIII for use in dose individualization. J Pharmacokinet Pharmacodyn. 2019; 46(5):411-426. DOI: 10.1007/s10928-019-09634-7.

Introduction

Hemophilia A is a genetic bleeding disorder caused by a deficiency of functional clotting factor VIII (FVIII), affecting 1 in 6,500 male births [62]. As a result, hemophilia patients are unable to form clots in response to vascular injury and are thus prone to bleeding episodes. Among the most severe patients (i.e. those with less than 1% of normal FVIII activity), bleeds may occur spontaneously, particularly in joints, resulting in debilitating arthropathy. Current hemophilia therapy consists of regular intravenous infusions of exogenous FVIII to maintain FVIII levels above a certain trough at all times. Often, the selected trough is 1% (or 10 IU L^{-1}), based on the observation that the rate of increase in joint score of moderate patients with endogenous FVIII activity between 1% and 3% was halved compared with those with

endogenous levels below 1% [7]. Furthermore, a correlation between time spent below the 1% threshold and the occurrences of bleeds and hemarthroses has been demonstrated [10].

Today, there is global consensus that prophylaxis should be initiated at a young age, before joint disease is apparent [11–13]. However, no optimal regimen has been determined due to a highly variable pharmacokinetic (PK) response between patients. High between subject variability (BSV) and relatively low interoccasion variability (IOV) suggests that FVIII dosing regimens ought to be tailored to the individual to ensure both the safety of the patient and the responsible use of expensive clotting factor concentrates [70,167]. The classic approach to PK-based dose tailoring has been difficult to apply in a clinical setting, especially when trying to apply the approach recommended by the International Society of Thrombosis and Haemostasis (ISTH) for bioequivalence studies with new concentrates, which requires more than 10 samples taken over the course of 48 hours. More recent ISTH-issued guidelines detail the value of a population pharmacokinetic (PopPK) approach to PK studies oriented to dose individualization [169,181]. A PopPK model, which provides typical values of PK parameters (clearance [CL], central volume [V_1]) and quantifies the variability within the population based on patient covariates, can act as informative prior knowledge for the Bayesian estimation of individual PK parameters including half-life and time to target trough.

Table 7. Published PopPK models for standard half-life FVIII products

Reference	Product	Description
Abrantes [182]	ReFacto AF/Xyntha	2-compartment; combined RUV model; BSV on CL, F and baseline; IOV on CL and V_2; body weight on V_1 and V_2; age, inhibitor status, and study effect on CL; race on V_2; assay on F and RUV
Björkman [183]	Advate	2-compartment; additive RUV model; BSV on CL and V_1; body weight on CL, V_1, and V_2; age on CL
Nestorov [184]	Advate	2-compartment; combined RUV model; BSV on CL and V_1; body weight on V_1; study on RUV and V_2
Garmann [185]	Kovaltry	2-compartment; combined RUV model; BSV on CL and V_1; lean body weight on CL and V_1
Jimenez-Yuste [103]	NovoEight	1-compartment; combined RUV model; BSV on CL and V; body weight on CL and V; age on CL
Bolon-Larger [186]	Various recombinant and plasma-derived products	2-compartment; proportional RUV model; BSV on CL, V_1 and V_2; HIV status on V_1 *model for continuous infusion
Karafoulidou [187]	ReFacto	1-compartment; proportional RUV; BSV on CL and V; body weight on CL and V; HIV status on V
Hazendonk [188]	Various recombinant and plasma-derived products	2-compartment; combined RUV; BSV on CL and V_1; age on CL and V_1; blood group and major surgical procedure on CL; product type on F *some continuous infusion patients included

Several PopPK models for standard half-life (SHL) FVIII products have been published in the literature, and are summarized in Table 7. Each of the cited models is dedicated to one specific brand of FVIII, and FVIII products do vary in ways that may be clinically relevant. One such characteristic is the source of the FVIII concentrate, which may be plasma-derived (pdFVIII) or recombinant. Plasma-derived concentrates can be further categorized based on their

von Willebrand factor (vWF) content: intermediate purity (vWF:FVIII > 1), high purity (vWF:FVIII = 0.2–0.4), or immunopurified (vWF:FVIII < 0.1) [189]. The presence of a native FVIII-vWF complex in pdFVIII has been shown to impact the early phase of the PK profile, but does not appear to affect half-life [129]. Recombinant FVIII products can be classified according to their structure. In 2000, the first B-domain deleted recombinant FVIII product (BDDrFVIII) was released, followed by a B-domain truncated product in 2013 [190]. The purpose of this deletion was to improve production efficiency, with no changes to immunogenicity or pharmacokinetic profile. While some studies have found BDDrFVIII products to be bioequivalent to their full-length counterparts [94,95,97], others have found that half-lives are shorter after this modification [191,192]. This may be due to disrupted intermolecular interactions that impact the life span of FVIII [193]. Despite these differences in source and structure, variability seems to be greater across patients than across brands [167]. Thus, a generic PopPK model for SHL FVIII products can be a valuable tool, especially if one considers that hemophilia is a rare disorder with an abundance of similar products, all of which benefit from PK-tailored dosing regimens.

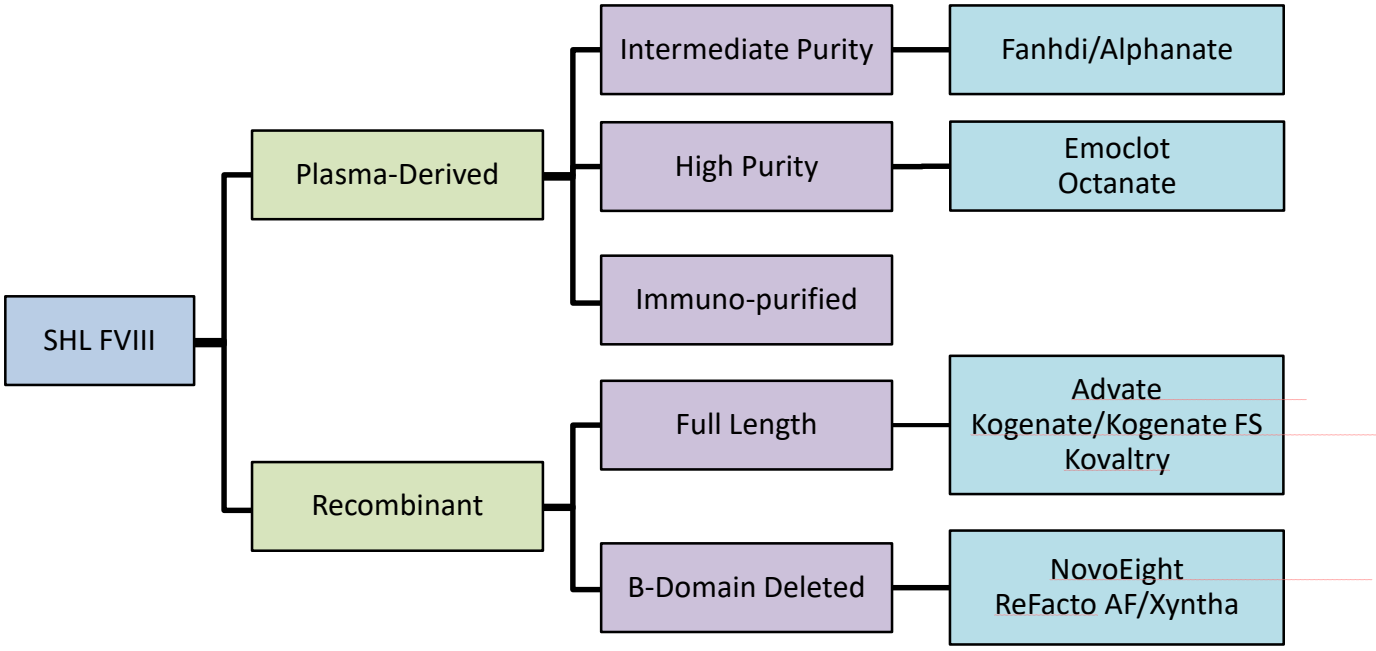


Figure 10. Sources and structures of the brands of SHL FVIII included in the modelling dataset

The aim of this study was to develop and evaluate a generic PopPK model for SHL FVIII products, both plasma-derived and recombinant, using data acquired through the Web Accessible Population Pharmacokinetic Service – Hemophilia (WAPPS-Hemo) project. The development of such a model will help to determine if there are distinct PK differences between FVIII brands and the clinical relevance of these differences. Further, the model will be incorporated into the WAPPS-Hemo platform, which tackles the issue of high BSV by using PopPK models for Bayesian forecasting to obtain individual PK estimates from relatively few patient samples. Clinicians provide 2-4 factor levels, along with demographic information, and are provided with individual estimates of relevant PK parameters (such as half-life, time to 1% FVIII activity, or FVIII activity at 72 hours). Furthermore, the wide covariate space of the generic model may

permit its use for patients on brands of SHL FVIII that are not included in the modelling dataset, making it particularly useful in cases where a dedicated, brand-specific PopPK model is lacking.

Methods

Patient Data

Data for recombinant and plasma-derived SHL FVIII was collected from multiple industry sources through the WAPPS-Hemo project. The model was developed using FVIII activity measurements from 310 densely sampled patients (one infusion per patient), consisting of between 4 and 12 factor levels (median: 10). All samples were measured using the one-stage clotting assay. All patients had either severe or moderate hemophilia (<1% or 1-5% of normal FVIII activity, respectively) and did not present detectable inhibitors at the time of PK analysis. The lower limit of quantification (LLOQ) varied among studies, ranging between 4 and 12.5 IU L^{-1} (median = 10 IU L^{-1}) and samples that were below limit of quantification (BLQ) comprised 6.9% of the dataset. Demographic details of the modelling dataset can be found in Table 8.

Table 8. Demographics of the patient population used to develop the generic SHL FVIIII model

Sampling Information				
Total Number of Patients	Total Number of Samples	Number of BLQ Samples (%)	Number of Samples per Patient	Duration of Sampling (h)
310	2760	191 (6.9%)	10 (4 – 12)	48.0 (3.25 – 96.25)
Patient Demographics				
Brand	n	Age (years)	Body Weight (kg)	Fat-Free Mass (kg)
Advate	79	20 (1.1 – 62)	66.9 (10.6 – 132.5)	53.5 (8.1 – 82.7)
Emoclot	14	33 (14 – 55)	70 (40 – 93)	55 (35 – 66.9)
Kogenate	64	19 (5 – 54)	69.15 (16.6 – 124.2)	55.4 (14.2 – 84.3)
Kovaltry	31	31 (12 – 61)	70 (46 – 124.2)	53.2 (39.2 – 76.5)
NovoEight	55	11 (1 – 54)	42.7 (11.7 – 107)	35.9 (10 – 71.4)
Octanate	35	18 (3 – 54)	53 (18.5 – 89)	45.8 (14 – 67.1)
ReFacto AF	32	24 (14 – 57)	78.5 (50.7 – 117.2)	59.35 (43.9 – 75.2)
TOTAL	**310**	**21 (1 – 62)**	**66.0 (10.6 – 132.5)**	**53.0 (8.1 – 84.3)**

Population Modelling

PopPK model building was performed using non-linear mixed effects modelling techniques implemented in NONMEM and PDxPop (v7.3 and v5.2, respectively; ICON Development Solutions, Ellicott City, MD, USA). Graphical analysis was conducted in MATLAB (R2017b, Mathworks, Natick, MA, USA). Samples that were BLQ were handled using the M3 method [173].

First, the structural component of the model was developed. The model describes not only the exogenous dose administered, but also endogenous FVIII production and any residual FVIII from prior doses as trials did not necessarily include a washout period.

$$C(t) = Ae^{-\alpha t} + Be^{-\beta t} + endogenous\ FVIII + (predose - endogenous)e^{-\beta t}$$

The endogenous FVIII component was considered to be constant; when the endogenous level was unknown or unmeasurable, it was assumed to be half of the LLOQ. Residual exogenous FVIII decayed according to the terminal rate constant; if no predose measurement was taken, it

was assumed that there was no exogenous FVIII remaining when the dose was administered. The standard 1- and 2-compartment models were tested. For each, three different residual error models were explored: additive, proportional, and combined additive/proportional.

Following this step, between subject variability (BSV) terms were added to PK parameters using an exponential form. For example:

$$CL_i = CL_{pop} \cdot e^{\eta_j}$$

where CL_i is an individual's clearance, CL_{pop} is the population value for clearance, and η_j is the individual's deviation from population value. The η values follow a normal distribution with a mean of zero, such that the PK parameters are log-normally distributed. Decision-making during these steps was driven by changes in the objective function value (ΔOFV) and shrinkage of the random effects.

The inclusion of explanatory covariates helps to minimize unpredictable BSV. Only covariates that were consistently available for all data sources were investigated; these included body weight, fat-free mass (calculated from body weight, age, and height using the maturation model defined by Al-Sallami et al [194]), age, and brand. Preliminary covariate analysis consisted of examining plots of η-values versus each covariate. Covariates were then added to the model in a stepwise manner, and either kept or removed based on their effect on OFV, BSV, and parameter estimates. Body weight, fat-free mass, and age were incorporated prior to brand so that demographic differences between datasets were not falsely attributed to brand. Body size metrics were modelled using allometric functions; a variety of functions were considered to model the age effect. Power, linear, and piecewise linear relationships are shown below:

$$CL_i = CL_{pop} \cdot \left(\frac{cov_i}{cov_{med}}\right)^{\theta_{cov-CL}} \cdot e^{\eta_i}$$

$$CL_i = CL_{pop} \cdot \left(1 + \theta_{cov-CL} \cdot \frac{cov_i - cov_{med}}{cov_{med}}\right) \cdot e^{\eta_i}$$

$$CL_i = CL_{pop} \cdot \left(1 + \theta_{cov-CL} \cdot max\left(0, \frac{cov_i - cov_{med}}{cov_{med}}\right)\right) \cdot e^{\eta_i}$$

where cov_i is the individual's value for the covariate, cov_{med} is the median value for the covariate, and θ_{cov-CL} is the estimated effect of the covariate on CL.

After taking body size and age effects into account, the effect of brand was explored. Initially, each brand was modelled with its own covariate effect either on CL and V_1.

$$CL_i = CL_{pop} \cdot (1 + \theta_{Brand1-CL} \cdot Brand_1) \cdot (1 + \theta_{Brand2-CL} \cdot Brand_2) \cdots$$
$$\cdot (1 + \theta_{Brand7-CL} \cdot Brand_7) \cdot e^{\eta_i}$$

where $Brand_i = 1$ if the individual was dosed with Brand i; otherwise, $Brand_i = 0$. Subsequently, brands were grouped together according to results of the previous runs, or based on their source (e.g. plasma-derived, recombinant) and structure (e.g. full-length, B-domain deleted) in an effort to reduce the number of model parameters. Grouping schemes are delineated in Table 9.

Table 9. Modelling of brand as a covariate

Scheme	Rationale	OFV (ΔOFV)	Reference Group	Group Details	
1	Individual effect for each brand	25321 (-89)	Advate	Each brand as its own group	
2	Based on results of Scheme 1	25325 (-85)	Advate	Effect of Kovaltry removed from CL Effect of Kogenate, NovoEight, and Octanate removed from V_1	
3	Source	25389 (-21)	Advate Kogenate Kovaltry NovoEight ReFacto AF (Recombinant)	Octanate Emoclot (Plasma-derived)	
4	Source and structure	25357 (-53)	Advate Kogenate Kovaltry (Full-length recombinant)	NovoEight ReFacto AF (BDD recombinant)	Emoclot Octanate (Plasma-derived)

OFV: objective function value; ΔOFV: change in OFV compared to model with FFM and AGE.

Model Evaluation

The final SHL FVIII model was evaluated in several steps. First, graphical techniques were employed to assess the model's goodness-of-fit and to assure that all model assumptions were met. Bootstrap analysis was also performed to assess estimated parameters and their associated confidence intervals. One thousand datasets consisting of 400 individuals were generated by randomly sampling the original dataset with replacement; to ensure all groups were represented in the bootstrap datasets, the original dataset was stratified according to age and brand. Parameter estimation was performed for each dataset and the median parameter estimates and corresponding 95% confidence intervals were calculated.

To evaluate the model for use in Bayesian forecasting, internal cross validation and limited sampling analysis were performed. A 5-fold cross validation was performed, meaning the dataset was randomly split into 2 subsets, one containing 80% of the data (the learning subset) and the other the remaining 20% (the validation subset). Relative error on individual PK parameters was calculated using the following equation:

$$Relative\ Error = \frac{P_{CV} - P_{full}}{P_{full}}$$

where P_{CV} is the individual parameter estimate (CL, V_1, half-life) obtained during the cross validation using Bayesian forecasting and P_{full} is the "true" value estimated from the initial dataset.

Limited sampling analysis can be used to determine how well the model can predict individual PK parameters from sparse samples. Using a method similar to that described by Brekkan [178], a population of 1000 virtual subjects was generated from the final SHL FVIII model using covariate distributions from the original dataset. For each virtual subject, a treatment regimen corresponding to 50 IU kg^{-1} on a Monday-Wednesday-Friday schedule was simulated for 4 weeks, with the last Friday dose being used for analysis. Different limited sampling schemes consisting of convenient sampling times (e.g. predose, peak, 24 hours post-infusion) are described in detail in Table S5; a total of 34 designs were tested. Each design was used to obtain estimates of individual PK parameters using Bayesian forecasting, and estimates from the limited sampling strategies were compared to those obtained from the full sampling design using the following equation:

$$Relative\ Error = \frac{P_{LSS} - P_{full}}{P_{full}}$$

where P_{LSS} is the parameter estimate from the limited sampling strategy and P_{full} is the parameter estimate from the rich sampling design.

External evaluation was performed using data extracted from the WAPPS-Hemo database. Using Bayesian forecasting, PK outcomes including clearance, central volume, half-life, and time with FVIII activity above 2% (TAT2%) were estimated using both the generic SHL FVIII model and a brand-specific model. The evaluation dataset was extracted on September 14, 2018 and contained PK for 394 patients on three brands of factor product: Kovaltry (full-length rFVIII), ReFacto AF (BDDrFVIII), and Fanhdi/Alphanate (intermediate purity pdFVIII, not included in the modelling dataset). PK data for Xyntha, a BDDrFVIII products produced using the same manufacturing process as ReFacto AF but calibrated using a different assay, was also included in the evaluation; Xyntha doses were scaled by a factor of 1.38 to account for the difference in calibration, as done in Abrantes et al [182]. Patients ranged between <1 and 78 years of age and weighed between 10.6 and 138.8 kg.

Results

An abridged log of model building steps is found in Table S3. A 2-compartment structure with a combined residual error model and random effects on clearance (CL) and central volume (V_1) was found to be the superior base model; random effects were not included on intercompartmental clearance (Q) or peripheral volume (V_2) due to high shrinkage (>40%). Of the two body size metrics available, fat-free mass had the strongest correlation with η_{CL} and η_{V_1}(0.558 and 0.808, respectively) and provided the greatest improvement to the model in terms of both OFV (ΔOFV = -485) and unexplained BSV on CL ($\Delta\omega_{CL}$ = -10.5%) and V_1 ($\Delta\omega_{V1}$ = -

28.1%). The addition of a fat-free mass effect on V_2 further reduced the OFV by 116. Based on covariate plots (shown in Figure S1), the effect of age on CL was explored. Power, linear, and piecewise linear functions for the age effect were investigated. Ultimately, a piecewise linear function with no age effect below the median age was selected.

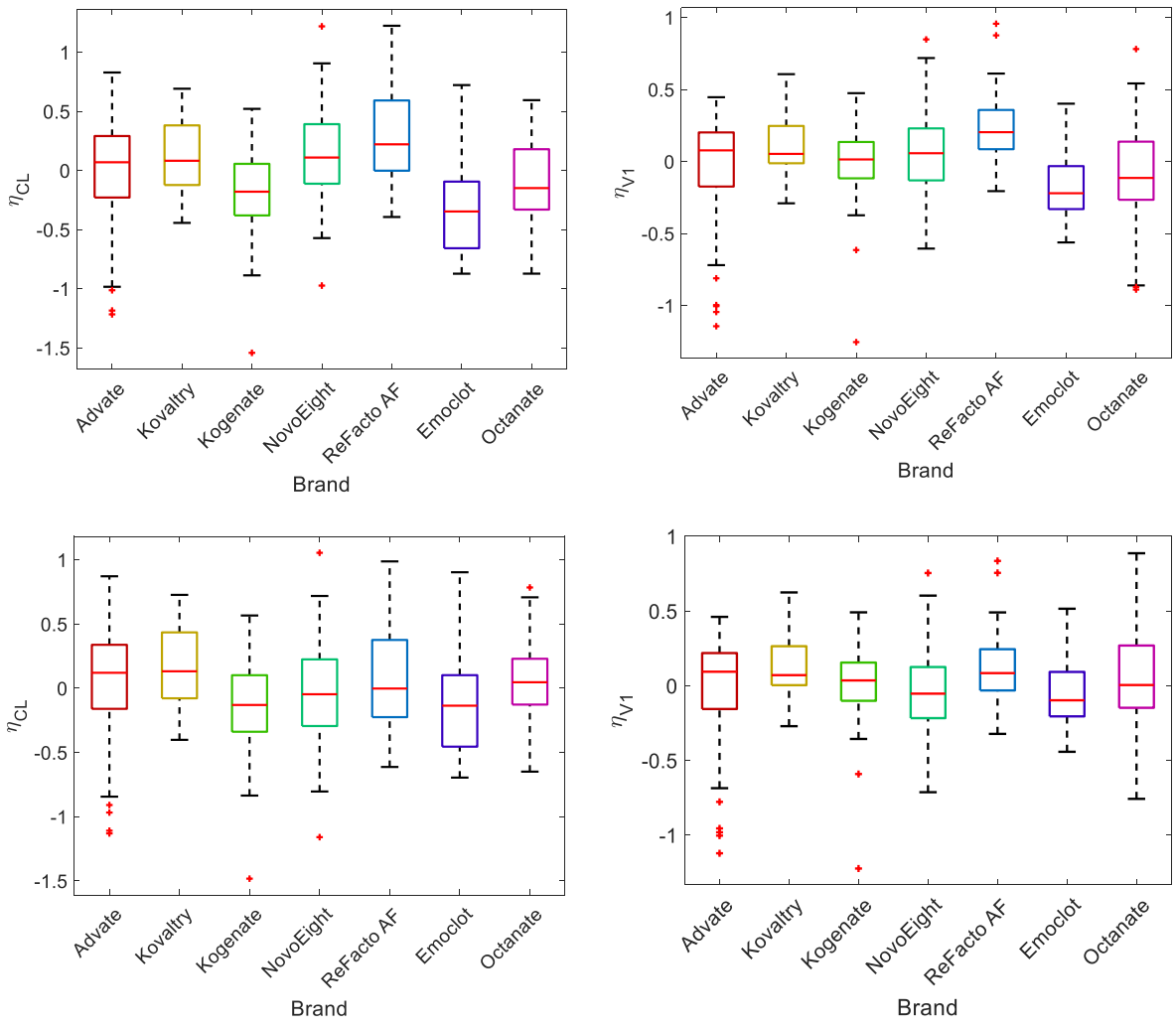


Figure 11. Boxplots of $\boldsymbol{\eta}$-values for clearance (left) and central volume (right) across brands before (top) and after (bottom) inclusion of brand as a covariate.

After accounting for body size and age, there still appeared to be significant differences across brands as shown in Figure 11. Initially, a unique effect for CL and V_1 was estimated for each brand. In an effort to reduce the number of model parameters, brands were grouped in a number of ways. We began by estimating an individual effect on CL and V_1 for each of the brands included in the dataset, using Advate as the reference brand (Table 9, Scheme 1). Based on the results of this run, effects were either removed (when effect sizes were below 10%) or combined (when effect sizes were within 10% of one another). The brand-specific effects of Kovaltry on CL and of Kogenate and Octanate on V_1 were removed (Table 9, Scheme 2). We also explored a grouping scheme based on the source and structure (Table 9, Schemes 3 and 4, respectively) of the factor products. While grouping scheme 2 produced the lowest OFV (25325), grouping scheme 4 was ultimately selected for the final model. This decision was driven by the objective of building a model that can be used for all SHL FVIII products. Since grouping scheme 4 is based on the source and structure of the product, choosing a group for a product not included in the model dataset is intuitive. Although OFV is somewhat increased for this scheme (ΔOFV = +64), parameter estimates (including BSV) were relatively unchanged. The final model is summarized by the following expression:

$$\begin{cases} CL = CL_{pop} \cdot \left(\frac{FFM}{53.0}\right)^{\theta_{FFM-CL}} \cdot \left(1 + \theta_{AGE-CL} \cdot max\left(0, \frac{AGE - 21.0}{21.0}\right)\right) \cdot (1 + \theta_{PD-CL} \cdot PD) \cdot (1 + \theta_{BDD-CL} \cdot BDD) \cdot e^{\eta_{CL}} \\ V_1 = V_{1\,pop} \cdot \left(\frac{FFM}{53.0}\right)^{\theta_{FFM-V_1}} \cdot \left(1 + \theta_{PD-V_1} \cdot PD\right) \cdot \left(1 + \theta_{BDD-V_1} \cdot BDD\right) \cdot e^{\eta_{V_1}} \\ Q = Q_{pop} \\ V_2 = V_{2\,pop} \cdot \left(\frac{FFM}{53.0}\right)^{\theta_{FFM-V_2}} \end{cases}$$

where $PD = 1$ for plasma-derived products and $BDD = 1$ for B-domain deleted products. Final model parameter estimates are shown in Table 10.

Table 10. Parameter estimates for the final SHL FVIII model

Parameter	Estimate	%RSE	95% Confidence Interval
Clearance, CL (L h^{-1})	0.238	3.6%	(0.221, 0.254)
FFM effect on CL (θ_{FFM-CL})	0.794	6.1%	(0.699, 0.883)
Age effect on CL (θ_{AGE-CL})	-0.205	14.5%	(-0.259, -0.145)
Central volume, V_1 (L)	3.01	2.5%	(2.85, 3.14)
FFM effect on V_1 (θ_{FFM-V_1})	1.02	4.2%	(0.940, 1.11)
Intercompartmental clearance, Q (L h^{-1})	0.142	14.4%	(0.107, 0.186)
Peripheral volume, V_2 (L)	0.525	7.0%	(0.457, 0.600)
FFM effect on V_2 (θ_{FFM-V_2})	0.787	16.5%	(0.557, 1.07)
Plasma-derived on CL (θ_{PD-CL})	-0.126	54.0%	(-0.232, 0.023)
Plasma-derived on V_1 (θ_{PD-V_1})	-0.104	52.2%	(-0.195, 0.017)
BDD on CL (θ_{BDD-CL})	0.309	23.3%	(0.175, 0.461)
BDD on V_1 (θ_{BDD-V_1})	0.159	32.4%	(0.060, 0.262)
BSV on CL (%)	41.1%	4.9%	(37.3%, 44.9%)
BSV on V_1 (%)	32.4%	7.0%	(28.3%, 37.2%)
CL-V_1 Correlation	0.703	5.2%	(0.624, 0.765)
Proportional error (%)	17.4%	4.8%	(16.0%, 19.3%)

Goodness-of-fit plots indicate that the model described the data well, with R^2 values of 0.748 and 0.945 for the population and individual predictions, respectively (Figure 12). Plots of the residual errors suggest that all assumptions of normality are followed, and the pcVPC demonstrates an adequate description of both median values and variability across all time points (Figure 13). Bootstrap analysis demonstrated the model's stability; RSE% was ≤35% for all parameters except those associated with the PD brand group (RSE% ≈50%; Table 10, Figure S2, Figure S3) and the additive error component (≥90%), which was subsequently removed. Internal cross-validation was performed to evaluate the model's utility for Bayesian forecasting. The results summarized in Table S4 and Figure S4 show low errors (95^{th} percentile of error <2%) on all parameters of interest (CL, V_1, half-life, and TAT2%). Optimal sampling analysis further evaluated the model's ability to accurately estimate PK parameters from sparsely sampled data. Estimates of half-life and time to 2% (TAT2%) from sampling designs containing as few as two levels were generally within 25% of those obtained from the rich sampling design (Figure 14).

Errors appear to be largest in sampling schemes that contain only a 72h point in tail of the curve, as this sample is likely to be BLQ for a significant proportion of the virtual population. The full results of the optimal sampling analysis can be found in Table S5.

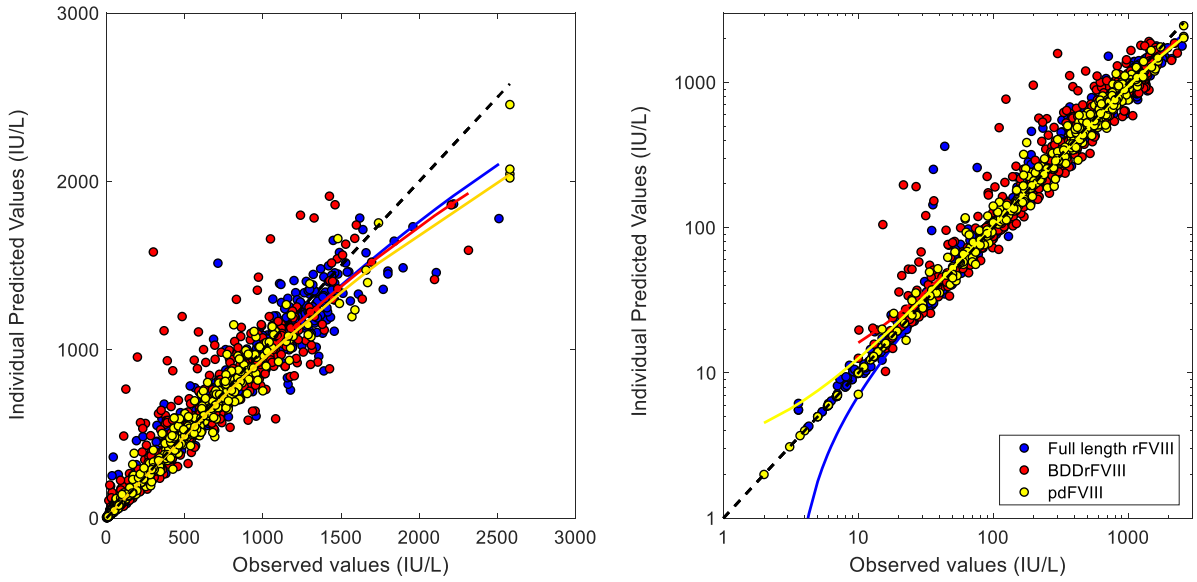


Figure 12. Individual predicted values from the final SHL FVIIII model versus observed values by brand group on linear (left) and log (right) scale. Samples that were BLQ are not depicted.

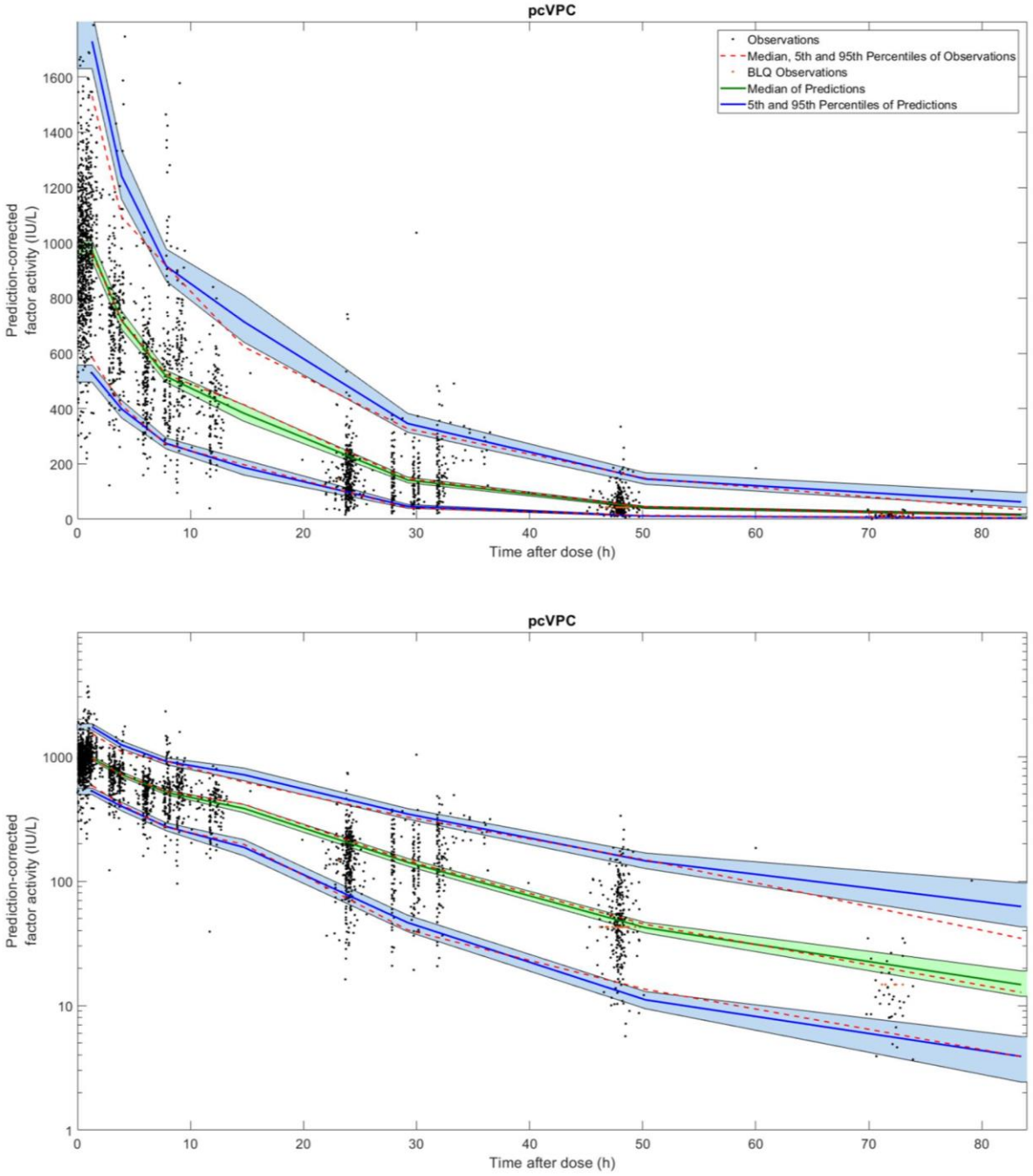


Figure 13. Prediction-corrected visual predictive check (pcVPC) for the final SHL FVIII model shown on linear (top) and log (bottom) scales. Shaded regions are the 90% confidence intervals for the simulated percentiles.

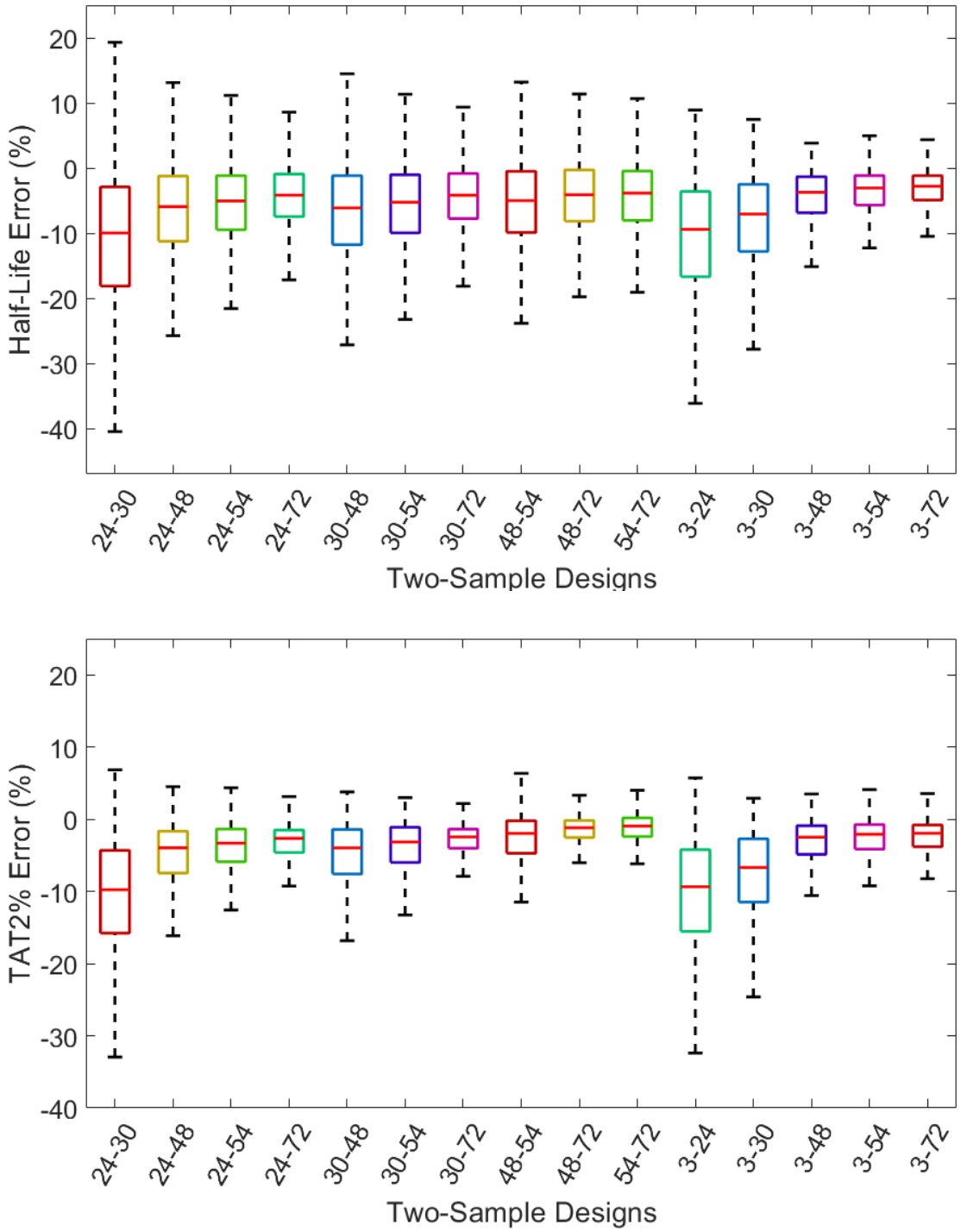


Figure 14. Errors on estimates of half-life (top) and time to 2% activity (TAT2%, bottom) from limited sampling strategies consisting of 2 post-infusion samples

Using data from the 394 patients collected through the WAPPS-Hemo network, we compared the performance of the generic SHL FVIII model to brand-specific models for brands of FVIII products in each of the brand groups of the final model: Kovaltry (full-length rFVIII), ReFacto AF/Xyntha (BDDrFVIII), and Fanhdi/Alphanate (pdFVIII). In each case, Bayesian forecasting produced similar estimates of clearance, central volume, half-life, and TAT2% (Figure 15) from both models (Kovaltry: $R^2 = 0.94 – 0.97$; ReFacto AF/Xyntha: $R^2 = 0.86 – 0.94$; Fanhdi/Alphanate: $R^2 = 0.94 – 0.99$); the correlation is slightly poorer in the ReFacto AF comparison as the comparator model has a one-compartment structure.

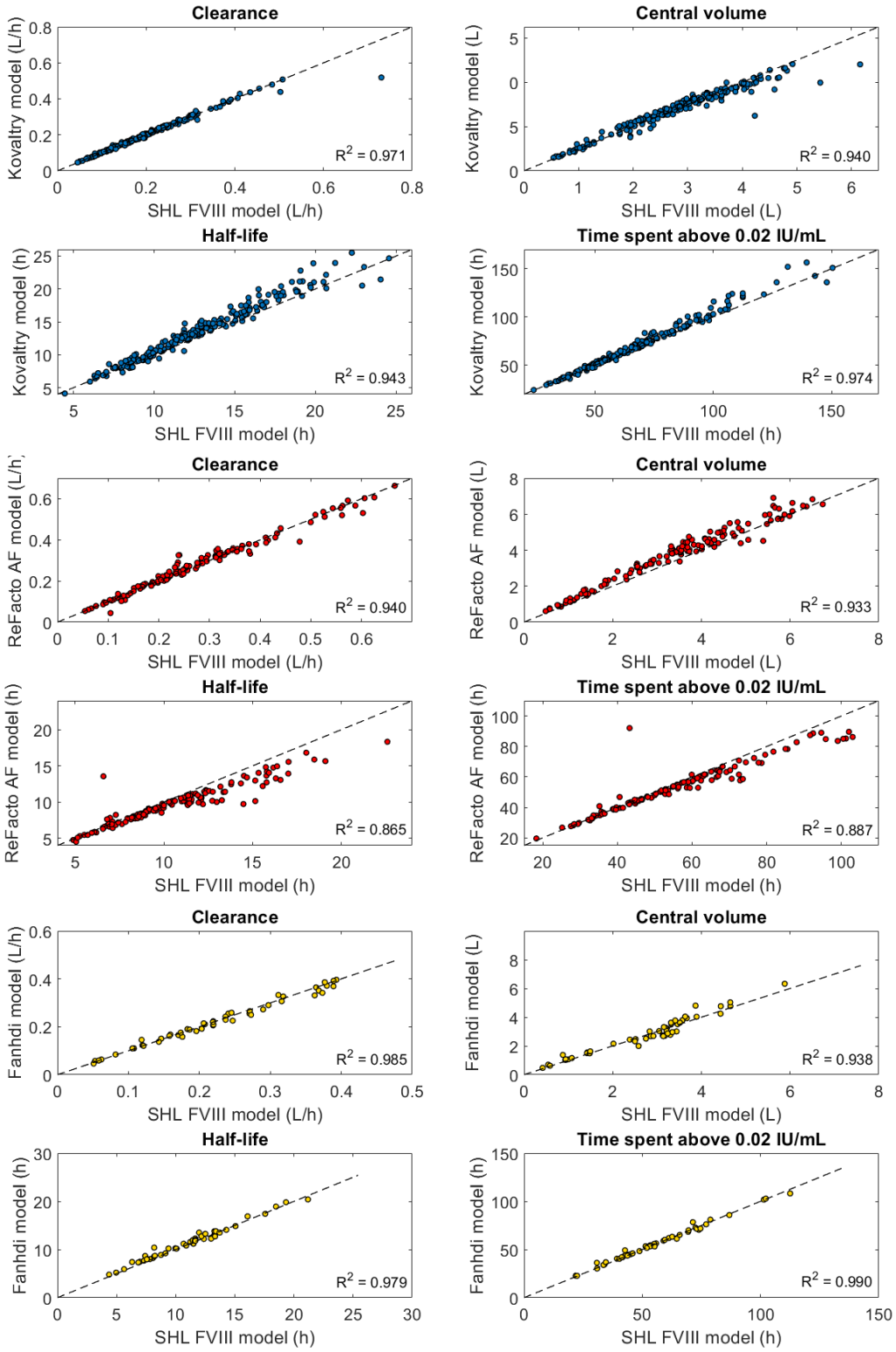


Figure 15. Comparison of PK parameter estimates generated from the SHL FVIII model and brand specific models for Kovaltry (blue, $\boldsymbol{n}$ = 213), ReFacto AF/Xyntha (purple, $\boldsymbol{n}$ = 132), and Fanhdi/Alphanate (orange, $\boldsymbol{n}$ = 49).

Discussion

This study describes the development and evaluation of a generic PopPK model for SHL FVIII products, built on a dataset containing data measured using the one-stage assay for seven different brands of SHL FVIII. The estimates of population PK parameters were similar to those reported in published brand-specific models despite differences in modelling data, approach, and objective (Table 11).

Table 11. Summary of parameter estimates from published PopPK models for SHL FVIII

Reference	Product	Parameter Estimates				BSV
		CL (L h^{-1})	V_1 (L)	Q (L h^{-1})	V_2 (L)	
Generic SHL FVIII model	All SHL FVIII products	rFVIII: 0.237 BDDrFVIII: 0.324 pdFVIII: 0.210	rFVIII: 3.00 BDDrFVIII: 4.10 pdFVIII: 2.72	0.143	0.522	41.1% [CL] 32.4% [V_1]
Abrantes [182]	ReFacto AF/ Xyntha	0.276	2.45	2.51	0.923	30.5% [CL] 13% [F]
Bjorkman [183]	Advate	0.193	2.22	0.147	0.73	30% [CL] 21% [V_1]
Nestorov [184]	Advate	0.253	3.46	0.055	0.494	30.4% [CL] 16.2% [V_1]
Garmann [185]	Kovaltry	0.188	3.00	0.190	0.637	37.0% [CL] 11.2% [V_1]
Jimenez-Yuste [103]	NovoEight	0.302	3.46	---	---	---
Karafoulidou [187]	ReFacto	0.393	4.86	---	---	38.9% [CL] 13.0% [V_1]

Fat-free mass explained a significant portion of the BSV on CL and V_1 and was likely superior to total body weight due to its better correlation to plasma volume. Age was also found to be a significant covariate for CL, possibly acting as a surrogate for changes in levels of vWF [119], an important predictor of FVIII clearance [145]. It has been shown that vWF levels increase with age in hemophilia A patients [120], resulting in lower clearance and longer half-

lives among these patients. The choice of a piecewise linear function to describe this age effect was primarily due to high correlation between FFM and age in children and teenagers. Including both covariates for patients in this age range produces a falsely high estimate of the FFM effect on CL, which in turn results in half-life estimates decreasing when FFM increases. As in the brand-specific models developed for WAPPS-Hemo, the median age (21 years) was selected as the cut-off for the age effect; this value is physiologically plausible, as vWF levels are fairly stable up to this age [120] and the correlation between age and FFM is much weaker after puberty (Figure S5). Unfortunately, vWF could not be directly included in the model because patient vWF levels were not available for all brands in the modelling dataset; blood group (which can also act as a surrogate for vWF) was also not available consistently and therefore could not be included. However, published models for FVIII that include vWF or blood group as covariates on CL had similar unexplained BSV on CL; moreover, unexplained BSV on CL only decreased by 5-8% after adding vWF or blood group compared to the base or structural model [184,188]. For the final SHL FVIII model described here, unexplained BSV on CL and V_1 remained high (42% and 31%, respectively) in the final SHL FVIII, even after incorporation of explanatory covariates; one possible explanation for this observation is inter-laboratory variability, as the modelling dataset was compiled from numerous sources. For the one-stage assay, this variability has been estimated to be around 10% for peak levels, but closer to 35% at levels below 50 IU L^{-1} [195–198].

This model was developed with two purposes in mind. First, the model is intended for use in Bayesian analysis to produce accurate estimations of relevant PK parameters from sparse patient data. To evaluate the model for this purpose, 5-fold cross-validation and optimal sampling analysis were performed, the results of which indicate the model is well-suited for this

purpose. Secondly, it was hoped that by combining data from a variety of SHL FVIII products, we could develop a model that performs Bayesian estimation accurately for all brands of SHL FVIII, including those not included in the modelling dataset. To assess this capability, we compared the estimates of PK parameters for 49 patients on Fanhdi/Alphanate (a plasma-derived SHL FVIII) produced by the generic SHL FVIII model and a dedicated Fanhdi/Alphanate model. Agreement between the estimates from each model was good ($R^2 \geq 0.94$ for $y = x$ regression for all parameters), suggesting that the models produce similar predictions of the parameters of interest. Based on these results, the model seems capable of predicting PK for brands outside the original covariate space, and may prove to be especially valuable for brands for which there is no dedicated PopPK model. An additional strength of the model is the ability to leverage pediatric data from other products when brand-specific pediatric data is unavailable.

Although it performed well in all evaluations, the model does have some limitations and there may be some instances in which a brand-specific model is more appropriate. For example, the covariate model of the SHL FVIII model was limited to values that were available across all seven brands. It is well known that additional covariates such as vWF, blood group, and hematocrit can be useful for predicting the PK of FVIII; a brand-specific model may allow for the incorporation of these covariates, resulting in lower unexplained BSV. Additionally, the modelling dataset does not contain pediatric PK data for all of the included brands; NovoEight alone represents over 60% of the data for children under the age of 5. If estimating PK in young patients, a brand-specific model may be preferable, provided that the model is built on enough patients, with an adequate proportion of the data coming from children. On the contrary, the SHL FVIII model allows for the leveraging of pooled pediatric data and may prove extremely useful in cases where data in young patients is lacking.

Conclusions

In summary, we have developed a generic PopPK model for plasma-derived and recombinant SHL FVIII products measured using the one-stage assay. Fat-free mass, age, and brand of factor product were found to significantly influence PK parameters. All evaluation steps suggest that the model is fit for Bayesian forecasting and capable of accurately predicting individual PK for SHL FVIII, including brands outside the original covariate space.

Chapter 5: Development of a population pharmacokinetic model for recombinant factor IX and its use in evaluating limited sampling strategies for pediatric patients

This chapter is reflective of an original manuscript prepared by the Ph.D. candidate (Alanna McEneny-King) for submission to *Journal of Thrombosis and Haemostasis*. All pertinent dialogue in this chapter was written by the Ph.D. candidate.

Introduction

Hemophilia B is an inherited bleeding disorder resulting from a deficiency of functional clotting factor IX (FIX). Consequently, hemophilia B patients have a lowered clotting ability and are thus prone to bleeding episodes, which may occur spontaneously among the most severe patients (i.e. those with <1 IU dL^{-1} or 1% of normal FIX activity). Joints are particularly prone to such bleeding events and irreversible joint damage can occur, severely impacting physical activity and quality of life. Although recent evidence suggests that hemophilia B may not be as severe as hemophilia A [199], several studies have demonstrated the dramatic reduction in overall, spontaneous, and joint bleeds achieved with prophylaxis as compared with on-demand treatment [49,200,201]. Thus, regular prophylaxis is considered to be the optimal approach for preventing bleeds and preserving joint function in hemophilia B patients.

A common goal of hemophilia therapy is to maintain clotting factor levels above 1 IU dL^{-1} throughout the week, thereby converting the patient from a severe to moderate phenotype. Though this target is largely based on observations in hemophilia A patients [7,10,68], there was no difference found in bleeding phenotype between young hemophilia A and B patients to suggest an alternative approach to prophylaxis is warranted [202]. Despite the unanimous

recommendation that prophylaxis be initiated at a young age [11,203–207], a ‘one size fits all’ dosing regimen is difficult to define due to wide inter-patient variability in pharmacokinetics (PK) and, particularly in the case of hemophilia B, a lack of evidence in support of a specific therapeutic target [208].

The high between subject variability (BSV) and relatively low inter-occasion variability (IOV) observed for FIX [18,209,210] suggests that FIX dosing regimens should be tailored to the individual for reasons of both safety and cost-effectiveness [211]. While the classic approach to PK-tailoring has been hampered by intense sampling requirements, recent guidance from the International Society of Thrombosis and Haemostasis advocates for a population PK (PopPK) approach to dose individualization [169]. The PopPK technique uses Bayesian methods to estimate individual PK parameters, with a PopPK model providing informative prior knowledge of typical values of PK parameters, estimates of variability, and influential covariates.

The first recombinant FIX (rFIX) product (nonacog alfa, BeneFIX®) was approved by the US Food and Drug Administration in 1997; two other standard half-life (SHL) recombinant products (nonacog gamma [RIXUBIS®] and trenonacog alfa [IXINITY®]) followed in 2013 and 2015, respectively. While all three rFIX products are similar to plasma-derived FIX, small differences exist between the brands. IXINITY corresponds to the Thr-148 polymorph of plasma-derived FIX, while BeneFIX and Rixubis contain the less common Ala-148 variant [212]. Also, the amount of activated factor IX was found to be significantly lower for Rixubis as compared to BeneFIX [213]. Despite these differences, pharmacokinetic studies have demonstrated that both new rFIX products are bioequivalent (RIXUBIS) or non-inferior (IXINITY) to BeneFIX [33,40]. However, there appears to be a clear difference between plasma-

derived and recombinant FIX products, particularly with regards to clearance [214], suggested to arise from biochemical variations (e.g. under-carboxylation).

For these reasons, we aimed to develop and evaluate a generic PopPK model for SHL rFIX products, using data collected through the Web Accessible Population Pharmacokinetic Service – Hemophilia (WAPPS-Hemo) project. A second aim of the study was to use the model to perform a limited sampling analysis, the results of which are applicable to an ongoing phase 3/4 study (NCT03855280) to evaluate IXINITY PK in pediatric (<12 years old) patients, as pediatric sample volume limitations may prevent centres from collecting all intended blood samples.

Methods

Patient data

Pharmacokinetic data for SHL rFIX was collected from both industry sources and routine hemophilia care through the WAPPS-Hemo project. The model was developed using FIX activity levels from 99 patients, measured using the one-stage assay. The median number of samples per infusion was 9 (range: 1 – 13). Samples that were below the limit of quantification (BLQ) comprised 1.5% of the dataset. A summary of the sampling characteristics and patient demographics of the full modelling dataset can be found in Table 12.

Table 12. Demographics of the patient population used to develop the generic SHL rFIX model. Data are presented as median (range) where appropriate.

Sampling Information					
Total number of patients	**Total number of samples**	**Number of BLQ samples**		**Number of samples per patient**	**Duration of sampling (h)**
99	840	13 (1.5%)		9 (1 – 13)	72.4 (23.8 – 188.1)
Patient Demographics					
Brand	***n*** **(% from WAPPS)**	**Age (years)**	**Body weight (kg)**	**Fat-free mass (kg)**	**Dose (IU/kg)**
BeneFIX	48 (52%)	28.3 (3.8 – 68.7)	71 (18.5 – 187)	54.6 (14.3 – 99.6)	49.8 (21.7 – 120)
IXINITY	37 (0%)	23.7 (4 – 64.5)	78 (14 – 145)	59.5 (11.5 – 85.6)	75.1 (52.3 – 83.4)
Rixubis	14 (100%)	39.1 (6.3 – 72.2)	69 (31 – 85)	53.6 (24.8 – 59.8)	44.0 (22.0 – 130.1)
TOTAL	99 (39%)	28.2 (3.8 – 72.2)	72 (14 – 187)	54.8 (11.5 – 99.6)	51.4 (21.7 – 130.1)

Population modelling

PopPK model building, employing non-linear mixed effects modelling techniques, was implemented in NONMEM and PDxPop (v7.3 and v5.2, respectively; ICON Development Solutions, Ellicott City, MD, USA). Data and graphical analyses were conducted in MATLAB (R2017b, Mathworks, Natick, MA, USA). Samples that were BLQ were handled using the M3 method [173].

The structural component of the model was developed first, and describes not only the exogenous dose, but also endogenous FIX production and residual FIX from prior doses.

$$C(t) = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t} + endogenous\ FIX + (predose\ FIX - endogenous\ rFIX) \cdot e^{-\beta t}$$

The endogenous FIX component was assumed to be constant. When the endogenous level was unknown or BLQ, it was assumed to be half of the LLOQ, or 0.5 IU dL^{-1}. Residual exogenous

FIX (i.e. greater than baseline FIX activity measured immediately before dose) decayed according to the terminal rate constant (β); if no predose measurement was available, it was assumed that only endogenous FIX remained at the time of dosing. The standard 1-, 2-, and 3-compartment models were tested, with three different residual error (RUV) models (additive, proportional and combined) explored for each. Between subject variability (BSV) terms were added to PK parameters using an exponential form as follows:

$$CL_i = CL_{pop} \cdot e^{\eta_i}$$

where CL_i is the clearance value for patient i, CL_{pop} is the typical value of clearance for the population, and η_i represents the individual's deviation from the population value. The η-values for each parameter are normally distributed with a mean of zero, such that the PK parameters follow a log-normal distribution. Selection of the structural, BSV and RUV models was driven by changes in goodness-of-fit metrics (objective function value [OFV], Akaike information criterion [AIC], or Bayesian information criterion [BIC]), diagnostic plots, plausibility of parameter estimates, and shrinkage of random effects.

To minimize unpredictable BSV, explanatory covariates were incorporated into the model. All data sources provided total body weight, height, age, and brand; fat-free mass (FFM) was calculated from body weight, age, and height using the maturation model defined by Al-Sallami et al [194]. Plots of η-values versus each covariate were used for preliminary analysis. Covariates were then added in a stepwise manner, and kept based on their effect on OFV, BSV, and parameter estimates. Body size metrics were modelled using power functions; age effect was explored using both power and linear functions.

Model evaluation

The final SHL rFIX model was evaluated using a variety of techniques. First, diagnostic plots were used to assess the model's goodness-of-fit and to ensure that that all model assumptions were met. A prediction-corrected visual predictive check (pcVPC) was generated to evaluate the model's predictive potential. Next, bootstrap analysis was performed with replacement to evaluate model stability and estimate confidence intervals around PK parameters. Internal cross-validation and limited sampling analysis (LSA) were used to evaluate the model for use in Bayesian forecasting. In more detail, a 10-fold cross-validation was performed; 90% of the data was used to build the model (learning subset) and the remaining 10% was used for evaluation (validation subset). Relative error on individual PK parameters was calculated using the following equation:

$$Relative\ Error = \frac{\varphi_{CV} - \varphi_{full}}{\varphi_{full}}$$

where φ_{CV} is the parameter estimate obtained from the cross-validation and φ_{full} is the "true" value estimated from the complete modelling dataset. This process was repeated with 100 random splits of the data to avoid any biases.

Finally, LSA was used to determine which samples are most critical for the estimation of various parameters by Bayesian forecasting. A population of 1000 virtual individuals was simulated, with the same demographic and PK parameter distributions as the original modelling dataset. The simulated dosing regimen consisted of a 5-minute infusion of 50 IU kg^{-1} (rounded to the nearest 250 IU to account for available vial sizes) every Monday and Thursday. The regimen was simulated for 4 weeks to ensure steady state was reached, and the final Thursday dose was used for analysis. A full sampling scheme containing 11 sampling times (Predose and 0.5, 1, 3, 6,

12, 24, 36, 48, 72, and 96 h post-infusion) was used as the reference case. A total of 31 limited sampling designs containing 2-3 samples each were tested. Estimates of individual PK parameters from the limited sampling strategies were compared to those from the full sampling design as follows:

$$Error = \frac{\varphi_{LSS} - \varphi_{full}}{\varphi_{full}} \times 100\%$$

$$Absolute\ Error = |Error|$$

where φ_{full} and φ_{LSS} represent the estimates of PK parameter φ estimated by the rich and limited sampling strategies, respectively.

Limited sampling analysis for pediatric clinical trial

A second LSA was performed to determine if any of the planned sampling times for the IXINITY pediatric trial could be omitted without compromising the accuracy of the parameter estimates. To do so, three populations (n = 1000 each) were simulated, representing 2-, 5-, and 11-year-old boys. Body weight and height distributions were taken from the NHANES database for calculation of fat-free mass [215]. The intended sampling schedule is based on guidance from the European Medicines Agency (EMA), and contains samples at predose, 0.25–0.5 h, 4–6 h, 24–26 h, and 46–50 h; limited sampling designs were created by systematically removing these timepoints (the full set of designs is described in Table S6). Pharmacokinetic outcomes of interest for the trial include area under the plasma concentration-time curve (AUC), terminal half-life, maximum post-infusion plasma concentration (C_{max}), incremental recovery (IVR), clearance, and volume of distribution at steady-state (V_{ss}); estimates of trough levels (C_{72}, C_{96})

and time to 2% and 1% factor IX activity (TAT2% and TAT1%) were also assessed. Strategies were evaluated using error and absolute error.

As model-independent methods are more commonly used for PK analysis in clinical trials than the PopPK approach [216], we explored how noncompartmental methods would fare in the same limited sampling conditions. Noncompartmental analysis (NCA) was performed using the SimBiology® app in MATLAB. To calculate the terminal rate constant (λ_z), a minimum of 3 observations in the terminal phase spanning at least 2 half-lives is recommended [217]. Based on these guidelines, only the intended EMA-based sampling strategy and the five 4-sample subsets thereof were assessed using NCA. Estimates of half-life, clearance, V_{ss}, and AUC were evaluated against the same rich 11-sample scheme as in the Bayesian method as the reference value.

Results

Model development and evaluation

The final base model consisted of a 2-compartment structure with proportional RUV and random effects on clearance and central volume (V_1). Of the body weight metrics available, fat-free mass had the strongest correlation with both η_{CL} and η_{V_1} (0.3415 and 0.5941, respectively) and its inclusion in the model significantly decreased both OFV (ΔOFV = –193) and BSV on CL ($\Delta\omega_{CL}$ = –16%) and V_1 ($\Delta\omega_{V1}$ = –23%). Addition of fat-free mass terms on Q and V_2 resulted in further decrease of the OFV (ΔOFV = –133).The estimate of fat-free mass effect on V_2 had high standard error but, due its physiologic relevance, was kept in the model with a fixed exponent of 1.0, without significantly increasing OFV. Age and brand were not found to be significant

covariates. The final model is summarized by the following equation, with parameter estimates presented in Table 13:

$$\begin{cases} CL = CL_{pop} \cdot \left(\frac{FFM}{54.8}\right)^{\theta_{FFM-CL}} \cdot e^{\eta_{CL}} \\ V_1 = V_{1\,pop} \cdot \left(\frac{FFM}{54.8}\right)^{\theta_{FFM-V1}} \cdot e^{\eta_{V_1}} \\ Q = Q_{pop} \cdot \left(\frac{FFM}{54.8}\right)^{\theta_{FFM-Q}} \\ V_2 = V_{2\,pop} \cdot \left(\frac{FFM}{54.8}\right)^{\theta_{FFM-V2}} \end{cases}$$

Table 13. Parameter estimates for the final SHL rFIX model

Parameter (unit)	Estimate	% RSE	95% Confidence Interval[a]
Structural Model			
CL_{pop} (dL h^{-1})	3.36	3.4%	(0.314, 0.360)
$V_{1\,pop}$ (dL)	78.4	2.5%	(7.46, 8.24)
Q_{pop} (dL h^{-1})	3.01	8.1%	(0.261, 0.358)
$V_{2\,pop}$ (dL)	61.6	8.2%	(5.36, 7.34)
Covariate Effects			
FFM effect on CL	0.765	10.5%	(0.594, 0.922)
FFM effect on V_1	0.893	8.7%	(0.762, 1.06)
FFM effect on Q	0.688	26.4%	(0.303, 1.00)
FFM effect on V_2	1.00 (FIXED)		
Between Subject Variability			
ω_{CL}	30.3%	8.5%	(25.1%, 34.9%)
$Corr_{CL-V1}$	0.696	11.3%	(0.522, 0.819)
ω_{V1}	25.1%	12.2%	(19.0%, 30.5%)
Residual Unexplained Variability			
CV of proportional RUV	14.7%	9.1%	(12.1%, 17.3%)

[a]From 1000 bootstrap runs

Goodness-of-fit plots demonstrate that the model describes the data well, with R^2 values of 0.904 and 0.975 for the population and individual predictions, respectively (Figure 16), and residual plots confirm that all assumptions of normality are followed. The pcVPC suggests that the model is able to adequately describe the central tendency and the variability of the data across all time points (Figure 17).

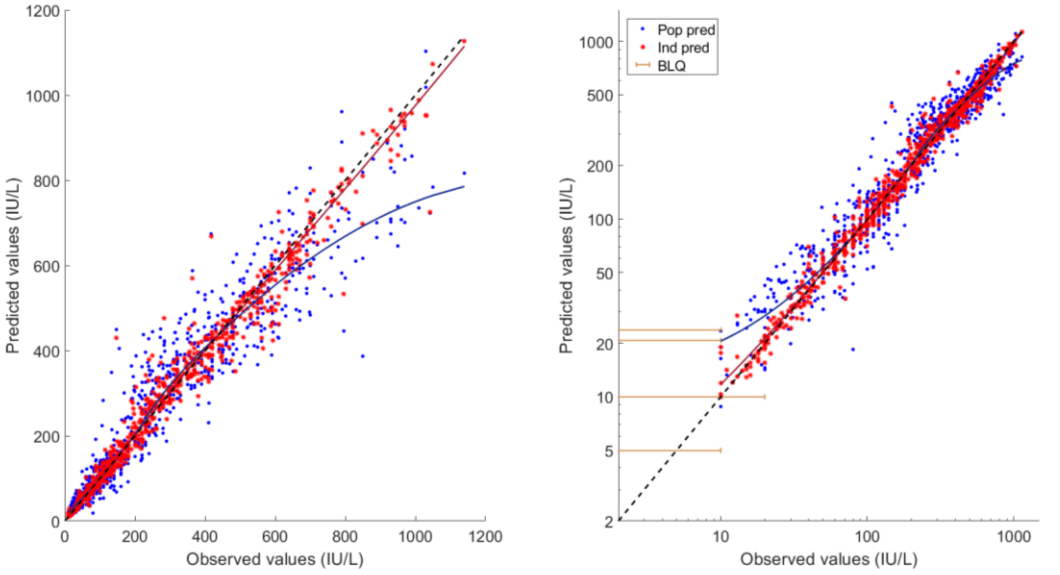


Figure 16. Goodness-of-fit plots on linear (left) and log (right) scales.

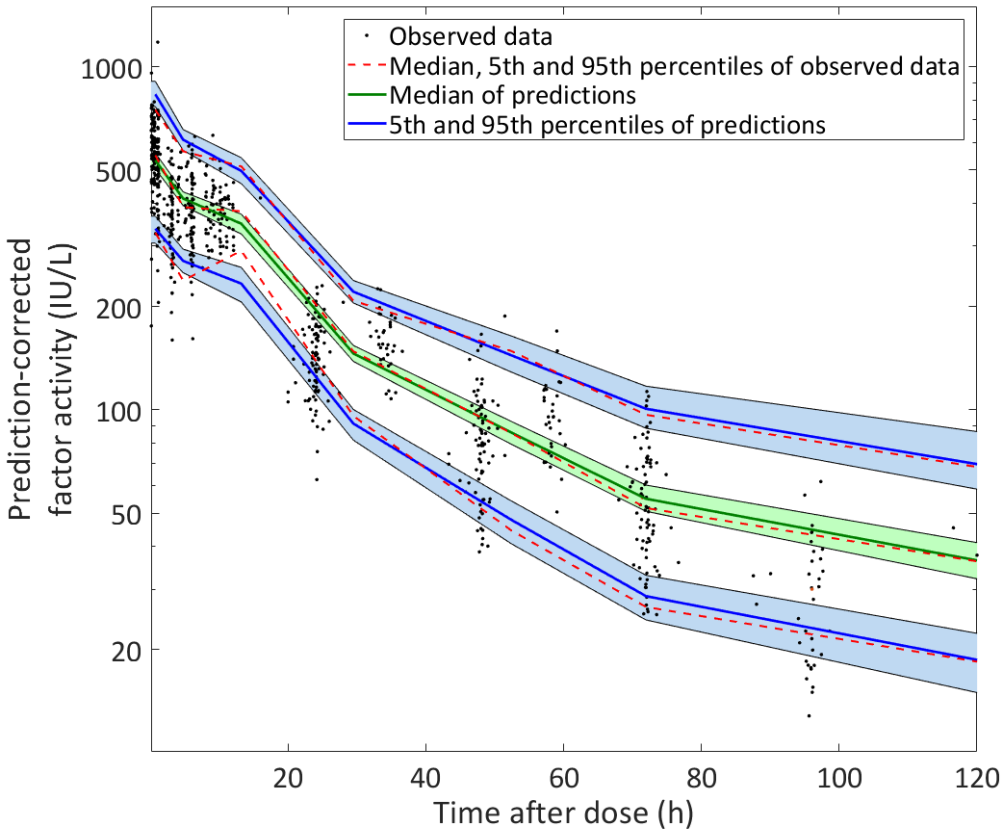


Figure 17. Population prediction-corrected visual predictive check (pcVPC) for the final SHL rFIX model. Shaded regions are the 90% confidence regions for the simulated percentiles.

Bootstrap analysis showed estimates of all model parameters are stable; RSE% was below 15% for all parameters except the FFM effect on Q (Table 13). The model's utility in Bayesian forecasting was evaluated using cross-validation, which resulted in low errors (median <1.5%; 95th quantile <5.0% [Figure 18]) on all parameters explored (CL, V_1, half-life, and TAT2%). Its ability to provide accurate estimates from sparsely sampled data was assessed using optimal sampling analysis, which revealed that key PK outcomes such as half-life and TAT2% can be well estimated when sample times are chosen appropriately.

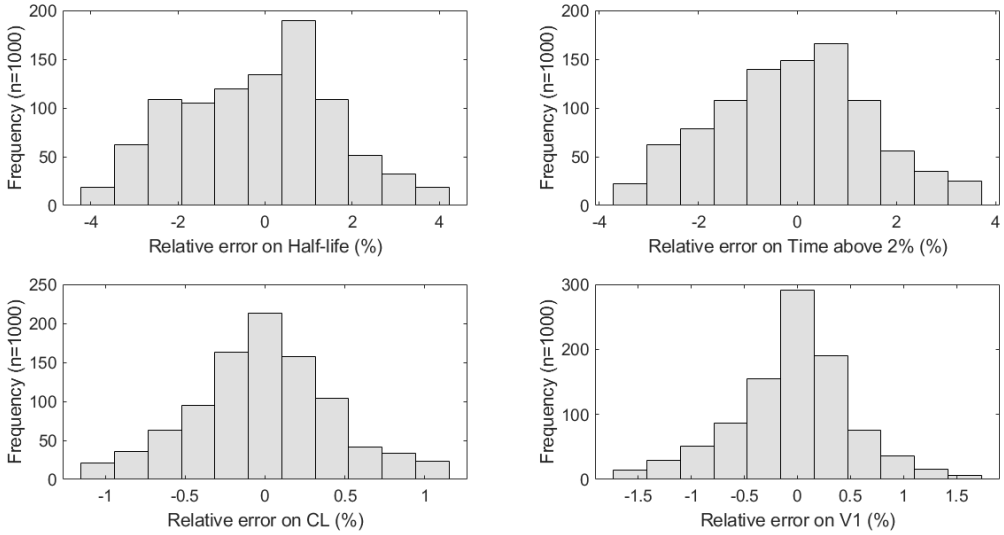


Figure 18. Histograms of prediction errors on half-life, TAT2%, CL, and V_1 estimates

Limited sampling analysis for pediatric clinical trial

Results of the limited sampling analysis were very similar for all simulated ages; for this reason, only the results from the 2-year-old population are shown Table 14, Table 15, Figure 19 and Figure 21. Full results for all nine PK outcomes from all 26 limited sampling designs can be found in Table S6. Compared to the 11-sample reference sampling scheme, the median [95th quantile (Q95)] absolute error on PK parameters estimated by the 5-sample EMA-based sampling scheme was below 6% [<16%], with the highest error being on trough levels and central volume.

Omitting a single sample from EMA-based sampling

When only one sample is eliminated from the sampling design, the estimates for most PK parameters are largely unchanged compared to the reference EMA-based design (Table 14, Figure 19 [top]). The most crucial times to include are the predose sample (which greatly affects estimates of clearance, trough levels, and to a lesser degree, half-life) and the peak sample (which impacts central volume and, by consequence, C_{max}) Omitting any one of the 6-, 24-, or 48-hour samples results in low errors (median [Q95] absolute error <8% [<23%]) for all outcomes.

Table 14. Error (presented as mean (range) in percent) and absolute error (presented as median [Q95] in percent) relative to the 10-sample design using Bayesian estimation

Error	Parameters			
Sampling Design	**Half-Life**	**Clearance**	**V_{ss}**	**AUC**
EMA-Based	-0.2 (-15.7, 11.9)	0.1 (-10.8, 18.1)	-0.4 (-14.1, 17.3)	0.2 (-15.3, 12.1)
Pre-0.5-6-24	0.5 (-17.4, 13.8)	-0.7 (-15.3, 20.5)	-0.4 (-14.2, 17.2)	1.1 (-17.0, 18.0)
Pre-0.5-6-48	0.04 (-17.1, 15.1)	-0.3 (-13.7, 20.7)	-0.5 (-14.6, 16.1)	0.6 (-17.2, 15.9)
Pre-0.5-24-48	-0.3 (-15.4, 12.3)	-0.1 (-15.6, 16.9)	-0.7 (-20.4, 21.0)	0.4 (-14.5, 18.5)
Pre-6-24-48	-0.4 (-18.1, 14.0)	0.1 (-14.5, 21.8)	-0.3 (-24.3, 37.1)	0.3 (-17.9, 17.0)
0.5-6-24-48	-3.4 (-22.9, 13.7)	3.9 (-12.7, 33.3)	-0.8 (-14.8, 17.2)	-3.3 (-25.0, 14.5)
Pre-0.5-6	1.1 (-20.3, 18.6)	-1.5 (-19.2, 29.7)	-0.6 (-14.8, 16.1)	2.0 (-22.9, 23.8)
Pre-0.5-24	0.4 (-17.4, 14.0)	-1.0 (-17.1, 22.8)	-0.8 (-20.5, 20.0)	1.4 (-18.6, 20.7)
Pre-0.5-48	-0.03 (-16.8, 14.6)	-0.6 (-16.4, 22.7)	-0.9 (-21.2, 21.7)	0.9 (-18.5, 19.6)
Pre-6-24	0.3 (-19.5, 15.9)	-0.7 (-18.6, 31.2)	-0.4 (-24.1, 36.9)	1.2 (-23.8, 22.8)
Pre-6-48	-0.2 (-19.1, 15.7)	-0.4 (-17.6, 23.2)	-0.6 (-26.1, 32.4)	0.9 (-18.9, 21.3)
Pre-24-48	-0.9 (-17.9. 11.8)	-0.8 (-24.1, 28.4)	-2.0 (-33.0, 29.6)	1.4 (-22.1, 31.7)
0.5-6-24	-4.4 (-33.0 16.9)	5.7 (-18.6, 66.2)	-0.9 (-15.3, 16.2)	-4.4 (-39.8, 22.9)
0.5-6-48	-3.8 (-27.8, 14.3)	4.7 (-16.5, 44.7)	-0.7 (-14.9, 15.9)	-3.8 (-30.9, 19.8)
0.5-24-48	-3.4 (-23.1, 13.8)	3.9 (-13.7, 33.6)	-0.8 (-20.7, 19.6)	-3.3 (-25.1, 15.9)
6-24-48	-3.5 (-23.4, 12.7)	3.9 (-14.0, 40.8)	-0.7 (-24.0, 34.9)	-3.2 (-29.0, 16.3)

Absolute Error	Parameters			
Sampling	**Half-Life**	**Clearance**	**V_{ss}**	**AUC**
EMA-Based	2.8 [7.8]	3.1 [9.5]	3.1 [9.5]	3.2 [9.3]
Pre-0.5-6-24	3.4 [9.9]	4.2 [11.5]	3.1 [9.7]	4.3 [11.8]
Pre-0.5-6-48	3.0 [8.5]	3.7 [10.0]	3.2 [9.7]	3.6 [10.5]
Pre-0.5-24-48	2.8 [8.1]	3.5 [9.8]	3.7 [11.0]	3.5 [10.0]
Pre-6-24-48	3.1 [8.7]	4.1 [12.5]	5.8 [17.3]	4.1 [12.0]
0.5-6-24-48	4.2 [13.0]	4.8 [16.1]	3.1 [9.4]	4.7 [13.9]
Pre-0.5-6	4.1 [11.4]	5.3 [13.7]	3.2 [9.7]	5.4 [14.8]
Pre-0.5-24	3.4 [10.1]	4.6 [12.7]	3.7 [11.1]	4.7 [13.3]
Pre-0.5-48	3.0 [8.7]	4.1 [11.3]	3.8 [11.3]	4.1 [12.0]
Pre-6-24	3.6 [10.8]	5.0 [14.1]	5.8 [17.5]	5.0 [14.1]
Pre-6-48	3.2 [9.0]	4.7 [13.3]	5.8 [17.7]	4.7 [13.2]
Pre-24-48	3.3 [9.5]	5.3 [14.7]	7.7 [22.0]	5.4 [14.7]
0.5-6-24	6.3 [17.9]	6.9 [24.4]	3.2 [9.6]	6.8 [19.8]
0.5-6-48	4.6 [15.1]	5.7 [20.0]	3.2 [9.6]	5.6 [16.8]
0.5-24-48	4.2 [12.9]	5.1 [16.7]	3.7 [11.1]	5.0 [14.5]
6-24-48	4.3 [13.3]	5.4 [18.0]	5.9 [17.3]	5.3 [15.4]

Omitting multiple samples from EMA-based sampling

Several 3-sample designs perform well, particularly for the estimation of half-life (Figure 19 [middle]) and AUC (median [Q95] absolute error ≤7% [<20%] for all 3-sample designs). The Predose-0.5h-48h strategy resulted in the lowest absolute errors across all outcomes of interest (median [Q95] <6.5% [<19%]), with the Predose-0.5-24h design producing similar results (Table 14). Estimates of central volume, peak and trough levels, and times to critical factor levels were the most sensitive to sample timing, with the 95th percentile of error greater than 30% for some sampling schemes (Table S6).

A number of 2-sample strategies also produce reasonably accurate estimates of most PK parameters. In particular, half-life is still well-estimated (median absolute error <7%) for all strategies except for 0.5h-6h (Figure 19 [bottom]). Of particular note, the Pre-0.5h strategy produces PK parameter estimates that are similarly accurate to the optimal 3-sample design (median <7%; Q95 ≤20% - Table S6); however, errors on trough levels are slightly elevated (9% [25%] at 72 hours; 9% [27%] at 96 hours).

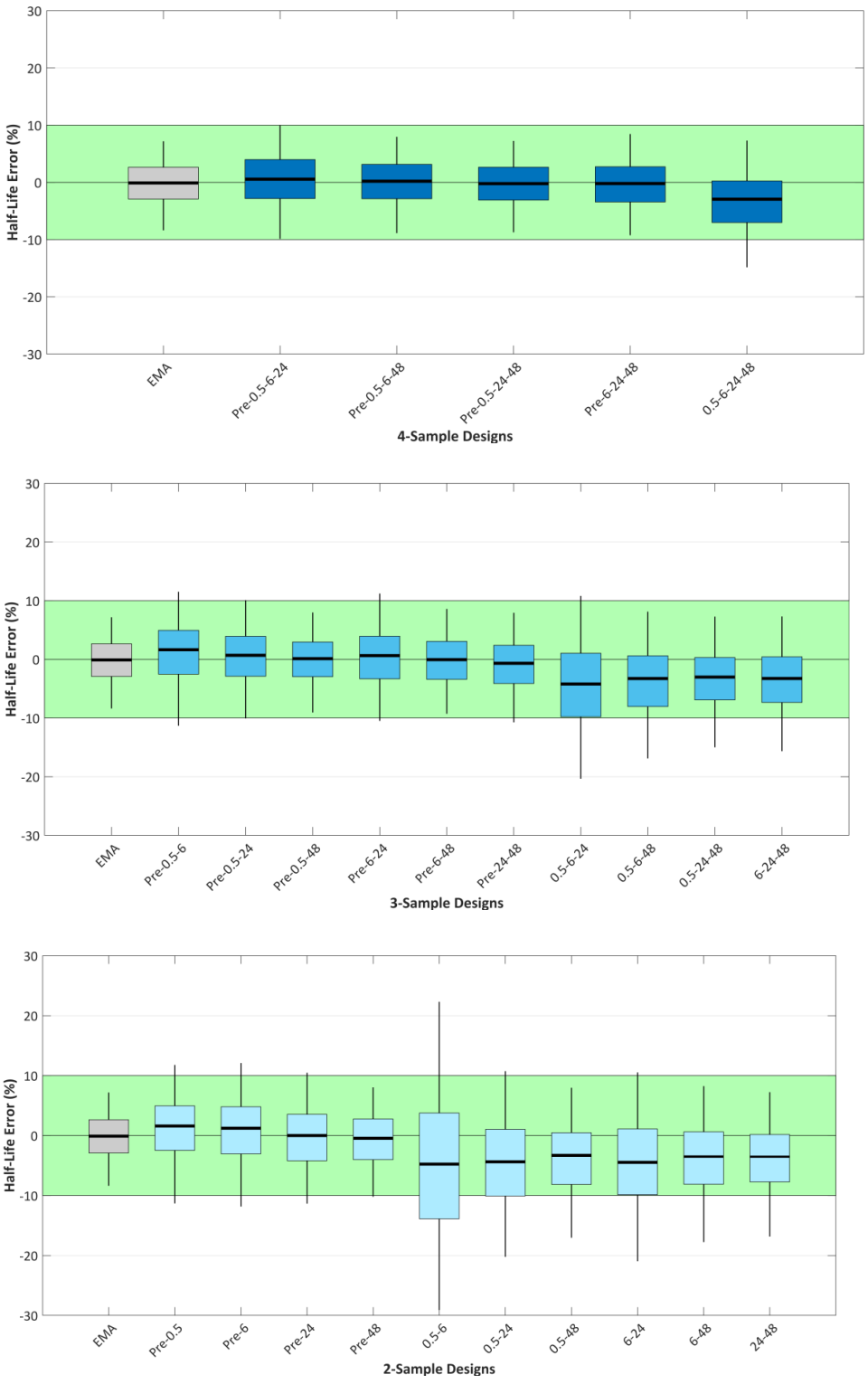


Figure 19. Half-life error relative to the rich 10-sample design for 4- (top), 3- (middle), and 2- (bottom) sample designs.

Noncompartmental analysis

Results from the noncompartmental analysis are shown in Table 15. Relative to the rich 11-sample design, estimates of CL, V_{ss}, and $AUC_{0\text{-}inf}$ obtained from the EMA-based sampling design are fairly precise (median [Q95] absolute error <6% [≈20%]). However, half-life estimates are significantly under-predicted using this sampling strategy (mean error: –24.6%).

Omitting the 48-hour sample has the greatest impact on PK parameter estimates. With the exception of V_{ss}, which was relatively unchanged, all other outcomes had substantially higher errors. Without this late sample, AUC is considerably underestimated (mean error: –44%), resulting in an overestimation of clearance (mean error: +27%); half-life was also severely underestimated, with mean error of –48%. Omission of the predose sample – which resulted in the largest error on most PK parameters when Bayesian methods were used – produced almost identical estimates to the full EMA-based sampling scheme when NCA was used. This is due to a difference in how the predose sample is used between the two methods. For NCA, the predose simply acts as a starting point (C_0) while in Bayesian estimation, predose acts as a late time point (72–96 hours, depending on the day of the PK study) when knowledge of the previous dose is available, as was assumed in this study. Of the post-infusion samples, exclusion of the 6-hour point produced the most accurate estimate of clearance and $AUC_{0\text{-}inf}$; half-life was best predicted from the strategy omitting the 24-hour sample, while the steady state volume of distribution was well estimated when the 48-hour sample was removed.

Table 15. Error (presented as mean (range) in percent) and absolute error (presented as median [Q95] in percent) relative to the 10-sample design using noncompartmental analysis

Error	Parameter			
Sampling Design	**Half-Life**	**Clearance**	**V_{ss}**	**AUC**
EMA-Based Sampling	-24.6 (-73.3, 65.2)	4.0 (-19.2, 61.8)	-3.7 (-23.9, 27.0)	-3.0 (-38.2, 23.7)
Pre-0.5-6-24	-48.3 (-80.7, 37.6)	26.7 (-22.5, 161.7)	7.5 (-18.6, 91.8)	-19.4 (-61.8, 29.1)
Pre-0.5-6-48	-29.9 (-75.2, 35.3)	-8.1 (-38.5, 67.5)	-19.8 (-44.1, 23.1)	10.8 (-40.3, 62.6)
Pre-0.5-24-48	-33.2 (-76.4, 83.8)	-5.2 (-40.9, 58.6)	-16.5 (-47.8, 15.7)	7.2 (-36.9, 69.2)
Pre-6-24-48	-20.7 (-73.3, 65.2)	19.6 (-9.0, 83.8)	16.1 (-6.7, 51.0)	-15.6 (-45.6, 9.9)
0.5-6-24-48	-24.6 (-73.3, 65.2)	4.0 (-19.2, 62.1)	-3.6 (-23.8, 27.1)	-3.1 (-38.3, 23.7)
Absolute Error	Parameter			
Sampling Design	**Half-Life**	**Clearance**	**V_{ss}**	**AUC**
EMA-Based Sampling	27.2 [56.4]	6.1 [20.5]	5.4 [14.6]	6.2 [17.3]
Pre-0.5-6-24	49.9 [69.0]	24.4 [58.0]	7.4 [25.5]	19.6 [36.7]
Pre-0.5-6-48	30.3 [56.6]	11.1 [27.3]	20.2 [34.1]	12.1 [35.8]
Pre-0.5-24-48	34.0 [58.5]	9.3 [24.0]	16.7 [32.9]	9.6 [30.5]
Pre-6-24-48	24.0 [54.4]	18.9 [38.3]	15.3 [32.5]	15.9 [27.7]
0.5-6-24-48	27.2 [56.4]	6.2 [20.6]	5.3 [14.6]	6.2 [17.4]

Due to the significant bias observed in half-life estimates for all sampling strategies, we investigated the impact of including a later time point. If the EMA-based sampling design is supplemented with a 72 h or 96 h sample, the mean error on half-life improves from –24.6% to –2.5% and +2.0%, respectively, without significantly altering the estimates of other PK parameters of interest (Figure 20). If the addition of another sampling time is problematic, recall that the omission of predose has little impact on estimates obtained using NCA. Therefore, an alternative strategy consisting of samples at peak (0.5 h), 6 h, 24 h, 48 h, and either 72 or 96 hours may be more appropriate to accurately estimate all relevant PK parameters when noncompartmental methods are employed.

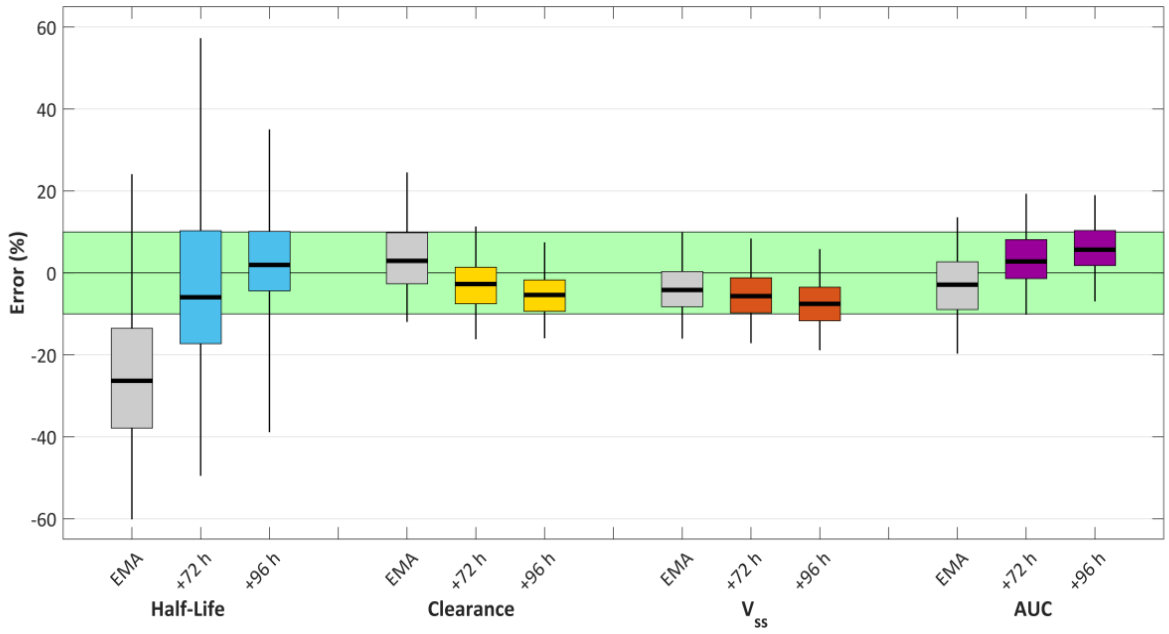


Figure 20. Impact of inclusion of a late sampling tie (72 or 96 h) on PK parameter estimates compared to EMA-based sampling strategy when using NCA

Discussion

We report the development and evaluation of a PopPK model for all currently available SHL rFIX products, built on data measured using the one-stage clotting assay and amassed from both industry trials and routine hemophilia care through the WAPPS-Hemo project. The structural and covariate models, and parameter estimates, are within the space of those reported in the literature [178,218–220], despite differences in both modelling objectives and approaches, and in the products included in the modelling datasets across the referenced studies. Inclusion of fat-free mass explained substantial portions of the BSV on both CL (ΔBSV_{CL} = –16.1%) and V_1 (ΔBSV_{V1} = –23.0%), and resulted in a greater reduction in OFV compared to total body weight (ΔOFV = –193 for FFM; ΔOFV = –164 for BW). The objective function value was further decreased by addition of FFM terms on Q and V_2 (ΔOFV = –121), but age was not found to be a significant covariate on CL or V_1. The ability to assess brand differences was limited. Patients on

RIXUBIS comprised only 14% of the dataset, and all RIXUBIS data was very sparsely sampled (mean number of samples per infusion: RIXUBIS – 3.0; BeneFIX – 8.4; IXINITY – 11.1). When an effect for IXINITY was included on CL and $V_{1,}$ the OFV did not substantially decrease, the magnitude of the covariate effect was small (<10% difference from the reference group), and unexplained BSV on CL and V_1 were effectively unchanged. For these reasons, no brand effect is included in the final model. This may change in future updates to the model, as the WAPPS-Hemo project continues to collect data from all three products to facilitate this comparison.

This model was built to tackle two objectives. The first is development of a model for Bayesian forecasting for use on the WAPPS-Hemo platform; this requires a model that can produce accurate estimations of all relevant PK parameters from sparsely sampled patient data. To evaluate the model in this capacity, 10-fold cross-validation and limited sampling analysis were performed, and the results demonstrate that the model is well-suited to this purpose. The second aim of the study was to determine how omission of one or two of the intended samples would affect the estimation of key PK outcomes in a clinical trial investigating the PK of IXINITY in pediatric patients. When Bayesian forecasting is used to estimate the PK parameters, one sample can be removed without compromising the accuracy of the outcomes of interest. In fact, several 3-sample designs also maintain low errors (absolute error Q95 <15%). However, these results do not hold when noncompartmental methods are used. In fact, even the intended EMA-based sampling results in considerably underestimates half-life; an additional sample at 72 or 96 h post-infusion is needed for its accurate estimation. This is not unexpected, as it has been noted that FIX requires relatively long sampling to obtain sufficient data to accurately estimate PK [221]; many studies sampling for 48 hours report a half-life of less than 20 hours, while analyses with later sampling times (72–96 hours) report longer half-life (25–35 hours) [26,38].

Despite this shortcoming, AUC, clearance, and V_{ss} were estimated quite accurately using the EMA-based sampling strategy for NCA (Table 15). This limited sampling analysis serves to highlight the robustness of the Bayesian approach, and its flexibility with respect to missing samples, in comparison to the traditionally used noncompartmental methods (Figure 21).

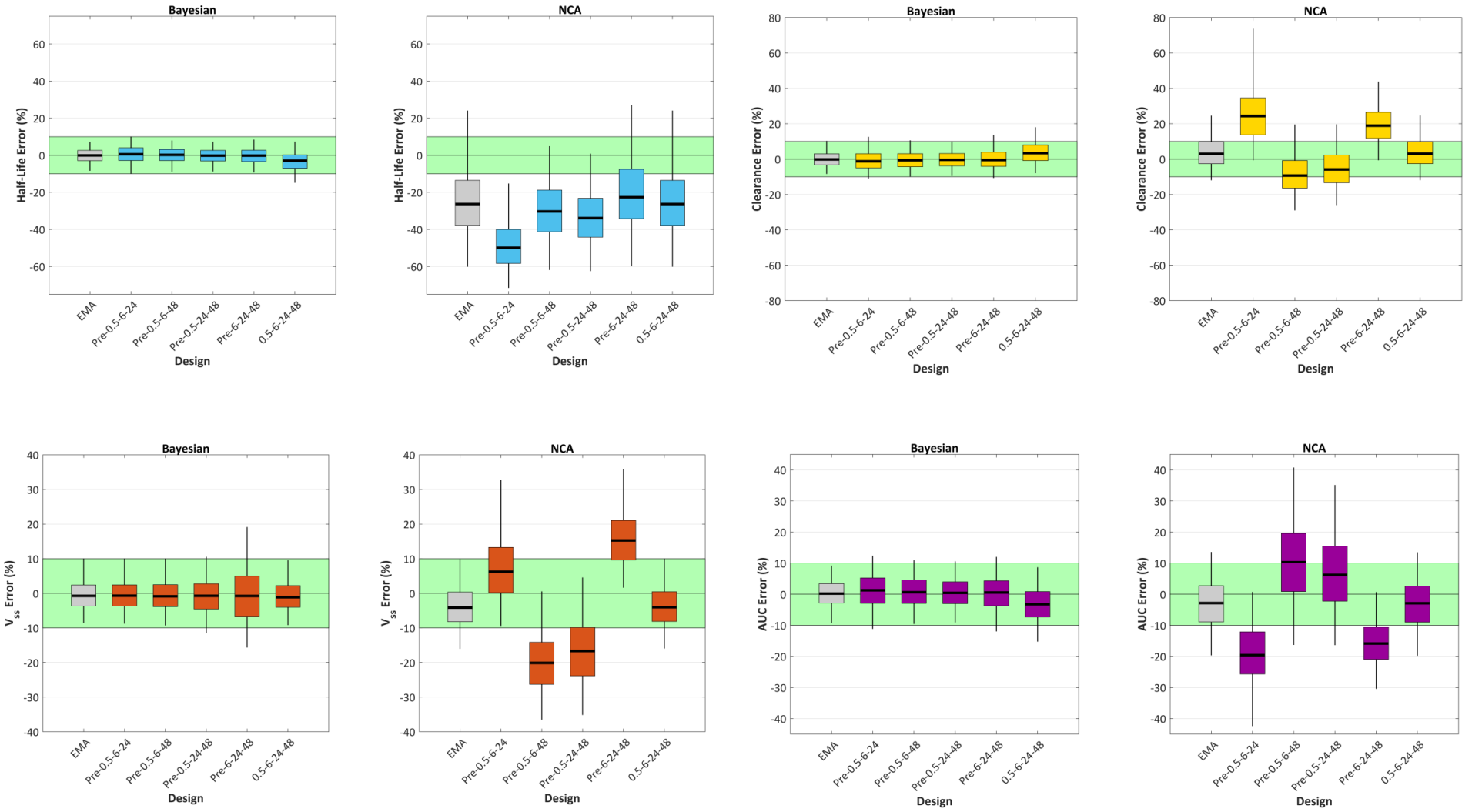


Figure 21. Comparison of errors on estimates of half-life (blue), clearance (yellow), V_{ss} (orange), and AUC (purple) from Bayesian estimation (left) and NCA (right)

Conclusions

We developed a PopPK model for rFIX and evaluated it for use in Bayesian forecasting on the WAPPS-Hemo platform. The model was built using data for three different brands of rFIX collected from both clinical trials and routine hemophilia care, and leveraged pooled pediatric PK data to address a practical problem when brand-specific pediatric data was unavailable. To this end, a limited sampling analysis was performed using the model to determine which sampling times can be omitted if pediatric sample volume limitations prevent participating centres from collecting samples at all the intended times. When Bayesian forecasting is used, accurate estimates of all PK outcomes of interest can be obtained from as few as 3 samples; if NCA is used, there is little flexibility to omit samples while maintaining the accuracy of relevant PK parameters.

Chapter 6: Limited sampling strategies for accurate determination of extended half-life factor VIII pharmacokinetics in severe hemophilia A patients

This chapter is reflective of an original manuscript prepared by the Ph.D. candidate (Alanna McEneny-King) for submission to *Haemophilia*. All pertinent dialogue in this chapter was written by the Ph.D. candidate.

Introduction

Hemophilia A is a rare and hereditary coagulation disorder characterized by a lack of functional coagulation factor VIII (FVIII). This lowered clotting ability can result in internal bleeding, often into the joints, resulting in debilitating arthropathy. In severe hemophilia A patients (i.e. those with less than 1 IU dL^{-1}, or 1% of normal FVIII activity), these bleeds may occur spontaneously. It has long been noted that maintaining FVIII activity levels above 1 IU dL^{-1}, even modestly, can greatly improve patient outcomes. Ahlberg observed that the chronic joint damage observed in severe hemophilia patients was rare among those with factor activity above 2–3 IU dL^{-1} [7]; Nilsson and colleagues later found that patients who spent more time with factor levels above 1 IU dL^{-1} had improved joint function [8]. More recently, bleeding rate has been shown to be correlated with time per week with FVIII < 1 IU dL^{-1} in both adults and children [10,222]. The concept of prophylactic factor replacement therapy is derived from these clinical observations, and is the only known method for the prevention of joint damage [9].

Although the prophylactic use of clotting factor concentrates has greatly improved clinical outcomes among hemophilia patients, long-term prophylaxis is not without challenges.

The half-life of conventional FVIII products is relatively short (approximately 12 hours [167]), necessitating frequent injections in order to maintain FVIII activity levels above 1 IU dL^{-1}. This demanding infusion schedule often results in the need for central venous lines in young children and reduced adherence among adolescents [223,224].

In an attempt to alleviate this treatment burden, a number of strategies have been employed to improve the pharmacokinetic (PK) profile of recombinant FVIII (rFVIII). One approach is the fusion of rFVIII to the Fc domain of IgG_1, taking advantage of the endogenous IgG recycling pathway [225]. The resulting product (rFVIII Fc fusion protein [rFVIIIFc], or efraloctacog alfa) demonstrates, on average, a 1.5-fold increase in half-life over standard half-life rFVIII products [108]. This more favourable PK profile allows for a dosing interval of 3–5 days, compared to the 2–3 day interval for rFVIII.

Although rFVIIIFc demonstrates an extended PK profile compared to rFVIII, it also exhibits the wide and unpredictable variability in PK response observed in its standard half-life counterparts [226]. The mean (95% confidence interval) half-life of rFVIIIFc was reported to be 18.8 (14.3–24.5) hours for adults [106], 14.9 (12.0 – 17.8) hours for children between the ages of 6 and 11 [110], and 12.7 (11.2 – 14.1) hours for children from 2 to <6 years of age [110]. As a result, dosing regimens should be tailored to the individual patient. To obtain a tailored dose using a classical PK approach, the International Society of Thrombosis and Hemostasis recommends at least 10 post-infusion samples after a washout period; this method is both burdensome and risky for the patient, and impractical for routine use. The population PK (PopPK) approach allows for the determination of individual PK parameters from fewer samples than traditional methods. The combination of a Bayesian PK approach, which estimates individual PK parameters using prior knowledge in the form of a PopPK model of historical data,

and a limited sampling strategy (LSS) has been shown to produce accurate estimations of individual activity levels for conventional FVIII [57,227,228]. Optimal sampling windows have been suggested for both conventional and EHL clotting factor concentrates; current recommendations for EHL factors suggest using the guidelines for conventional FVIII (i.e. 4–8h, 16–28 h, and 40–60 h) and adding a sample between 60 and 84 hours post-infusion [169]. However, formal investigations of LSSs for EHL FVIII products are lacking.

The aim of this study was to identify LSSs for rFVIIIFc that allow for accurate determination of relevant PK parameters. Furthermore, the predictions generated from the LSS should result in consistent clinical decisions as compared with densely sampled PK study.

Methods

Software

Factor activity profile simulation and statistical analyses were performed in Matlab (R2017b, Mathworks, Natick, MA, USA). Individual PK estimates of the simulated populations were obtained using Bayesian forecasting in NONMEM (v7.3, ICON Development Systems, Ellicott City, MD, USA).

Population pharmacokinetic model

The PopPK model used to generate estimates of individual PK parameters was developed by Nestorov et al [184] using data measured with the one-stage clotting assay. For this study, we used the parameters from the base model, as the final model included a von Willebrand factor effect on clearance and a hematocrit effect on central volume. The base model describes rFVIIIFc PK using a two-compartment structure and includes a body weight effect on central

volume. Further description of the model can be found in Table 16. This rFVIIIFc model was developed on richly sampled data from 180 previously treated severe hemophilia A patients between 12 and 65 years of age, weighing between 42.0 and 127.4 kg. The base model also included a study effect on the additive residual error component; since the population in the phase 3 study (n = 164) was considerably larger than the phase 1/2a study ($n = 16$), the additive error corresponding to the phase 3 study was used here.

Table 16. Details of the base rFVIIIFc model developed by Nestorov et al [184]

Parameter	Estimate	Covariate Effects	BSV (%)	IOV (%)
Clearance, CL (dL h^{-1})	1.72		31.1	21.9
Central volume, V_1 (dL)	36.4	$TV(V_1) \cdot \left(\frac{BW}{73}\right)^{0.498}$	13.8	10.5
Intercompartmental clearance, Q (dL h^{-1})	1.15		Corr: 0.461	Corr: 0.558
Peripheral volume, V_2 (dL)	5.79			
Additive error (IU dL^{-1})	0.264			
Proportional error (%)	14.6			

Simulated populations and activity profiles

An adult population of 1000 virtual 25-year-olds was generated, with PK parameter distributions taken from the published PopPK model described above [184]. Endogenous FVIII activity was assumed to be 0.25 IU dL^{-1} as all patients were reported to be severe and the assay's lower limit of quantification (LLOQ) was reported to be 0.5 IU dL^{-1}. For each individual, a treatment regimen of 50 IU kg^{-1} (rounded to the nearest 250 IU to account for available rFVIIIFc vial sizes) administered on Mondays and Thursdays was simulated, with infusion duration of 5

minutes. This regimen was simulated for four weeks to ensure steady state was reached, and the fourth Thursday dose or fifth Monday dose was used for analysis. Finally, the LLOQ for the simulations was defined as 1 IU dL^{-1} as this is the most common LLOQ in practice [229].

Next, adolescent (12-year-olds) and pediatric (2-year-olds) populations were generated. Body weight distributions for each age were based on the empirical distributions found in the NHANES database [215], and PK parameters were simulated using the estimates from the Nestorov model. A description of the model derivation data and the populations simulated in this study can be found in Table 17. In the younger populations, where it was common for the Thursday or Monday predose to be below the limit of quantification (BLQ), an alternative dosing schedule was used, consisting of three weeks of Monday-Thursday dosing followed by doses on Monday and Wednesday in the fourth week, with the Wednesday dose being used for analysis; in this scenario, the predose sample is now 48 hours after the previous dose rather than 72 (Thursday) or 96 hours (Monday).

Table 17. Demographic data of rFVIIIFc studies used for development of Nesterov model and of simulated populations.

	Study Populations		Simulated Populations		
	Phase 1/2a [106]	Phase 3 [108]	Adults	Young Adolescents	Children
n	16	164	1000	1000	1000
Weight (kg)	82.7 (54–111)	71.60 (42.0–127.4)	86.9 (63.6–127.9)	47.2 (33.4–79.6)	14.0 (11.5–17.5)
Age (years)	34.6 (23–61)	30 (12–65)	25	12	2
Doses (IU kg^{-1})	25 and 65	25, 30, 35, 40, 45, 50, 60, 65	50		
Number of Samples per Patient	13–16	5–8	6		
Sampling Duration (h)	168–240	96–120	96		

Phase 1/2a study data presented as mean (range). Phase 3 study data presented as median (range). Simulated population data presented as median (range).
Age was not explicitly simulated as it is not a covariate in the model.

Design of limited sampling strategies

We began with a rich sampling scheme that included a predose measurement (-0.5 h) and samples at 1, 24, 48, 72, and 96 hours post-infusion. Limited sampling schedules were created by systematically excluding sample points from this rich sampling scheme. Overall, twenty-one sampling schemes were created. Each scheme had between 1 and 5 samples. The details of the sampling schemes are described in Table 18.

In addition to the timing of post-infusion samples, we also investigated the importance of handling a predose measurement appropriately. We compared two methods of predose handling: in Method A, no knowledge of prior doses was used for the estimation; in Method B, information about the date and dose of the previous infusion was incorporated. Additionally, we looked at the timing of this predose sample relative to the prior dose. For example, if the patient is on a Monday-Thursday dosing schedule, a PK study performed on Monday will have a predose

sampled at 96 hours after the last dose while a PK study performed on Thursday will have a predose sampled at 72 hours after the last dose. For the two younger patient groups, the Wednesday PK study described above was also simulated. With all combinations of predose handling and study day, a total of 126 sampling strategies were explored. Bayesian estimation was performed in NONMEM to obtain individual PK estimates from each sampling strategy, for each population.

Table 18. Details and nomenclature of tested limited sampling strategies. All doses were 50 IU kg^{-1} (rounded to the nearest 250 IU vial) unless otherwise indicated

Timing of Samples[a] (h)	Handling of Predose[b]					
	Predose Method A			Predose Method B		
	Study Day [c]					
	Thursday (72 h)	Monday[d] (96 h)	Wednesday (48 h)	Thursday (72 h)	Monday[d] (96 h)	Wednesday (48 h)
-0.5, 1, 24, 48, 72, 96	AT1		AW1	BT1		BW1
-0.5, 1, 24, 48, 72	AT2	AM2	AW2	BT2	BM2	BW2
-0.5, 1, 24, 48, 96	AT3		AW3	BT3		BW3
-0.5, 1, 24, 72, 96	AT4		AW4	BT4		BW4
-0.5, 1, 48, 72, 96	AT5		AW5	BT5		BW5
-0.5, 1, 72, 96	AT6		AW6	BT6		BW6
-0.5, 1, 48, 96	AT7		AW7	BT7		BW7
-0.5, 1, 24, 96	AT8		AW8	BT8		BW8
-0.5, 1, 48, 72	AT9	AM9	AW9	BT9	BM9	BW9
-0.5, 1, 24, 72	AT10	AM10	AW10	BT10	BM10	BW10
-0.5, 1, 24, 48	AT11	AM11	AW11	BT11	BM11	BW11
-0.5, 1, 24	AT12	AM12	AW12	BT12	BM12	BW12
-0.5, 1, 48	AT13	AM13	AW13	BT13	BM13	BW13
-0.5, 1, 72	AT14	AM14	AW14	BT14	BM14	BW14
-0.5, 1, 96	AT15		AW15	BT15		BW15
-0.5, 1	AT16	AM16	AW16	BT16	BM16	BW16
1, 24	AT17	AM17	AW17	BT17	BM17	BW17
1, 48	AT18	AM18	AW18	BT18	BM18	BW18
1, 72	AT19	AM19	AW19	BT19	BM19	BW19
1, 96	AT20		AW20	BT20		BW20
1	AT21	AM21	AW21	BT21	BM21	BW21

[a] -0.5 h indicates a predose sample. [b] 'Handling of Predose' refers to whether information about the previous dose was incorporated into the estimation. [c] 'Study Day' refers to the day the dose is given for the PK study; the length of time since the last dose is indicated in parentheses. [d] The reference sampling scheme for the AM and BM series does not contain the 96 h sample, since the next dose would be given at 72 h post-infusion in this scenario. Consequently, LSSs with the 96 h sample were not considered for this subset.

Evaluation of limited sampling strategies

Each of the limited sampling strategies was evaluated by assessing the error and absolute error (AE) of the estimated PK parameters (clearance, central volume, half-life) and predicted activity at 72 and 96 hours post-infusion (C_{72} and C_{96} – troughs corresponding to a Monday-Thursday dosing schedule), as defined in the equations below:

$$Error_i = \frac{\varphi_{i,LSS} - \varphi_{i,full}}{\varphi_{i,full}} \times 100\%$$

$$Absolute\ Error_i = |Error_i|$$

where $\varphi_{i,full}$ and $\varphi_{i,LSS}$ represent the estimates of PK parameter φ estimated by the rich and limited sampling strategies, respectively, for individual i.

Trough levels at 72 and 96 hours guide clinical decision-making and drive dose adjustments, so accurate prediction of FVIII activity at these times is critical. In this study, we aimed to keep FVIII activity above 1 IU dL^{-1} at all times. If the predicted troughs (i.e. C_{72} and C_{96}) were both above this value, the regimen was deemed appropriate; otherwise, the regimen required a dose adjustment. If the regimen decision was inconsistent between an LSS and the rich sampling strategy (i.e. if a trough predicted by an LSS was greater than 1 IU dL^{-1} but less than 1 IU dL^{-1} for the rich sampling strategy - or vice versa), it would result in an inappropriate dosing adjustment. This inappropriate dosing adjustment rate ($IDAR_{72}$, $IDAR_{96}$) was also used to evaluate the different strategies.

Results

A complete listing of the AE on half-life and trough levels, as well as the IDARs, for all sampling strategies in all populations can be found in Supplementary Table S7, Table S8,

Table S9 and Table S10. A summary of the simulated PK outcomes for each age group (including a comparison to previously reported values for half-life) is given in Table 19.

Table 19. Pharmacokinetic outcomes for simulated populations from the rich sampling design. Results are presented as mean (standard deviation). Age is presented as median (range), except in the adult case where the median for the PK subgroup was unknown. Simulated values of PK parameters are presented as mean (SD); reported values of half-life are presented as mean (95% CI).

	Adults		**Adolescents**		**Children**	
	Simulated	Reported	Simulated	Reported	Simulated	Reported
Age	20	(12–65)	12	8.0 (6 – 11)	2	4.0 (1 – 5)
PK Outcome						
Half-Life (h)	18.3 (4.8)	19.0 (17.0–21.1) [108]	15.2 (4.0)	14.88 (11.98–17.77) [110]	10.0 (2.3)	12.67 (11.23–14.11) [110]
C_{72} (IU dL^{-1})	6.7 (5.1)		3.1 (2.7)		0.5 (0.3)	
C_{96} (IU dL^{-1})	3.2 (3.0)		1.4 (1.4)		0.3 (0.1)	
TAT1% (h)	125.4 (33.3)		97.1 (27.8)		48.6 (14.2)	

Adult Population

A summary of the results for the 2- and 3-sample schemes is shown in Table 20. If we first consider the AT series of sampling designs (i.e. those using Method A for predose handling, with the study taking place on Thursday), most strategies – including several 2-sample designs – perform well, with mean AE below 7% and 17% for PK parameters and trough levels, respectively (Figure 22). The IDARs were generally low (<8%) for most sampling schemes. Of particular note, designs AT9 (Pre-1-48-72h) and AT14 (Pre-1-72h) were the best 4- and 3-sample designs, respectively; AT19 (1-72h) produced similar results to 3- and 4-sample designs

and performed the best of the 2-sample designs, emphasizing the importance of the 72-hour sample in this population. Additionally, central volume was well-estimated (mean AE <5%) for all designs as all sampling strategies included a peak level; future references to PK parameters refer to half-life and clearance only.

The exceptions to this largely good performance are designs AT16 (predose and peak) and AT21 (peak only). These designs performed equally poorly, with mean [95th percentile] AE greater than 20% [52%] on PK parameters and greater than 75% [290%] on trough levels. The IDAR, while still low at 72 hours, increased to more than 20% at 96 hours. Furthermore, all cases of inappropriate dose adjustment overpredicted the trough level, resulting in a failure to increase dose and time spent below 1 IU dL^{-1}. Strategies where the latest sample was taken at 24 hours post-infusion (AT12 and AT17) also performed poorer than most (mean AE ≈10% for PK parameters, ≈25% for trough levels; $IDAR_{96}$ = 8%). Finally, strategies AT15 (Pre-1-96 h) and AT20 (1-96 h) performed well for PK parameter estimation but had slightly elevated $IDAR_{96}$ (7.5% for both).

Table 20. Comparison of predose methods A and B for 2- and 3-sample designs following Thursday sampling in adults. Absolute errors are presented as mean [95th percentile].

Method A	PK Parameters			Trough Levels		IDAR	
Design	Half-Life	Clearance	Central Volume	C_{72}	C_{96}	72 h	96 h
AT6	1.3 [3.7]	3.6 [9.4]	3.2 [7.9]	5.3 [15.3]	5.2 [14.9]	0.9%	0.9%
AT7	2.2 [6.0]	3.3 [8.6]	2.7 [6.4]	6.2 [17.2]	7.1 [18.1]	0.2%	2.9%
AT8	3.1 [9.0]	3.7 [10.2]	1.3 [3.2]	8.8 [25.9]	10.0 [28.1]	0.5%	3.4%
AT9	2.7 [7.3]	3.0 [8.0]	2.9 [7.1]	6.2 [15.1]	7.6 [19.3]	0.0%	1.0%
AT10	3.5 [9.5]	3.4 [9.2]	1.6 [4.2]	8.4 [20.8]	10.1 [24.9]	0.6%	1.6%
AT11	5.8 [15.0]	4.4 [13.0]	2.1 [5.2]	12.6 [30.5]	15.8 [38.6]	0.1%	2.9%
AT12	10.9 [26.8]	10.9 [31.5]	2.3 [5.7]	26.3 [64.2]	32.0 [77.3]	0.7%	6.6%
AT13	6.1 [15.8]	6.1 [17.1]	3.0 [7.3]	14.2 [35.0]	17.3 [42.2]	0.2%	4.1%
AT14	3.7 [10.3]	4.9 [13.1]	3.2 [8.0]	9.8 [24.1]	11.2 [28.2]	0.8%	1.6%
AT15	3.8 [11.4]	5.6 [15.7]	3.3 [8.2]	12.8 [38.4]	13.1 [40.5]	1.5%	4.4%
AT16	21.1 [52.6]	23.3 [60.7]	3.4 [8.8]	73.9 [279.6]	88.3 [339.2]	1.5%	13.1%
AT17	10.7 [25.8]	9.9 [22.9]	4.7 [12.2]	26.2 [66.7]	32.6 [85.9]	0.5%	6.5%
AT18	6.2 [15.8]	6.8 [16.6]	4.7 [12.0]	14.0 [35.3]	17.3 [43.5]	0.2%	4.0%
AT19	4.4 [11.9]	6.6 [16.2]	4.7 [11.7]	9.9 [24.1]	11.3 [27.6]	0.8%	1.7%
AT20	4.3 [11.4]	7.0 [17.1]	4.6 [11.3]	13.0 [38.3]	13.1 [40.6]	1.5%	4.4%
Method B	**PK Parameters**			**Trough Levels**		**IDAR**	
Design	Half-Life	Clearance	Central Volume	C_{72}	C_{96}	72 h	96 h
BT6	0.9 [2.4]	2.9 [6.8]	3.0 [7.0]	3.8 [10.2]	3.6 [10.0]	0.7%	0.8%
BT7	1.5 [3.9]	2.4 [6.0]	2.6 [6.4]	4.3 [10.8]	4.8 [11.8]	0.2%	1.5%
BT8	1.9 [5.0]	2.3 [5.8]	1.1 [2.7]	5.5 [15.2]	6.2 [16.4]	0.2%	1.5%
BT9	2.0 [5.3]	2.2 [5.5]	2.9 [7.1]	4.4 [10.2]	5.4 [13.1]	0.2%	0.6%
BT10	2.4 [6.3]	2.1 [5.2]	1.6 [4.3]	5.8 [14.3]	7.0 [16.9]	0.6%	1.4%
BT11	3.2 [8.6]	1.8 [5.0]	1.9 [5.2]	7.1 [18.0]	8.9 [23.5]	0.1%	2.3%
BT12	4.2 [10.7]	3.4 [8.6]	2.0 [5.2]	10.1 [25.4]	12.2 [30.2]	0.3%	2.9%
BT13	3.3 [8.8]	3.0 [7.7]	3.0 [7.3]	7.7 [19.3]	9.4 [23.5]	0.2%	2.5%
BT14	2.5 [6.6]	3.3 [7.9]	3.1 [7.7]	6.6 [15.9]	7.5 [18.4]	0.7%	1.2%
BT15	2.0 [5.7]	3.6 [8.7]	3.0 [7.2]	6.7 [19.1]	7.0 [19.2]	0.8%	2.6%
BT16	4.4 [11.8]	4.8 [12.2]	3.1 [7.8]	11.6 [29.5]	13.4 [33.4]	0.9%	3.7%
BT17	10.1 [23.9]	9.2 [25.6]	2.8 [6.7]	25.3 [60.7]	30.4 [72.2]	0.8%	6.3%
BT18	6.0 [15.0]	5.7 [15.9]	3.1 [7.6]	14.5 [36.1]	17.4 [42.7]	0.1%	4.1%
BT19	3.8 [10.1]	4.8 [13.1]	3.1 [8.0]	10.4 [26.8]	11.8 [29.3]	0.9%	2.2%
BT20	4.1 [12.1]	5.7 [16.4]	3.1 [7.9]	13.8 [40.9]	14.4 [41.9]	1.6^	4.5%

Results were fairly similar using both methods of predose handling for most designs (Table 20), except for the case of the predose-peak sampling strategy (AT16 vs. BT16). Briefly, mean AE on half-life and clearance decreases from greater than 20% to below 5%, mean [95th percentile] AEs on trough levels decrease from over 75% [≈300%] to less than 13% [≈30%]. $IDAR_{96}$ also drops from over 20% using Method A to 3.3% for Method B (Figure 2). Though the

difference was less dramatic, strategies using Method B and lacking a 48- or 72-hour sample (i.e. BT8, BT12, and BT15) also had lower mean AE than their respective AT counterparts.

Due to the higher percentage of patients with BLQ factor levels at 96 hours compared to 72 hours (21.2% vs. 2.6%; $p < 0.0001$), we hypothesized that the sampling day would be influential when Method B was used to handle predose. For most sampling schemes, the AEs on PK parameters and troughs were quite similar for both sampling days. However, major differences in these outcomes were observed for two designs: BM15 (predose, 1 h, 96 h) and BM16 (predose, 1 h); in these cases, errors were considerably higher when sampling on Monday as compared to Thursday (Figure 24). Furthermore, there were five designs that resulted in at least one IDAR that was significantly higher when sampling was done following a Monday dose rather than a Thursday dose: BM6 ($IDAR_{72}$ 1.6% vs. 0.5%, $p = 0.016$), BM11 ($IDAR_{96}$ 3.1% vs. 1.7%, $p = 0.041$), BM14 ($IDAR_{72}$ 1.6% vs. 0.6%, $p = 0.032$), BM15 ($IDAR_{72}$ 2.8% vs. 1.1%, $p = 0.006$), and BM16 ($IDAR_{72}$ 2.8% vs. 1.1%, $p = 0.006$; $IDAR_{96}$ 5.7% vs. 3.3%, $p = 0.001$).

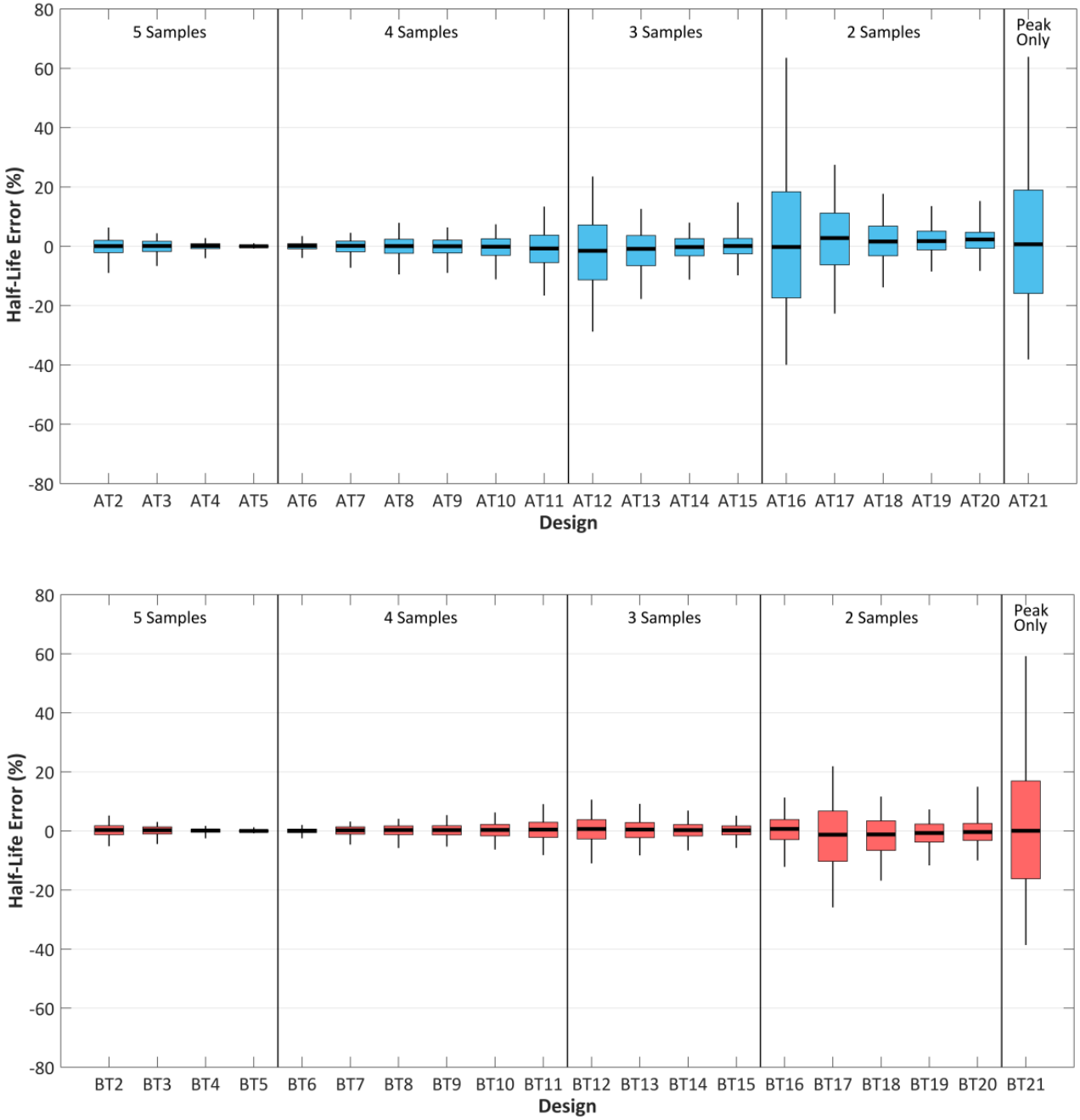


Figure 22. Boxplot of relative error on half-life estimates from limited sampling strategies using (top) Method A and (bottom) Method B for predose handling in a simulated adult population. Error is calculated relative to design AT1 and BT1, respectively.

Pediatric and Adolescent Populations

Next, we explored whether different LSSs are required when dealing with a younger population. When comparing the AT-series designs for the 2-year-old group, a number of designs still perform quite well; AT10 (Pre-1-24-72), AT12 (Pre-1-24), and AT17 (1-24) were the best 4-, 3- and 2- sample designs, with mean AEs below 10% on all PK parameters and troughs, and IDARs below 3%. In fact, $IDAR_{96}$ is below 1% for all designs in this series (

Table S9). However, this is due to the fact that most simulated 2-year-olds reach a factor activity level of 1 IU dL^{-1} well before 96 hours (mean ± SD time to 1 IU dL^{-1}: 49 ± 14 h) while around 80% of adults are still above this threshold at 96 hours (mean ± SD time to 1 IU dL^{-1}: 125 ± 33 h). Designs without a sample at 24 or 48 hours (i.e. AT6, AT14–AT16, and AT19–AT21) result in estimates with considerably higher mean [95^{th} percentile] AEs for both PK parameters (≈15% [>38%]) and 72-hour trough level (≈25% [>65%]).

The incorporation of predose data did not result in the same degree of improvement in the pediatric population as in the adults. Means and 95^{th} percentiles of AE were improved for the same sampling designs (i.e. BT8, BT12, BT15, and BT16), but with less dramatic results (Figure 23). Taking the BT16-series designs as an example, mean AE was reduced from 21% to 4% for half-life and from 87% to 13% for C_{96} in adults when prior dose knowledge was included; the same change in simulated 2-year-olds reduced half-life mean AE from 17% to 14% and C_{96} mean AE from 42% to 30%. We attributed this reduced impact to the higher proportion of BLQs at 72 hours (>90%) compared to adults (2.1%).

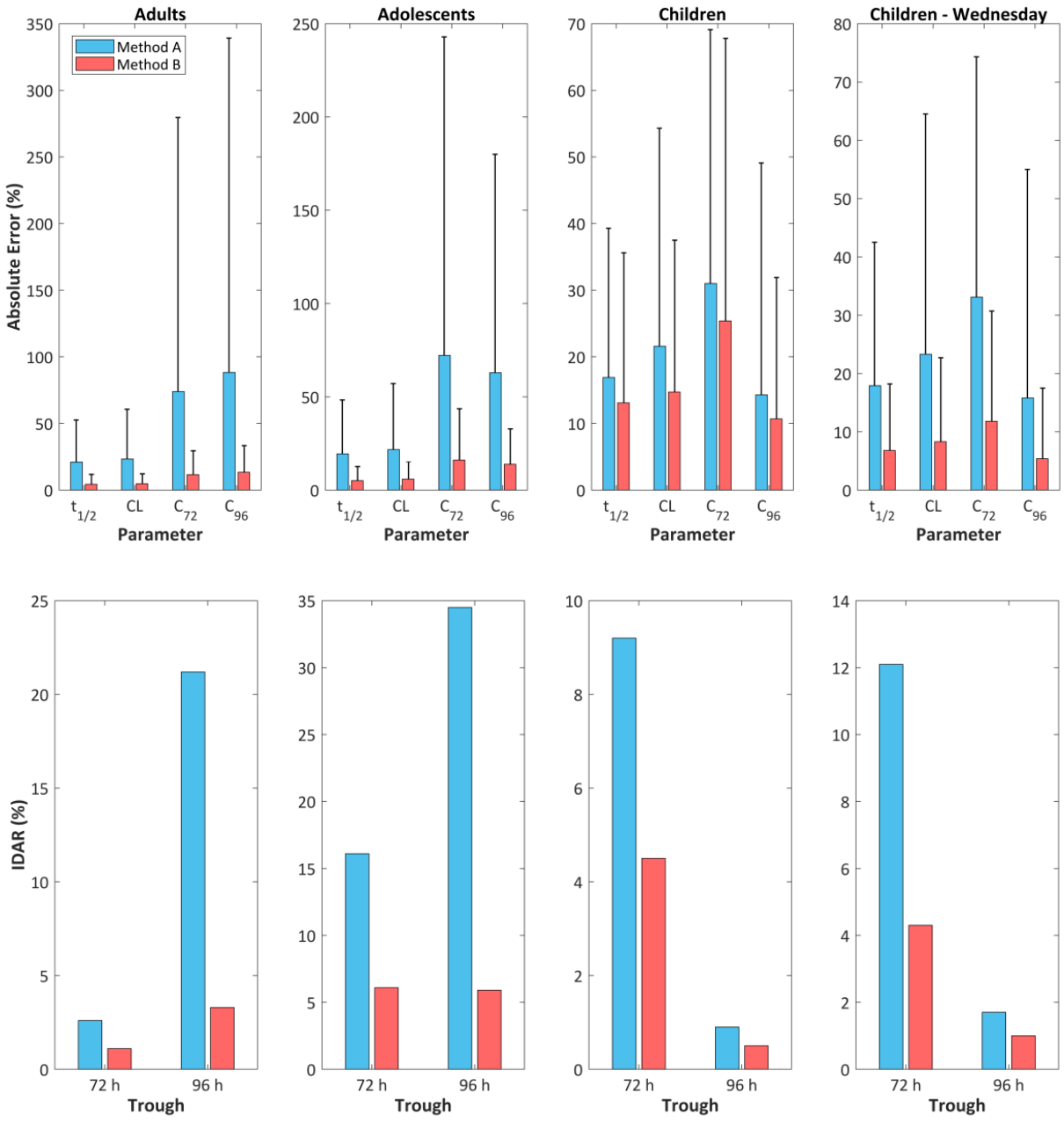


Figure 23. Effect of predose handling on estimates of PK parameters and IDAR for adults, adolescents, and children for the predose-peak sampling strategy

Due to an even higher proportion of BLQs than expected, additional simulations were performed for the pediatric population – one using a higher dose (80 IU kg^{-1}, as recommended in product guidelines) with the same sampling strategies, and another using the 50 IU kg^{-1} dose but collecting a predose sample that is 48 hours after the last dose rather than 72 or 96 hours (equivalent to performing the study on Wednesday for a Monday-Thursday regimen). Increasing the dose to 80 IU kg^{-1} did not result in any reduction in error on PK estimates, likely because this dose adjustment did not significantly reduce the proportion of patients who were BLQ at 72 (83.8%) or 96 (97.5%) hours. However, taking the predose sample at 48 hours (when less than half the population is BLQ) improved the error on half-life estimate considerably (Figure 23, Figure 24). For the predose-peak sampling strategy, mean [95^{th} percentile] AE on half-life decreases from 14% [38%] when sampled after the Thursday dose to 7% [20%] when sampled after the Wednesday dose. Estimates of trough levels are similarly improved at both 72 hours (12% [29%] vs. 29% [70%]) and 96 hours (5% [18%] vs. 14% [51%]). However, the IDARs obtained from Wednesday sampling are slightly higher than those from Thursday sampling ($IDAR_{72}$: 3.3% vs. 2.4%, $p = 0.23$; $IDAR_{96}$: 1.1% vs. 0.3%, $p = 0.032$).

Since there were striking differences between the adult and pediatric populations, we decided to investigate an intermediate population of adolescents. Similar to the adults, most sampling designs in the AT series estimate the PK parameters and troughs well (mean AE <10% for PK parameters and <15% for trough levels, Table S8), with strategies containing a 48- or 72-hour sample (e.g. AT13 and AT14 for the 3-sample case) outperforming those relying on a 24- or 96-hour sample (e.g. AT12 and AT15). As with both the adult and 2-year-old populations, AT16 and AT21 showed much worse performance than other strategies, with mean AE ≈20% on half-life and clearance and >60% for trough levels. While these errors were comparable to the adult

counterparts, the IDAR at both 72 and 96 hours was much higher for the adolescent group (16.1% and 34.5%, respectively) than for adults (1.5% and 13.1%; Figure 2, Figure 3). This can be attributed to the fact that the mean time to 1 IU dL^{-1} for the simulated 12-year-olds was 102 h (compared to 138 h for adults), so many patients will be very close to the target trough at 96 h post-infusion.

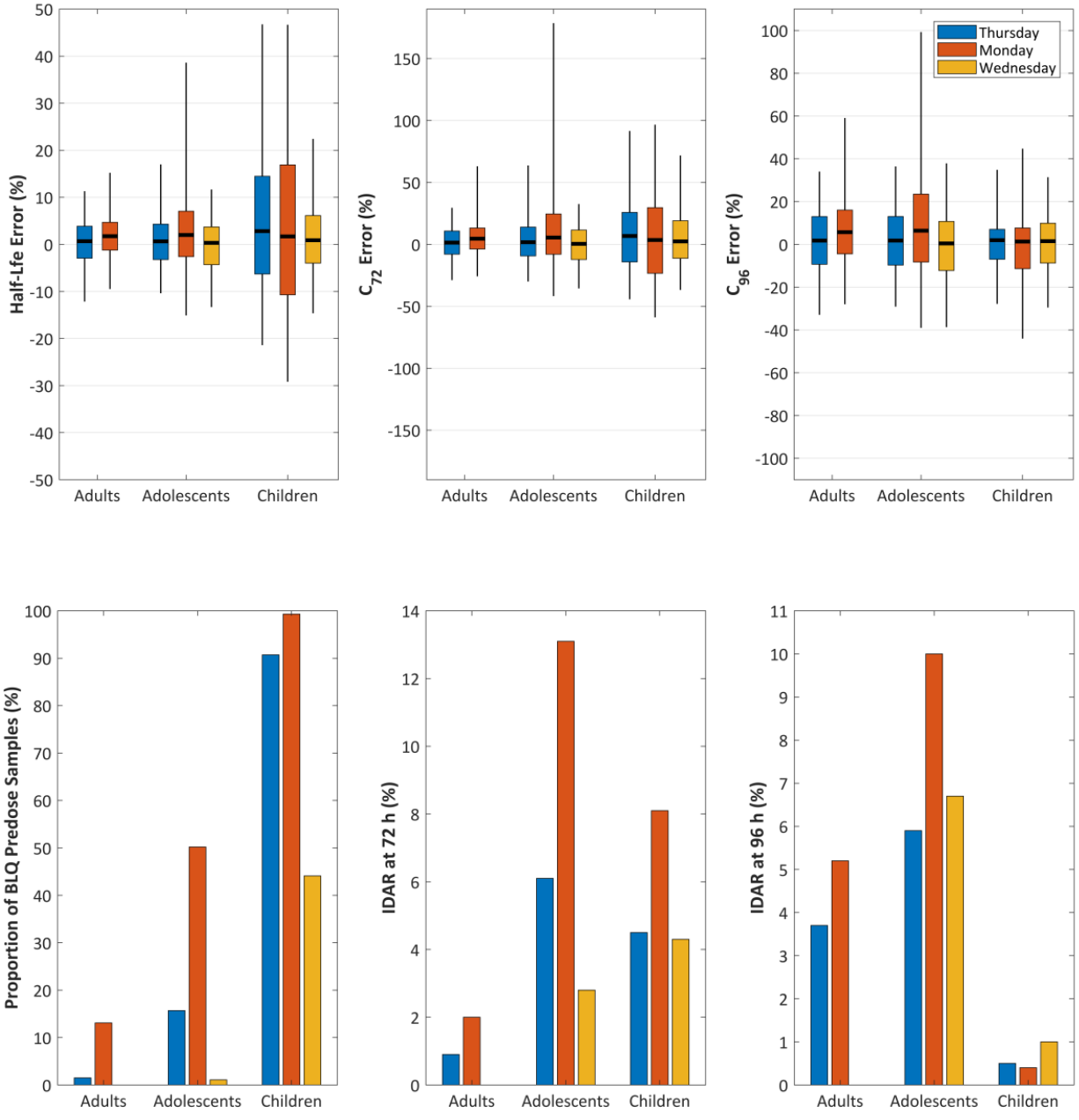


Figure 24. Influence of study day on PK parameters and IDAR for different simulated age groups. Predose is handled using Method B.

When predose was handled using Method B, the adolescent group was similar to the adults and saw dramatic improvement in error on PK parameters and in IDAR (Figure 23). Compared to the 2-year-old group, the proportion of adolescent patients who are BLQ at 72 hours is fairly low (≈15%). Consequently, performing the study on Wednesday or Thursday generally produces similar results (Figure 3). However, the $IDAR_{72}$ was significantly reduced when the PK study was performed on Wednesday for the eight sampling designs that include a predose sample but not a 48-hour point (i.e. BW4, BW6, BW8, BW10, BW12, BW14, BW15 and BW16) as compared to Thursday.

Discussion

The results of this study suggest that the pharmacokinetics of rFVIIIFc can be accurately estimated from as few as two samples using Bayesian forecasting. Of the strategies containing two post-infusion samples, those with a sample at 48 or 72 hours perform better than those with 24- or 96-hour samples in adult and adolescent populations. For these populations, a 24-hour point does not provide enough information about the late stages of the profile, resulting in greater error on clearance and slightly higher (albeit still quite low) IDARs. Conversely, choosing a point that is very late in the profile (i.e. 96 hours) increases the likelihood that the sample will be BLQ; while this has less impact on the estimation of clearance, it results in increased IDARs. However, the best strategies for young children were those containing the 24-hour sample as patients of this age reach FVIII levels that are BLQ much earlier.

We also sought to determine whether samples from a single clinic visit (i.e. 16-series designs, consisting of predose and peak measurements) can be used to accurately determine the

PK of rFVIIIFc, as this sampling strategy is not only more convenient for patients, but also reduces clinic resources compared to sampling at multiple visits over several days. The results of this sampling strategy are highly variable, and depend on two factors: (i) how the predose sample is handled, and (ii) the day in the regimen on which the PK study takes place. If the time and dose of the previous infusion are not taken into account (Method A), errors on half-life, clearance, and trough levels are high, as is the risk of overpredicting the 96-hour trough level and thereby failing to adjust the regimen. However, if prior dose information is available (Method B), errors on these parameters are greatly reduced and troughs are more accurately predicted, resulting in fewer instances of inappropriate dose adjustment. Handling predose in this way allows for the assessment of PK on two infusions per patient; when the time and amount of the previous dose are provided, the predose level serves as a trough for the prior dose, giving an additional late observation to assist with estimation. The benefit of handling predose in this manner was observed for all three of the investigated ages. Conversely, the ideal sampling day is more a function of half-life and thus depends on age, as children are known to have shorter rFVIIIFc half-lives than adults [230]. Since BLQ predose samples are less informative and all ages have a significant proportion of BLQs at 96 hours, performing the PK study around the Monday dose of a Monday-Thursday regimen is not ideal; it results in greater errors on all outcomes of interest and higher IDARs in almost all cases. Thursday sampling produces favourable results in the adult population, while Wednesday sampling is required to reduce the risk of a BLQ predose sample in the 2-year-old group. The results for the adolescent population are similar for both Wednesday and Thursday sampling, so it is likely not worth disrupting the patient's regimen to sample on Wednesday.

A major limitation of this exercise is the lack of a rFVIIIFc model that is validated for pediatric populations. As seen in Table 17, the youngest patients in the derivation dataset are 12 years old and models of this type are not usually suitable for extrapolation. However, half-life estimates obtained from the Nestorov model for the pediatric population (mean [95% CI] 10.0 [6.6 – 15.5] hours) are reasonable when compared with observed values for similarly aged children (Table 19).

Conclusions

In summary, we performed a limited sampling analysis using a published model for rFVIIIFc and found that PK parameters and key trough levels could be accurately predicted from as few as two sample points. For accurate estimation of all relevant PK parameters and trough levels, inclusion of a 72-hour, 48-hour, or 24-hour point is recommended for adult, adolescent, and pediatric patients, respectively. When trying to make the most of samples collected from a single clinic visit (i.e. predose and peak measurements), treatment providers should be conscious of the benefit of including knowledge of the timing and amount of the patient's previous dose, and of the importance of scheduling the PK study such that the most informative predose sample is collected.

Chapter 7: Clinical application of Web Accessible Population Pharmacokinetic Service – Hemophilia (WAPPS-Hemo): Patterns of blood sampling and patient characteristics among clinician users

This chapter is reflective of an original manuscript published by the Ph.D. candidate (Alanna McEneny-King) in *Haemophilia*. All pertinent dialogue in this chapter was written by the Ph.D. candidate.

McEneny-King A, Yeung CHT, Edginton AN, Iorio I, and Croteau SE. Clinical application of Web Accessible Population Pharmacokinetic Service – Hemophilia (WAPPS-Hemo): Patterns of blood sampling and patient characteristics among clinician users. Haemophilia. 2020; 26(1):56-63. DOI: 10.1111/hae.13882.

Introduction

While the success of prophylactic factor replacement for hemophilia A and B has been well established [9,166,231], the nuances of 'optimal' prophylaxis taking into account a patient's historical bleeding phenotype, target joints, type and intensity of physical activity, venous access, and the availability of factor concentrate are less well-defined. The significant annual cost of hemophilia therapy coupled with the expanding availability of newly designed factor concentrates [232] has created an opportunity for pharmacokinetic (PK) data to contribute to prophylaxis regimen decision-making. The addition of PK-tailored dosing may facilitate a provider's ability to individualize hemophilia prophylaxis [233–237], maximizing the musculoskeletal health of patients by increasing trough levels while minimizing factor concentrate consumption [169,234,238,239]. The well-described broad inter-patient variability in

the PK of both factor VIII (FVIII) and factor IX (FIX) clotting factor concentrates (CFCs) [167,168], suggests that empiric dosing regimens based on adult mean half-life and adjusted based on bleeding pattern or measured trough levels may be improved upon [181,240,241]. Within the hemophilia A population, an association has been demonstrated between an increased risk of musculoskeletal bleed events and duration of time (hours per week) an individual spends with FVIII activity levels below 1 IU/dL [10].

Implementation of population pharmacokinetics (PopPK) for hemophilia A and B has facilitated a practical approach to individualizing prophylactic factor replacement regimens informed by a patient's PK profile. Use of PopPK eliminates the need for a washout period and dense blood sampling following CFC infusion when performing PK analysis [181,242,243]. Historically, incorporation of PK into routine clinical practice has been impeded by the rigorous sampling required for classical PK methodology [244,245], limited access to provider-friendly tools for PK analysis, and provider and patient uncertainty about the magnitude of improvement to be gained by adding PK information to decision-making. Increasingly, PopPK models and interfaces have become available to clinicians. The required timing for blood samples, CFC-specific modelling, and regional availability of resources have impacted specific tool utilization by providers [170,234]. The Web Accessible Population Pharmacokinetic Service – Hemophilia (WAPPS-Hemo) is a globally available service with specific models for most commercially available FVIII and FIX CFCs. The WAPPS-Hemo network currently consists of over 400 centres, and the service has received over 1,200 infusions in the first half of 2019. Clinicians are able to submit de-identified patient covariates, factor infusion details, and post-infusion factor activity levels, and then receive a validated, individual PK profile report for their patient. The FVIII and FIX subcommittee of the International Society on Thrombosis and Haemostasis

(ISTH) recently published guidance on the use of PopPK in hemophilia management, suggesting post-infusion blood sampling windows for both standard half-life (SHL) and extended half-life (EHL) FVIII and FIX CFCs [169,181,246].

Success of PK-guided prophylaxis with respect to the potential for increased trough factor levels and reduction in factor concentrate utilization has been modeled and also demonstrated in small cohorts of primarily hemophilia A patients [234,237–239]. While the implementation of PK-tailored prophylaxis is increasingly featured at national and international congresses in both educational sessions and the scientific programs, utilization of PopPK profiles in clinical practice remains vague [245]. This retrospective study investigated the evolution of clinician use of WAPPS-Hemo for individual PK profile estimation by specifically examining the changes in laboratory data and patient characteristics submitted by all providers within the global WAPPS-Hemo Network. Secondarily, we assessed whether there was a difference in sampling strategies and patient characteristics for requested PK profiles between high-use centres (HUCs) and non-high use centres (non-HUCs).

Methods

Infusion data for pediatric and adult patients with hemophilia A or B, of all severities, submitted to WAPPS-Hemo from participating sites were extracted during two time periods: October 1, 2015 through September 30, 2016 (Period 1, early availability) and October 1, 2017 through September 30, 2018 (Period 2, recent use). Infusions were excluded if they were identified to be duplicates (based on timestamps) or were for a patient marked as positive for history of inhibitors as this may have altered the provider's decision-making for timing of post-

infusion blood sampling. Furthermore, we excluded infusions that appeared to contain a single confirmatory point, as these samples were collected with the intention of confirming a current prophylaxis regimen rather than for PK parameter estimation. Confirmatory points were defined as infusions that contained only one sampling point and had a more detailed PK profile for the same product within six months prior. The final dataset included de-identified patient characteristics (age, weight) as well as infusion details (factor product, assay method, infusion time, and FVIII or FIX activity levels with post-infusion timestamp). Details of whether data submitted was at steady state or following a single dose were not collected in WAPPS-Hemo during the timeframes investigated. CFCs were dichotomized into SHL or EHL product groups for both FVIII and FIX CFCs. EHL CFCs were defined as those with a moiety added (Fc-fusion, pegylation, or albumin) with the intention of reducing exogenous factor clearance.

Statistical analysis for continuous variables was performed using a T-test to describe differences between Period 1 and Period 2. Pearson's chi-square tests were employed for analysis of categorical covariates. Graphical representations of covariate distributions and plots of sampling times were created in MatLab (R2017b). Timing of blood samples was evaluated using the windows suggested in the ISTH PopPK guidance [169].

We analyzed the association between centre submission volume for PopPK analysis and the characteristics of patients and the number and timing of post-infusion blood samples. A de-identified list of centres participating in the WAPPS-Hemo Network and the cumulative number of infusions submitted since their initial participation were used to categorize centres according to the number of PK profiles requested. Only centres who had submitted at least one infusion were included in the analysis. HUCs were defined as the top 25^{th} percentile of unique patients

submitted at the time of analysis. T-tests compared HUCs versus non-HUCs with respect to patient and infusion characteristics during Period 2.

Results

Of the 1,931 eligible infusions entered into WAPPS-Hemo during the timeframes investigated, 468 were entered during Period 1 and 1,463 were entered during Period 2 (Table 21). There was a greater than 3-fold increase in the number of infusions entered during Period 2, driven by FVIII CFCs, particularly EHL FVIII. One-stage clotting assays for quantification of factor activity levels dominated during both periods. Infused doses (IU/kg) increased between Periods 1 and 2 (Figure 25) for both SHL FVIII ($p = 0.0074$) and EHL FIX CFC ($p = 0.021$). While PopPK was predominantly used in the adult population, patients entered were overall younger during Period 2 (Figure 26).

Table 21. Categorical covariate summary for final WAPPS-Hemo dataset for Period 1 and 2

COVARIATE	PERIOD 1	PERIOD 2	p-VALUE
Number of infusions	468	1,463	
Number of patients	413	1,286	
Number of centres	65	158	
Age (years), Mean (SD)	28.1 (17.8)	23.7 (17.0)	$p < 0.001$
Median [Range]	26 [0.5 – 77]	18 [0.5 – 81]	
Body weight (kg), Mean (SD)	64.6 (26.0)	61.8 (28.2)	$p = 0.042$
Median [Range]	68.3 [8.8 – 204]	65 [6.9 – 179]	
Hemophilia Type			
Hemophilia A	405 (86.5%)	1,248 (85.3%)	$p = 0.508$
Hemophilia B	63 (13.5%)	215 (14.7%)	
Product Type			
SHL FVIII	336 (71.8%)	826 (56.5%)	$p < 0.001$
EHL FVIII	69 (14.7%)	422 (28.8%)	$p < 0.001$
SHL FIX	16 (3.4%)	79 (5.4%)	$p = 0.085$
EHL FIX	47 (10.0%)	136 (9.3%)	$p = 0.631$
Assay Type			
One-Stage	406 (86.8%)	1,326 (90.6%)	
Chromogenic	57 (12.2%)	82 (5.6%)	
Two-Stage	5 (1.1%)	11 (0.8%)	
One-Stage & Chromogenic	0 (0.0%)	44 (3.0%)	

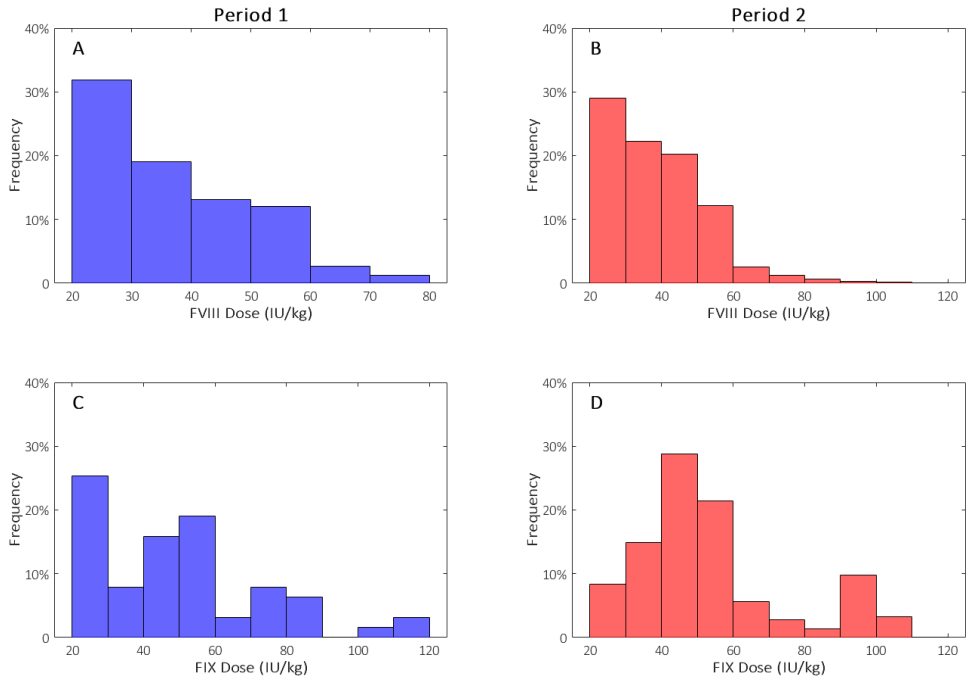


Figure 25. Dosages (IU/kg) of FVIII (A: Period 1; B: Period 2) and FIX (C: Period 1; D: Period 2) administered for infusions submitted for PK analysis

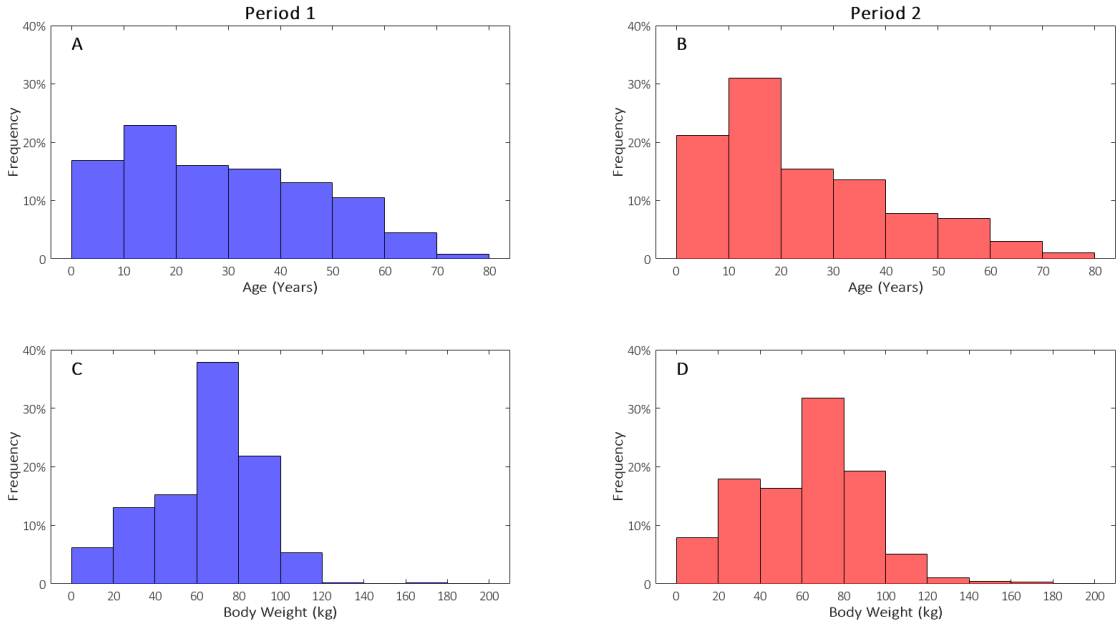


Figure 26. Age (A: Period 1; B: Period 2) and body weight (C: Period 1; D: Period 2) distributions of patients submitted for PK analysis

The post-infusion blood sampling strategy used by providers for PopPK estimation varied considerably. The median number of factor activity samples obtained per infusion decreased in Period 2 for FIX products (though not significantly), and did not change for FVIII products (Table 22, Figure 27).

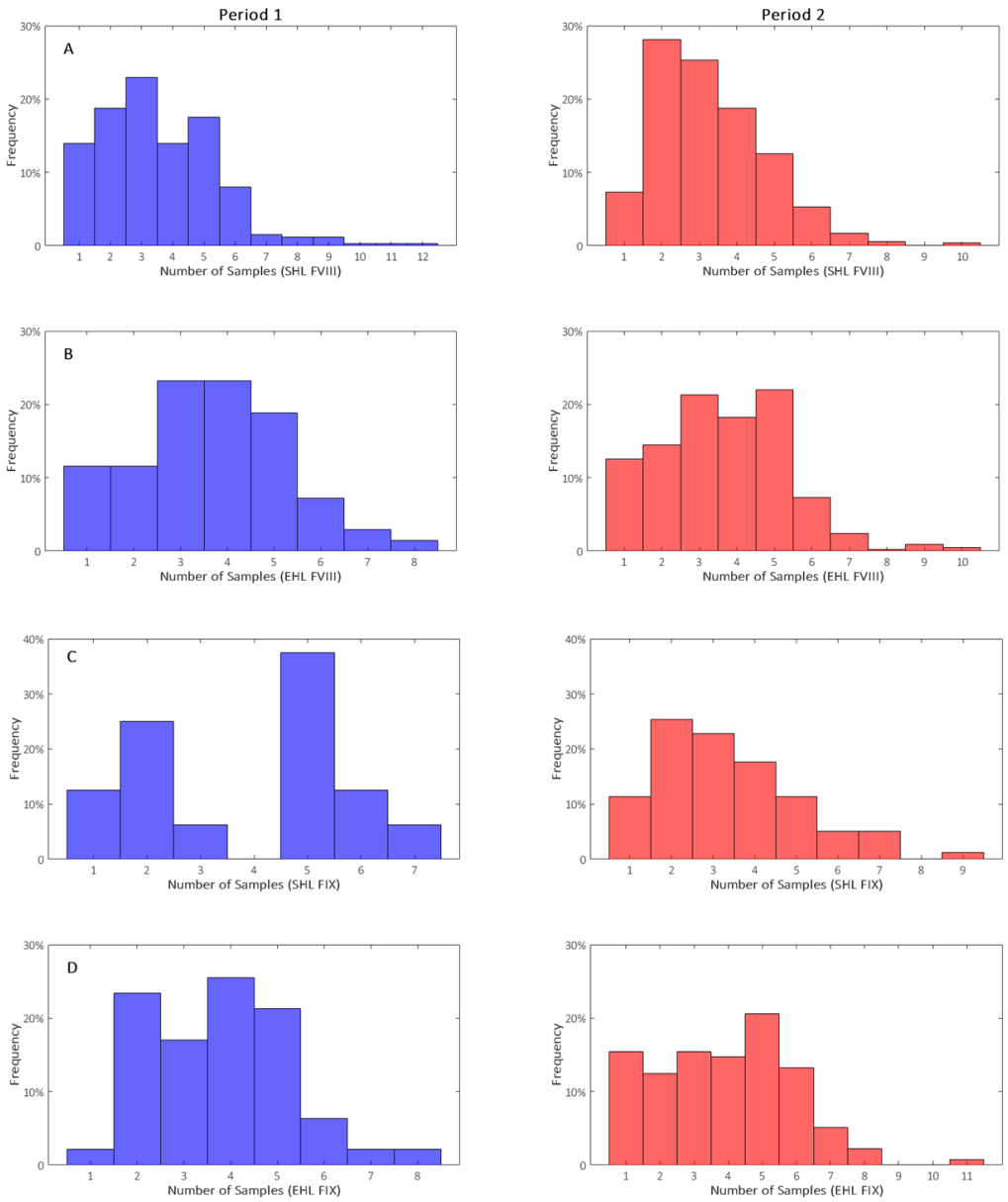


Figure 27. Number of samples collected per infusion for each of the product types (A. SHL FVIII; B: EHL FVIII; C: SHL FIX; D: EHL FIX) during Period 1 (left) and Period 2 (right).

Table 22. Mean and median number of blood samples per patient per infusion for each group of factor products during Period 1 and 2

Factor Product Group	Period 1	Period 2	p-value
SHL FVIII			
Mean (SD)	3.5 (1.9)	3.3 (1.5)	$p = 0.047$
Median [Range]	3 [1 – 12]	3 [1 – 10]	
EHL FVIII			
Mean (SD)	3.7 (1.6)	3.6 (1.7)	$p = 0.917$
Median [Range]	4 [1 – 8]	4 [1 – 10]	
SHL FIX			
Mean (SD)	3.9 (2.0)	3.4 (1.7)	$p = 0.336$
Median [Range]	5 [1 – 7]	3 [1 – 9]	
EHL FIX			
Mean (SD)	3.8 (1.5)	3.9 (2.0)	$p = 0.695$
Median [Range]	4 [1 – 8]	4 [1 – 11]	

Examination of the timing of blood samples for PK analysis demonstrated that peak levels continued to be frequently collected, despite ISTH recommendations; however, the timing of subsequent blood samples were consistent with suggested sampling windows based on CFC group (Figure 28). Of the non-peak samples submitted (n = 4,557), 71% were within recommended sampling windows for their respective product group. Among all of the 6,734 factor activity levels submitted during the study periods, the proportion of post-infusion levels within the ISTH recommended sampling windows were greater for FVIII CFCs (49% for SHL and 54% for EHL) compared to those for FIX CFCs (31% for SHL and 34% for EHL). Additionally, 83% of samples (5,592/6,734) were considered 'useful' in PopPK analysis (i.e. non-peak and not below level of quantification).

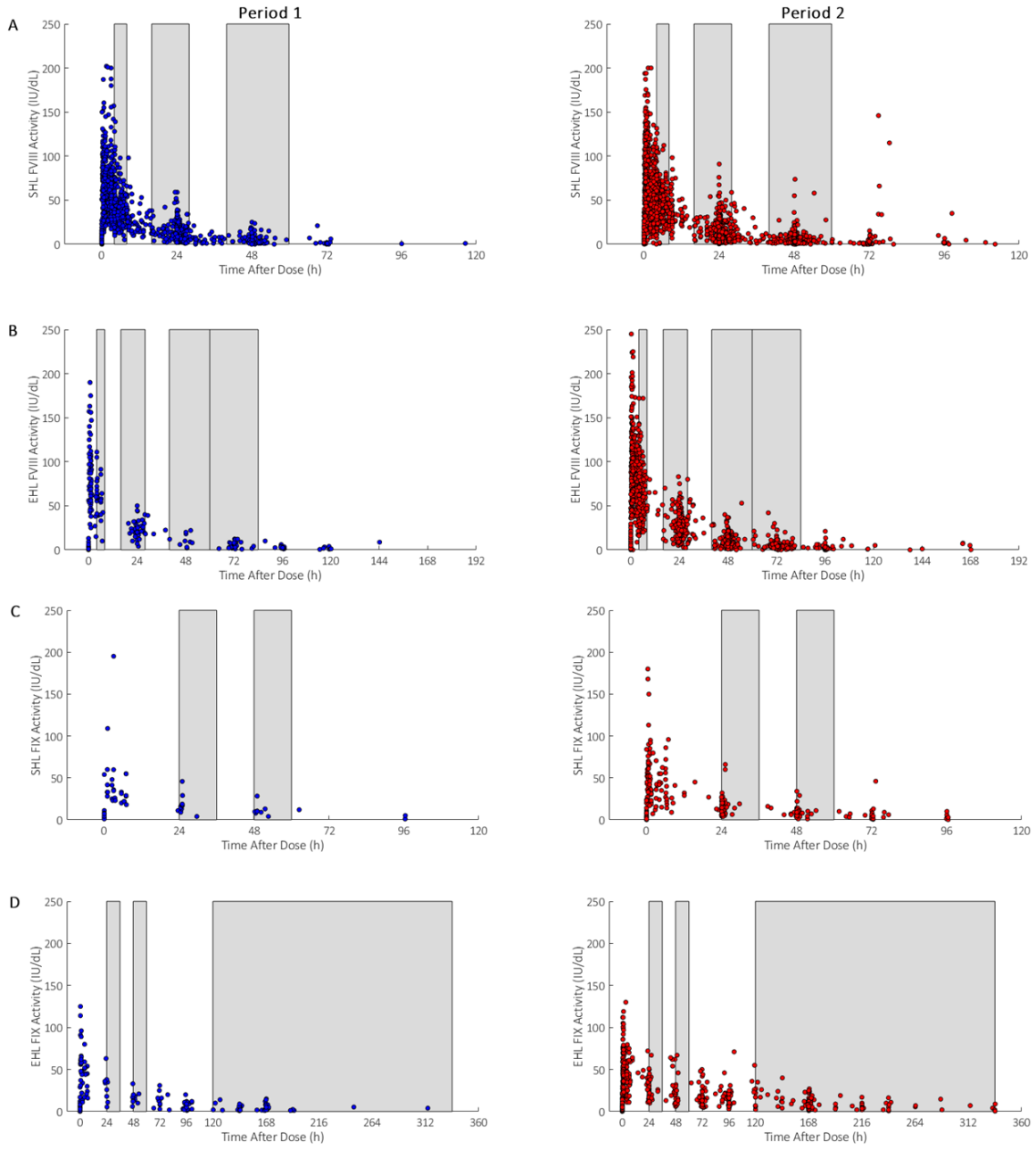


Figure 28. Post-infusion timing of factor activity levels for each of the product types (A. SHL FVIII, B. EHL FVIII, C. SHL FIX, and D. EHL FIX) collected during Period 1 (left) and Period 2 (right). Shaded regions represent sampling windows recommended by ISTH subcommittee for the specific product type: SHL FVIII: 4 – 8 h, 16 – 28 h, and 40 – 60 h, EHL FVIII add 60 – 84 h, SHL FIX: 24 – 36 h, and 48 – 60 h, EHL FIX: add 5 – 14 days.

Approximately 10% of infusions during the periods studied had only a single factor activity measurement submitted for PopPK analysis. The vast majority of these single factor level infusions were submitted in isolation, without evidence of another infusion with the same product and dosage within six months. The timing of the single post-infusion factor level varied by product group (Figure 29). The majority (70%) were obtained at least 24 hours post-infusion. The majority of single samples for SHL FVIII were obtained around 24 hours post-infusion, consistent with ISTH guidance, while single samples for EHL products tended to be taken at later timepoints. A minority of single samples (7.6%) were obtained less than 4 hours post-infusion and were not adequate for reliable PopPK analysis.

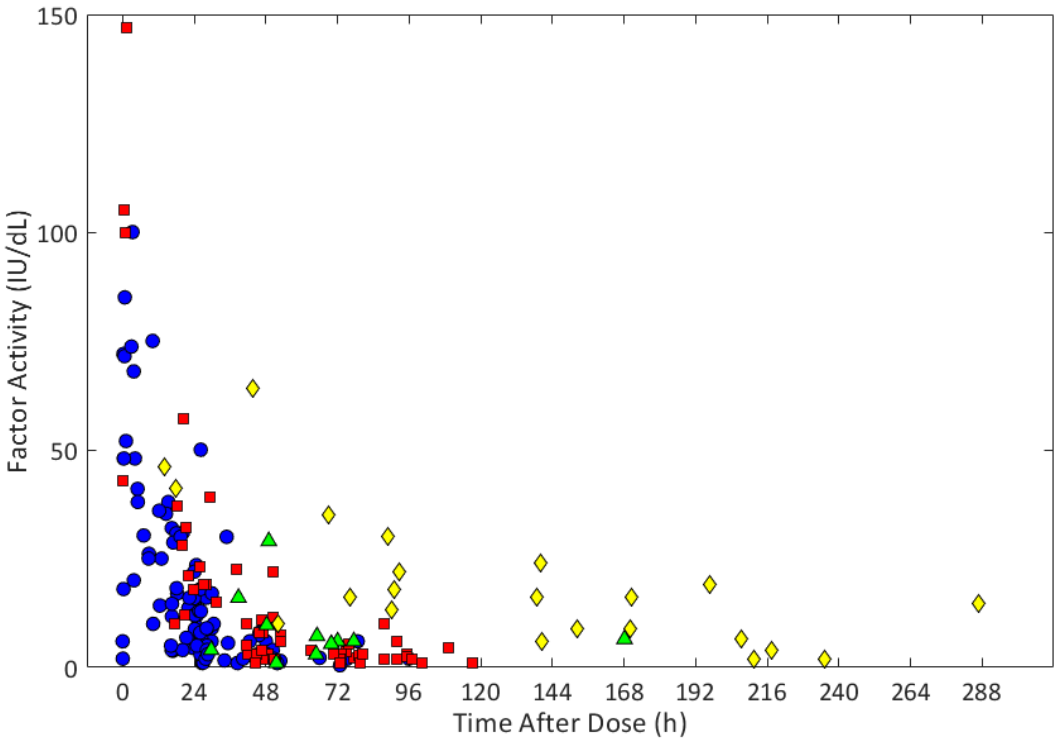


Figure 29. Factor activity levels for infusions containing a single sample, excluding confirmatory samples. SHL FVIII – blue circles, SHL FIX – red squares, EHL FVIII – green triangles, EHL FIX – yellow diamonds.

Across all WAPPS-Hemo sites (n = 204 at time of data extraction), the median number of individual patients with at least one infusion submitted was 8 [IQR 3–21]. The top 25th percentile was represented by 51 HUCs who submitted infusions for 22 patients or more. Of these 51 HUCs, 50 centres submitted infusions during at least one of the time periods studied and were included in the analysis. Of the 165 total centres who submitted infusion data during the time intervals studied, the median number of infusions entered per centre was 6 [IQR 3–13].

Across Period 1 and Period 2, the 50 HUCs contributed a total of 1,385 infusions (mean: 27 infusions per centre) compared to the 567 infusions submitted by the remaining 115 non-HUCs. Nearly 75% of the infusions submitted by HUCs during Period 1 were for SHL FVIII products, compared to about 60% of the submissions from non-HUCs during the same timeframe ($p = 0.105$). A full comparison of patient characteristics between HUCs and non-HUCs across both time periods can be found in Table S1.

During Period 1, infusions submitted by non-HUCs typically included more samples than those from HUCs (mean 4.3 for non-HUCs and 3.5 for HUCs, p = 0.009). Although this difference narrowed during Period 2, it continued to be statistically significant. Despite an overall trend toward fewer samples collected at HUCs, this was not consistently observed for different product types (Table S2). Although HUCs submitted fewer blood samples compared to non-HUCs for SHL products, the opposite trend was initially observed for EHL FVIII products. No differences were observed across time period or centre type for EHL FIX. Timing of post-infusion blood samples from both groups aligned well with the ISTH recommended sampling windows; however, direct comparison of timing of samples between HUCs and non-HUCs was limited by the small sample size for the non-HUCs.

Discussion

A wide range of sampling strategies was employed across providers for PK curve estimation, in terms of both number and timing of samples. It is reassuring to see a gradual decline in the average number of blood samples obtained. This may reflect increasing provider confidence with the reliability of fewer, well-timed samples for a PK estimation and also application of the international guidance supported by the ISTH subcommittee. The benefits of fewer sampling points for the patients are fewer venipunctures or accessing of a central line, and reduced time away from school or work for phlebotomy. Although the number of samples collected is decreasing, providers still tended obtain more than the minimum required sampling points and, in particular, continue to obtain peak levels. This may be due to interest in *in vivo* recovery or convenience if infusion occurred on site; however, this level is not included in the ISTH guidance for use of PopPK. It is important to note that all additional blood samples beyond the minimal proposed further improve accuracy of PK profile estimates for an individual patient but from a resource utilization perspective may not be necessary. Across both time periods, the timing of post-infusion factor activity measurements by clinicians was generally consistent with the timeframes recommended by the ISTH subcommittee. While the majority of post-infusion data points, excluding the peak values, fell into the windows first suggested by Björkman and others [57,178,243] and refined for EHL concentrates by the ISTH guidelines [169], it is important to note that blood samples were taken throughout those windows. This reinforces the value of flexibility in sampling timepoints in successful execution of PopPK in routine clinical practice.

PopPK was applied across the full age spectrum of hemophilia patients, ranging from infants to octogenarians, highlighting the importance of having PopPK clinical tools that are

validated across the age and weight range of the patient population. There is a trend toward application of PK profiles in younger patients; the median age and proportion of patients greater than 18-years-old decreased over time. This may represent increased interest in understanding the potential impact of PK for prophylaxis regimen tailoring among pediatric (27% of Period 2 infusions) and young adult (22% of Period 2 infusions) patients, or increased switching between SHL and EHL informed by PK occurring in younger age cohorts.

Given the higher prevalence of hemophilia A, it is not surprising that the majority of infusions were submitted for FVIII CFCs; however, compared to the anticipated ratio of hemophilia A to B patients, PopPK may be underutilized for those using FIX CFCs. This discrepancy may be due to the longer half-life of FIX CFCs, as well as the reduced bleed frequency in patients with severe hemophilia B compared to severe hemophilia A [199]. These factors may prompt providers to continue to rely predominantly on measured troughs and clinical phenotype to tailor FIX prophylactic dosing rather than use of PopPK. Another possible explanation for slower uptake of PopPK in hemophilia B may be that evidence supporting a particular therapeutic target is less established as compared to hemophilia A.

There are several limitations of our current study. Insight into a clinician's motivation or rationale for the submitted samples was not available. There may be many reasons for submitting a single sample, including lack of PopPK knowledge, self-editing (e.g. not including trough levels that are below limit of quantification), or patient refusal to return for more than one blood draw. We analyzed two timeframes to assess changes in use of PopPK, but this field and clinical practice continue to evolve; perhaps brand-specific analysis may be of interest in the future as the newer EHL CFCs achieve more widespread use globally. Despite emergence of non-factor therapies for hemophilia A prophylaxis, PopPK continues to be of importance for clinical care

globally as FVIII CFCs predominate for prophylaxis. Other PopPK tools are available from both manufacturer and academic groups, developed with different model assumptions and different requirements for post-infusion sampling. The patterns of use observed with WAPPS-Hemo cannot be extrapolated to other tools.

Conclusions

The use of PopPK in hemophilia treatment continues to expand with a greater than threefold increase in the number of infusions, patients and centres in the WAPPS-Hemo network between the timeframes queried. Infusions submitted during the recent use period contained fewer blood samples and younger patients than those from the early adoption phase. During both time periods, peak samples were frequently obtained but the remaining blood sample timepoints were well-aligned with the current guidance from ISTH.

Chapter 8: The effect of unmeasurable endogenous plasma factor activity levels on factor VIII dosing in patients with severe hemophilia A

This chapter is reflective of an original manuscript published by the Ph.D. candidate (Alanna McEneny-King) in *Thrombosis Research*. All pertinent dialogue in this chapter was written by the Ph.D. candidate.

McEneny-King A, Chelle P, Iorio A, Edginton AN. The effect of unmeasurable endogenous plasma factor activity levels on factor VIII dosing in patients with severe hemophilia A. Thromb Res. 2018; 170(2018):53-59. DOI: 10.1016/j.thromres.2018.08.004

Introduction

Hemophilia A is an X-linked bleeding disorder affecting 1 in 6,500 newborn males worldwide [62]. Genetic mutations responsible for hemophilia A number in the hundreds [247,248] but are all localized to the factor VIII (FVIII) gene (F8) and translate into lower than normal FVIII clotting activity. Manifestations of this lowered clotting ability include internal bleeding, particularly in joints, which, in more severe hemophilia A cases, is spontaneous (i.e. not caused by external trauma or injury). The severe hemophilia A phenotype is clinically assessed as <0.01 IU mL^{-1} FVIII activity level (<1% of normal) [69] and the prevalence of such severe cases in Canada is reported to be 31% of all hemophilia patients [62]. Treatment options include replacement of FVIII through intravenous injection, either on-demand or prophylactically on a regular schedule. Indeed, prophylactic treatment has been demonstrated to reduce the incidence of arthropathy in severe hemophilia A patients, a common and debilitating side effect of frequent joint bleeds [8,249].

The aim of the classical prophylactic treatment scheme is to maintain FVIII activity above 0.01 IU mL^{-1} through regular administration of FVIII. The choice of this pharmacokinetic biomarker is based on a 1965 study where the rate of increase in joint score (a positive measure of progressive arthropathy) was halved in patients with a baseline FVIII activity between 0.01 and 0.03 IU mL^{-1} as compared to patients with endogenous activity below 0.01 IU mL^{-1} [7]. Building on the results of this study, Nilsson et al [8] observed that the longer patients remained over the 0.01 IU mL^{-1} threshold, the lower their joint score (indicating improved joint function). Accordingly, FVIII concentrates are still labelled to be dosed between 15-50 IU kg^{-1} of total body weight, to be administered at a frequency of 2-4 times per week. The goal of this population-based dosing method is to maintain most patients above the 0.01 IU mL^{-1} threshold. The need for such a wide range of doses has been traditionally attributed to high variability of the FVIII PK in the population, and has driven the quest for individualized dose titration [167,234,250].

Historically, PK-based individualization of treatment has been hampered by the impracticality of drawing 10 or more blood samples as recommended by the International Society of Thrombosis and Haemostasis (ISTH) Scientific and Standardization Committee [250,251], and little effort was devoted to systematically using individual PK information to generate individual dosing regimens. With the advent and diffusion of the population pharmacokinetic (PopPK) approach, including Bayesian post hoc estimation to obtain individual profiles as recently recommended by the ISTH [169], far fewer blood samples are required and many more empirical sets of post-infusion samples have been collected through web-based PK software like the Web-Accessible Population Pharmacokinetics Service – Hemophilia (WAPPS-Hemo, www.wapps-hemo.ca) [170,252]. An excellent plain language description of this

individualized estimation technique from a PopPK model is presented in Bjorkman and Collins [243].

A system like WAPPS-Hemo offers a unique opportunity to explore the sources of variability of factor VIII (and IX) PK in the population. For example, analyzing the data collected via WAPPS-Hemo has prompted challenging the body weight metric currently used for dosing [253]. WAPPS-Hemo has an integrated clinical simulator module, which allows the clinician to predict the individual profile after any given combination of dose and infusion frequency, or to calculate the dose required to obtain a specific threshold after a given time. Implementing the algorithm used in the simulator has prompted the hypothesis that the amount of FVIII produced by individual patients in the range of 0 to 0.01 IU mL^{-1} is another determinant of the inter-individual PK variability.

Indeed, for many patients, the baseline value is reported as <0.01 IU mL^{-1}, either because the assay used to detect FVIII activity has a lower limit of quantification (LLOQ) of 0.01 IU mL^{-1}, or because the patient is phenotypically a severe hemophilia A patient (therefore defined as <0.01 IU mL^{-1} baseline), and the true baseline has not been measured or recorded. As a matter of fact, both the chromogenic and the one-stage clotting assays used to quantify FVIII activity typically had in the past a LLOQ of 0.01 IU mL^{-1}, which is why this value was selected as the cut-off for severity. Modern assays can measure FVIII activity as low as 0.004 IU mL^{-1}, which allows baseline values to be measured to lower values or defined as less than the LLOQ, for example <0.004 IU mL^{-1}.

The objective of the presented work was to assess the implications of imprecise knowledge of baseline to dosing regimen design in hemophilia. Towards this end, we used a

published PopPK model that describes FVIII PK to generate estimates of PK parameters (e.g. clearance, volume of distribution) for virtual patients. We then used these virtual patients to assess how different assumptions of their baseline value affect optimal dosing regimen design, both on a population level as well as during therapeutic drug monitoring and individual dose adjustment. We also used real patient data collected through the WAPPS-Hemo project to explore the sensitivity of PK outcomes (e.g. half-life, time to a specific FVIII level) to changes in the baseline assumption.

Methods

Matlab (R2017b) was used for re-creation of the PopPK model, population generation, simulations and graphical outputs.

Population pharmacokinetic modeling

The PopPK model used to generate individual estimates of PK parameters is a 2-compartment structure as described by Garmann et al [185] for a conventional recombinant FVIII. This model was built on a total of 183 subjects with body mass index (BMI) ranging from 13-38.3 kg m^{-2}. Details of the model structure are presented in Table 23.

Table 23. Details of the model developed by Garmann et al [185]

Parameter	Estimate	Covariate Effects[c]	BSV[d] (%CV)
Clearance (CL, dL h^{-1})	1.88	$\left(\frac{Lean\ Body\ Weight}{51.1}\right)^{0.610}$	37.0
Intercompartmental clearance (Q, dL h^{-1})	1.90		
Central volume (V_1, dL)	30.0	$\left(\frac{Lean\ Body\ Weight}{51.1}\right)^{0.950}$	11.2
Peripheral volume (V_2, dL)	6.37		
Proportional RUV[a] (%CV[b])	26.7		
Additive RUV (IU dL^{-1})	1.10		

[a]Residual unexplained variability; [b]Coefficient of variation; [c] $\theta_P$represents the population mean for parameter P; [d]Between subject variability

Development of simulated populations

Generated populations of 500 virtual individuals consisted of males with a uniform distribution of BMI in the range of 18-30 kg m^{-2}. Heights were derived from the distribution provided by the NHANES database [215]. The distribution of BMI's was simulated and the total body weights were calculated as the product of BMI and the square of height. Using these covariates, the PK parameters for each individual, as stated in Table 1, were generated from the model.

Simulations

The effect of the baseline assumption on optimal dose or frequency was assessed for 500 populations each containing 500 individuals. For each individual, the PK parameters were generated and individual FVIII activity levels were simulated following various administrations according to the following equation:

$$C(t) = \sum_{i=1}^{n} D_i \left(Ae^{-\alpha\left(t - t_{D_i}\right)} + Be^{-\beta\left(t - t_{D_i}\right)} \right) + baseline$$

where D_i is the dose administered, A, B, α and β are the macro-constants calculated from the parameters listed in Table 1, and t_{D_i} is the time at which dose D_i was given. The infusion time was assumed negligible (usually less than 5 minutes) and not included. Four weeks of prophylactic treatment were simulated in order to reach steady state, and the fifth week was used for analysis.

First, the doses required to maintain 95% of patients above troughs of 0.01 IU mL^{-1}, 0.03 IU mL^{-1} and 0.05 IU mL^{-1} at 48 hours post-administration (Q48h) were calculated. To do so, the dose was increased by increments of 1 IU kg^{-1} (to a maximum dose of 300 IU kg^{-1}) until 95% of each population maintained FVIII activity above the desired trough at steady state. Frequency was determined in a similar manner where, starting with a 40 IU kg^{-1} dose administered weekly, the dosing interval was decreased by 1 hour (down to a minimum of 12 hours or twice daily dosing) until 95% of each population maintained the desired trough activity of 0.01 IU mL^{-1}, 0.03 IU mL^{-1} and 0.05 IU mL^{-1}. To explore how the assumption of baseline affects both optimal dose and frequency, baseline was increased from 0 IU mL^{-1} to 0.01 IU mL^{-1} in steps of 0.001 IU mL^{-1}.

The effect of the baseline assumption on individual dose adjustments using trough levels was also performed. This scenario is akin to the common clinical situation of adjusting a therapeutic regimen, with a patient having a trough level taken after a given dose and the clinician adjusting the dose so as to achieve a different trough level. For this hypothetical

scenario, a dose of 2000 IU generated a trough of 0.01 or 0.02 IU mL^{-1} at a given time. The implication of the true baseline (and corresponding assumptions when the true baseline is unknown or unmeasurable) was assessed by scaling the exogenous trough level to achieve either double or half the observed trough (0.02 or 0.01 IU mL^{-1}, respectively). The exogenous (i.e. scalable) trough is the observed trough for a given dose ($Dose_{Observed}$) minus the baseline level. Three different baseline activities were explored for each scenario: 0, 0.005, 0.008 IU mL^{-1}. A new dose ($Dose_{New}$) was then calculated as:

$$Dose_{New} = \frac{Desired\ Trough - Baseline}{Observed\ Trough - Baseline} \cdot Dose_{Observed}$$

This exercise was also conducted using higher observed and desired troughs to demonstrate the reduced impact of baseline assumptions at higher FVIII levels.

Real patient data

Using the WAPPS-Hemo platform, we used a Bayesian approach to estimate individual PK parameters for three real patients collected in the database, varying each patient's baseline between 0 and 0.01 IU mL^{-1} by steps of 0.001 IU mL^{-1}. The estimates of terminal half-life and times to specific trough levels were compared for the different baseline values to assess the sensitivity of these outcomes to the baseline assumption.

Results

Regardless of the targeted trough level, there is a 40 IU kg^{-1} dose requirement drop from an assumed baseline of 0 to close to 0.01 IU mL^{-1} (Figure 30). For a targeted trough of 0.01 IU

mL^{-1}, the baseline assumption between 0 and 0.01 IU mL^{-1} means the difference between dosing at 40 IU kg^{-1} and 0 IU kg^{-1}, respectively. On a relative-to-dose basis, the importance of the baseline assumption is greatly reduced as the targeted trough increases. For example, when targeting 0.01 IU mL^{-1} and assuming a baseline of 0.005 IU mL^{-1} rather than 0 IU mL^{-1}, the calculated dose required drops by 50%, from 40 to 20 IU kg^{-1}. When targeting a 0.05 IU mL^{-1} trough, the same change in the assumption about baseline leads to only a 10% decline in the required dose.

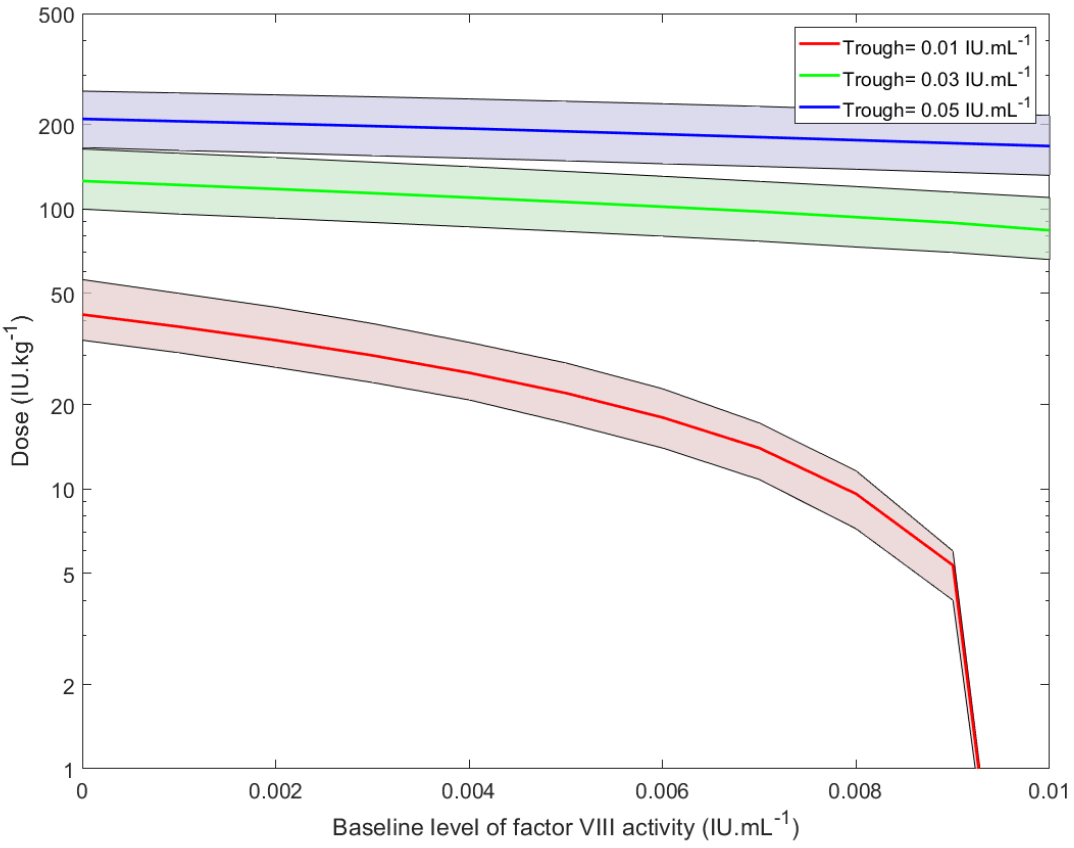


Figure 30. The Q48h dose required to keep 95% of individuals above the trough as a function of baseline value. The shaded region is the 95% confidence interval of the mean of 500 populations each containing 500 individuals.

Baseline also greatly affects the optimal frequency of dosing. If targeting 0.01 IU mL^{-1}, frequency is asymptotic as it approaches an assumed baseline of 0.01 IU mL^{-1}; this is expected since no drug needs to be given if the target is the same as the baseline (Figure 2). As baseline decreases, dosing interval decreases, and the drug needs to be administered more often. On the contrary, baseline assumptions between 0.007 and <0.01 IU mL^{-1} would result in a longer interval between infusions. Similar to dose, as the targeted trough increases, the baseline assumption has less and less influence on the frequency outcome. For dose optimization purposes on a population level, assuming a baseline of 0 IU mL^{-1} is the most conservative means for assessing optimal dose and/or frequency as it will always lead to the highest dose and the lowest frequency (Figure 30, Figure 31).

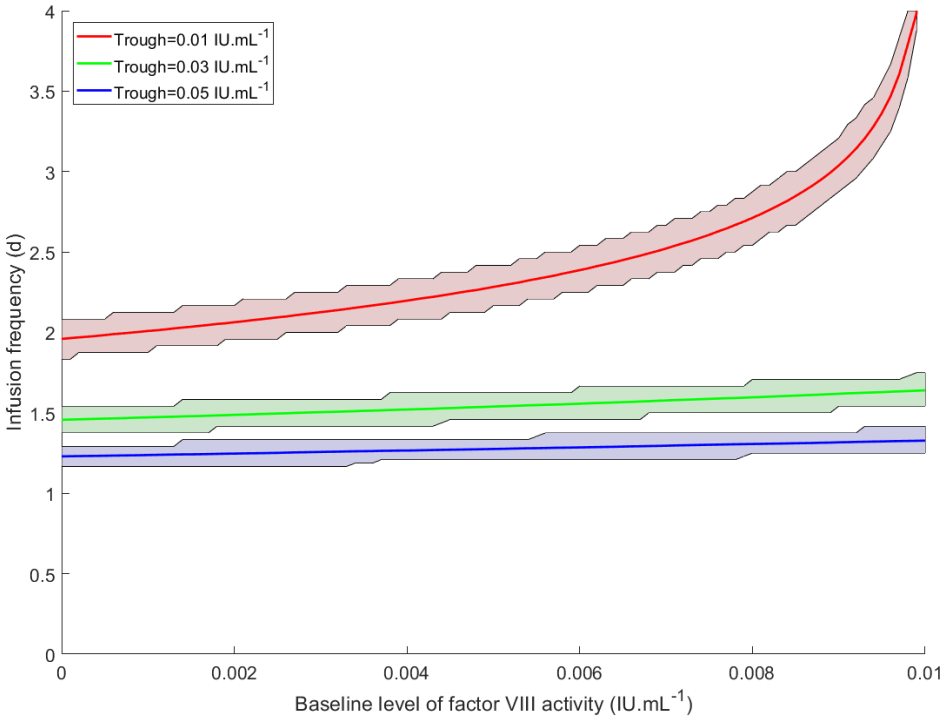


Figure 31. Effect of baseline assumption on the infusion frequency needed to keep 95% of individuals above a desired trough with a dose of 40 IU kg^{-1} when frequency is considered as a continuous variable. The shaded region is the 95% confidence interval of the mean of 500 populations each containing 500 individuals

For dose adjustment using an observed individual level, the importance of baseline is also evident. When a patient's baseline is known, any measurement can be partitioned into residual FVIII for prior doses and endogenous production; this step is critical for accurate dose adjustment since only the residual exogenous component is proportional to dose. The consequences of being unable to partition a FVIII measurement in this way are highlighted in Table 24. In this example, we suppose a trough of 0.01 IU mL^{-1} is observed following a 2000 IU dose, and a trough of 0.02 IU mL^{-1} is desired. If true baseline is 0 IU mL^{-1}, then the entirety of the observed trough is due to exogenous FVIII and dosing is linear; to double the trough, double the dose. However, if true baseline is 0.005 IU mL^{-1}, only 0.005 IU mL^{-1} of the observed trough can be attributed to the exogenous factor concentrate and, in order to achieve a trough of 0.02 IU mL^{-1}, we need to supplement the baseline activity by 0.015 IU mL^{-1} (i.e. triple the dose).

Table 24. The effect of baseline assumption on dose adjustments using a single observed level

Scenario	Baseline (IU mL^{-1})	Dose (IU)	Observed Trough (IU mL^{-1})	Exogenous Portion of Trough (IU mL^{-1})	Dose Required to Achieve a 0.02 IU mL^{-1} Trough (IU)
A	0	2000	0.01	0.01	$\frac{0.02-0}{0.01}\cdot 2000 = 4000$
	0.005	2000	0.01	0.005	$\frac{0.02-0.005}{0.005}\cdot 2000 = 6000$
	0.008	2000	0.01	0.002	$\frac{0.02-0.008}{0.002}\cdot 2000 = 12000$
					Dose Required to Achieve a 0.01 IU mL^{-1} Trough (IU)
B	0	2000	0.02	0.02	$\frac{0.01-0}{0.02}\cdot 2000 = 1000$
	0.005	2000	0.02	0.015	$\frac{0.01-0.005}{0.015}\cdot 2000 = 667$
	0.008	2000	0.02	0.012	$\frac{0.01-0.008}{0.012}\cdot 2000 = 333$
					Dose Required to Achieve a 0.03 IU mL^{-1} Trough (IU)
C	0	2000	0.05	0.05	$\frac{0.03-0}{0.05}\cdot 2000 = 1200$
	0.005	2000	0.05	0.045	$\frac{0.03-0.005}{0.045}\cdot 2000 = 1111$
	0.008	2000	0.05	0.042	$\frac{0.03-0.008}{0.042}\cdot 2000 = 1048$

The counterintuitive result that the patient with higher baseline activity requires a higher dose arises from having observed the same trough for both patients after giving the same dose; this observation means that the patient with no endogenous FVIII has a higher amount of exogenous FVIII at that time due to a better PK response. If the patient has a higher true baseline, the contribution of the administered dose to the measured trough is reduced, which results in a more extreme scaling factor (Table 24). Furthermore, if we perform this exercise using slightly higher troughs (as in Scenario C of Table 24), the impact of the baseline assumption is lessened; in this case, the calculated doses varied by about 150 IU (~12% difference).

When estimating PK parameters using a Bayesian approach, not all parameters are sensitive to baseline FVIII activity. For example, half-life is relatively unchanged across baseline values between 0 and 0.01 IU mL^{-1} (Table 3). Conversely, the time at which a patient will reach a trough of 0.01 IU mL^{-1} is extremely sensitive to baseline. In all three patients, this time varied by over 20 hours as baseline increased from 0 to 0.009 IU mL^{-1} – an important observation given the demonstrated correlation between time below 0.01 IU mL^{-1} and occurrence of bleeds [10]. However, the times to reach slightly higher troughs (i.e. ≥0.02 IU mL^{-1}) are much less affected (in most cases, varying by less than 3 hours).

Table 25. The effect of baseline assumption on estimated PK outcomes using a Bayesian approach

Observed Trough (IU mL^{-1})	Dose (IU kg^{-1})	Baseline (IU mL^{-1})	Half-life (h)	ΔHalf-life (%)	Estimated Time (h) to Specified Trough (IU mL^{-1})				
					0.01	**0.02**	**0.03**	**0.04**	**0.05**
0.01	58.42	0.000	10.18	---	57.49	47.31	41.36	37.13	33.86
		0.002	10.01	1.7%	59.71	48.00	41.62	37.21	33.84
		0.005	9.81	3.6%	65.05	49.50	42.27	37.51	33.95
		0.008	9.64	5.3%	76.59	51.66	43.23	38.02	34.24
		0.010	9.55	6.2%	n/a	53.62	44.06	38.48	34.51
		Range	**0.63**	**---**	**28.29**	**6.31**	**2.70**	**1.35**	**0.68**
0.015	46.15	0.000	9.42	---	56.09	46.66	41.15	37.24	34.21
		0.002	9.27	1.6%	58.14	47.29	41.38	37.29	34.17
		0.005	9.08	3.6%	63.03	48.63	41.94	37.53	34.24
		0.008	8.93	5.2%	73.67	50.60	42.79	37.97	34.47
		0.010	8.84	6.1%	n/a	52.39	43.55	38.38	34.71
		Range	**0.58**	**---**	**26.07**	**5.73**	**2.40**	**1.14**	**0.54**
0.02	57.97	0.000	8.47	---	56.46	47.99	43.04	39.53	36.80
		0.002	8.27	2.3%	57.85	48.17	42.90	39.26	36.47
		0.005	8.00	5.6%	61.38	48.70	42.80	38.92	36.02
		0.008	7.75	8.5%	69.75	49.72	42.94	38.75	35.71
		0.010	7.61	10.1%	n/a	50.82	43.21	38.76	35.60
		Range	**0.86**	**---**	**20.32**	**2.83**	**0.41**	**0.78**	**1.20**

These trends are also demonstrated in Figure 32, which shows the estimated FVIII activity profiles for one of the patients for each assumed baseline. The curve is almost identical for all baselines up until a level of approximately 0.02 IU mL^{-1} (Figure 32, left). The profiles begin to diverge at this point, with the truly zero baseline continuing in a linear fashion while the curves representing non-zero baselines begin to plateau (Figure 32, right).

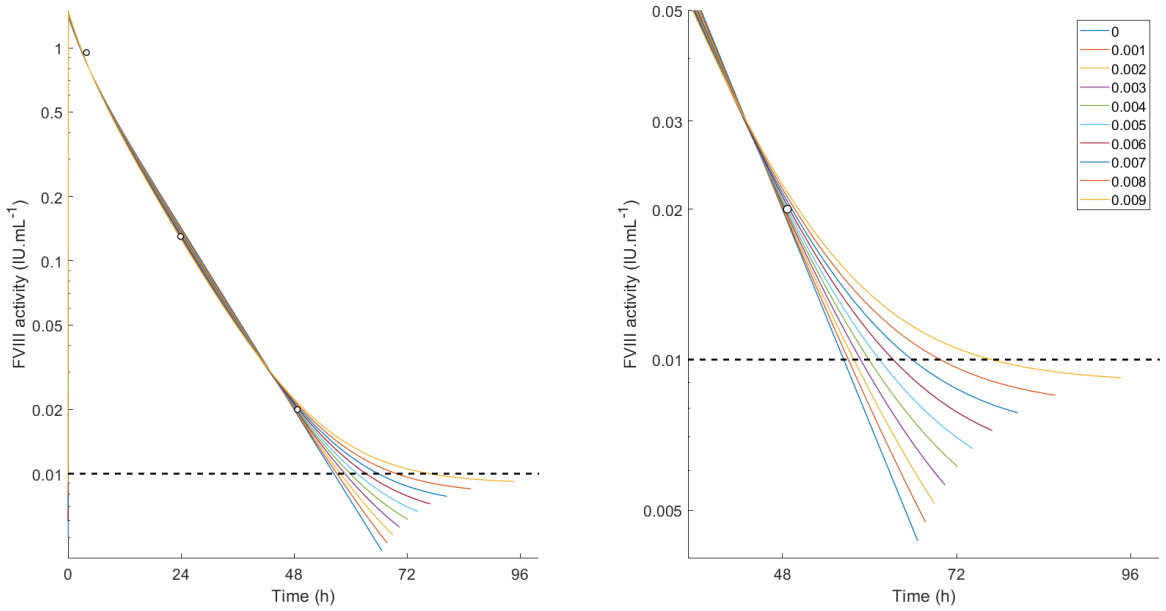


Figure 32. Estimated FVIII activity profiles following a dose of ~60 IU kg^{-1}, assuming different baseline levels (legend, IU mL^{-1}). White circles denote observed data

Discussion

The effect of imprecise knowledge about baseline on population level dose regimen design is increasingly pronounced as the target trough level approaches 0.01 IU mL^{-1}. When a trough of 0.01 IU mL^{-1} is targeted, within the range of realistic baseline values for severe hemophilia patients (0 through <0.01 IU mL^{-1}), the required dose changes from approximately 40 IU kg^{-1} to a theoretically negligible dose (practically approaching 0 IU kg^{-1}) Q48h – a drastic 100% difference depending on the assumed baseline. If baseline could be measured accurately, it would provide the clinician with a better starting point within this range when designing a regimen; for a patient with a baseline of 0.006 IU mL^{-1}, Figure 1 suggests a starting dose of 25 IU kg^{-1} while a patient with a baseline of 0.002 IU mL^{-1} may require closer to 40 IU kg^{-1} to maintain a trough of 0.01 IU mL^{-1}. When targeting a trough of 0.05 IU mL^{-1}, the effect of the baseline assumption on dose or frequency determination is minimal (approximately 10%). When targeting a 0.15 IU mL^{-1} target trough [254], baseline is essentially irrelevant, although standard half-life FVIII usage would be exceptionally high. In clinical practice, a trough of 0.01 IU mL^{-1} is often targeted using doses in the 15-40 IU kg^{-1} range and ignoring the true baseline value of the patient may well explain part of the variability in the dose response to FVIII and the need for such a wide range of doses.

To reduce this variability, we might examine data from clinical studies to understand the association between the variation in dose requirements and the actual baseline values in individual patients. In a single-centre study where the individual PK of hemophilia A and B patients was simulated for each of six years based on their actual prophylactic treatment, both doses (11-67 IU kg^{-1}) and trough levels were highly variable. With regards to troughs, the study showed that more than 80% of dosing schedules were at least as frequent as the recommendation

(3 times a week); however, 38% of the troughs were lower than 0.01 IU mL^{-1}, 27% of the troughs were between 0.01-0.03 IU mL^{-1}, and 35% of the troughs were higher than 0.03 IU mL^{-1} [255]. This variability is partially a function of the variation in baseline values of severe hemophilia patients.

When treating individual patients, the assumption of baseline affects dose adjustment. Björkman and Collins [243], on behalf of the Scientific and Standardization Committee of the ISTH, proposed that only those activities above 0.03 IU mL^{-1} be used in individual pharmacokinetic assessment as all values below are potentially inaccurate due to assay limitations and baseline. This was demonstrated here where the assumption of a 0 IU mL^{-1} baseline allows for linear kinetics to be used such that a doubling of a trough is precipitated by a doubling of the dose. As true endogenous baseline increases from 0 to 0.01 IU mL^{-1}, the assumption of linearity is no longer valid. However, when performing dose adjustments, the more conservative, and therefore safer, approach is to assume a 0 IU mL^{-1} baseline when decreasing trough, and a non-zero baseline of at least 0.005 IU mL^{-1} when increasing trough.

Replacement of an endogenous molecule that is missing or has limited endogenous production is not unique to hemophilia. Type 1 diabetes (insulin), Parkinson's disease (dopamine) and hormone replacement therapy for Addison's disease (e.g. hydrocortisone), menopause (estrogen) or stunted growth (human growth hormone) are all examples of replacement therapies. However, none suffer from an assay sensitivity that hinders assessment of a pharmacokinetic or pharmacodynamic target as in hemophilia. In diabetes, glucose level drives insulin dose and the assay sensitivity range for glucose (≈2-33 mM) covers the relevant glucose target levels (≈ 4-7 mM). For Addison's disease, while serum cortisol levels below the limit of detection of 20 nM strongly suggest adrenal insufficiency, replacement therapy aims to mimic

normal cortisol levels (55-690 nM depending on time of day), which are above the assay sensitivity limit [256]. The combination of a target trough that is the same or similar in value to the assay LLOQ makes dose individualization in hemophilia uniquely dependent on assay sensitivity.

The effect of baseline is independent from the nature of the test one uses to measure plasma FVIII activity levels. Two clotting assays are primarily used to assess FVIII activity in plasma: the chromogenic assay and the one-stage assay. According to a report by Chandler et al, the intra-assay imprecision was similar for both methods (CV = 13%) while inter-assay imprecision was lower for the one-stage (17.5%) compared to the chromogenic (26.7%) at low FVIII levels [257]. In recent peer-reviewed literature, the LLOQ for the chromogenic assay was 0.01 IU mL^{-1} (single-chain recombinant FVIII) [258], 0.01 IU mL^{-1} (B-domain deleted recombinant FVIII) [182], 0.015-0.03 IU mL^{-1} (full length recombinant FVIII) [185] and 0.004 IU mL^{-1} (recombinant FVIII Fc fusion protein) [106]. For the one-stage assay, the LLOQ was 0.005 IU mL^{-1} (recombinant FVIII Fc fusion protein) [106], 0.01 IU mL^{-1} (B-domain deleted recombinant FVIII) [182], 0.005 IU mL^{-1} (recombinant FVIII Fc fusion protein; lower limit of reportable range = 0.007 IU mL^{-1}) [184] and 0.005 IU mL^{-1} (recombinant FVIII) [184]. While there are LLOQ values below 0.01 IU mL^{-1} in the above referenced studies, the majority of participating clinical sites in WAPPS-Hemo report baseline as <0.01 IU mL^{-1}. This suggests that 0.01 IU mL^{-1} is the most common LLOQ in practice.

Shima et al [259,260] have been active in the area of FVIII activity assay sensitivity. Thirty-six hemophilia A patients defined as severe based on the above conventional assays (<0.01 IU mL^{-1}) were re-assayed using a more sensitive technique called clot waveform analysis with a LLOQ of 0.002 IU mL^{-1}. Of these patients, 23 (64%) had endogenous FVIII levels <0.002

IU mL^{-1} and 13 (36%) had levels between 0.002 and 0.01 IU mL^{-1} [260]. Another random sample of severe hemophilia A patients produced 12 (67%) patients with an endogenous FVIII level of <0.002 IU mL^{-1} and 6 (33%) with levels between 0.002 and 0.01 IU mL^{-1} [261]. Matsumoto went on to distinguish between those with a very low FVIII level (HA, <0.0025 IU mL^{-1}) and those patients with inhibitors (HA-ihb), who presumably have no FVIII activity. While there was some overlap between the maximum (HA-ihb) and minimum (HA) values of the two groups, for the most part, those patients with a <0.0025 IU mL^{-1} level had at least some FVIII activity, although it could not be directly quantified [262]. While different mutations have been correlated with the presence or absence of cross-reactive material (CRM) and thus immunologically measured amounts of FVIII with associated differential risk of inhibitor development [263], little is known about the clotting activity of those trace amounts. The potential for future studies correlating genotype with endogenous, not measurable, FVIII activity to translate clinical impact on dosing remains unknown. Furthermore, while F8 gene mutation is the largest determinant of baseline FVIII activity, other factors may contribute to baseline activity. In a study of mild/moderate hemophilia A patients, Loomans et al [264] found that patients with the same mutation had significantly different baseline activities (inter-individual variability = 45%). As a result, further development and implementation of assays that allow for detection at very low FVIII levels will be important to ensure both genotype/phenotype concordance for clinical purposes [259] as well as appropriate FVIII dosing.

Researchers have attempted baseline estimation using a PopPK modeling approach. Brekkan et al [178] (plasma-derived FIX) estimated baseline with a typical value of 0.016 IU mL^{-1} in a cohort of severe and moderate patients. Abrantes et al [182] (B-domain deleted recombinant FVIII) estimated a baseline of 0.0047 IU mL^{-1} (%CV = 7) and 0.0159 IU mL^{-1} for

the severe and moderate cohort, respectively. In Stass [228], recombinant FVIII baseline for each individual was estimated to be less than 0.01 IU mL^{-1} and in-line with their severe status. While this may provide for a population level data description of baseline, baseline level is not estimated in the Bayesian post hoc estimation step with sparse data and thus this population estimation is irrelevant when it comes to individualized dosing.

From a mathematical standpoint, it is interesting to contemplate an analytical solution to the quantification of an individual's baseline that is below the LLOQ. For this, one would require at least two FVIII levels at the same time point following different doses. With these two dose-level pairs, an analytical solution to baseline can be found by rearranging the equation used to scale dose based on an observed trough:

$$Dose_2 = \frac{Level_2 - Baseline}{Level_1 - Baseline} \cdot Dose_1$$

$$\downarrow$$

$$Baseline = \frac{Dose_1 \cdot Level_2 - Dose_2 \cdot Level_1}{Dose_1 - Dose_2}$$

Equivalently, one could plot these dose-level pairs and determine the intercept of the line drawn through them. For example, suppose a patient was given a dose of 1000 IU and a subsequent dose of 3000 IU. By comparing the 24-hour level following each dose, one could determine baseline. While this calculation appears simple, its application can be challenging as the slightest deviation from the expected activity level can have dramatic consequences for the baseline calculation. For example, inaccurate activities (even within 10% of the true value) can result in a negative value for baseline. Attempting this exercise using data from multiple doses in the same

real patients from the WAPPS-Hemo repository resulted in either negative values or impossibly high baseline activity (i.e. >20%) for severe hemophilia patients, suggesting that the imprecision of real data will not allow for an analytical solution to determining baseline. Based on available information, whether it be estimation of baseline from a PopPK modeling approach or a more direct assay approach, baseline in severe hemophilia patients is expected to be greater than zero. Assuming no endogenous production will therefore always overestimate dosing requirements on a population basis.

There are some limitations to our study approach. The PopPK model that was used to create the populations assumed a baseline of 0 for all patients and was partially based on FVIII activities below the limit of quantification (BLQ). In fact, the manuscript describing the model explicitly assessed PK estimation in the presence and absence of the BLQ data [185]. While the assumption of baseline FVIII activity during PopPK assessment in severe hemophilia is unlikely to greatly alter model parameters, simulation from the model to determine generic population doses is dependent on the baseline assumption. We have conducted this study using a single FVIII model, although there is no reason to imagine that similar results couldn't be replicated by using any other brand or model. Similarly, we anticipate that the uncertainty about the true baseline level of clotting factor will have the same consequences for factor IX, where the imprecision could be even larger, as the CRM positive cases are proportionally more.

As to clinical implications, all the above considerations about baseline do not diminish the importance of PopPK modeling and Bayesian estimation in deriving individual regimens, but emphasizes that the method is not helpful for deriving individual baselines. For all patients with measurable baseline levels, individualized dosing methods are obviously not affected by this uncertainty.

Conclusions

The dosing of factor concentrates in severe hemophilia is affected by the true unmeasurable level of endogenous FVIII activity. The sensitivities of FVIII activity assays are close to the commonly targeted trough level – a situation that is unique to hemophilia. On a population level, variation in baseline FVIII levels is a contributing factor to the overall variability in PK response to FVIII. On an individual basis, assumptions of baseline activity greatly impact the estimation of time to 0.01 IU mL^{-1} and the tailoring of a dosing regimen based on observed levels. Further reducing assay sensitivity below 0.005 IU mL^{-1} will decrease the proportion of patients for whom there is uncertainty with respect to baseline FVIII activity. Alternatively, clinicians may choose to target slightly higher troughs (0.02 IU mL^{-1} and above), as these are less sensitive to assumptions of baseline.

Chapter 9: Modeling of body weight metrics for effective and cost-efficient conventional factor VIII dosing in hemophilia A prophylaxis

This chapter is reflective of an original manuscript published by the Ph.D. candidate (Alanna McEneny-King) in *Pharmaceutics*. All pertinent dialogue in this chapter was written by the Ph.D. candidate.

McEneny-King A, Chelle P, Henrard S, Hermans C, Iorio A, Edginton AN. Modeling of body weight metrics for effective and cost-efficient conventional factor VIII dosing in hemophilia A prophylaxis. Pharmaceutics. 2017; 9(4):47. DOI: 10.3390/pharmaceutics9040047.

Introduction

Hemophilia A is an inherited bleeding disorder resulting from a deficiency in clotting factor VIII (FVIII), causing spontaneous and recurring joint bleeds, eventually leading to arthropathy and premature death if left untreated. The mainstay of severe hemophilia treatment is prophylactic replacement of the missing factor. The typical aim of prophylaxis is to maintain a clotting factor level of at least 1 IU dL^{-1}, based on the observation that patients with moderate hemophilia (i.e., those with baseline factor levels >1 IU dL^{-1}) are less prone to the spontaneous bleeds and subsequent arthropathy seen in more severe cases [69]. In a study of 65 boys with severe hemophilia A, only regular prophylactic infusions were shown to prevent joint damage as compared to on-demand treatment [9]. While there is global unanimity that prophylaxis should be initiated before joint disease is sustained [12,13], the implementation of this approach is quite variable [14]. No optimal dosing regimen has been identified; instead, an individualized approach that accounts for the patient's physical activity, current (and accepted future) musculoskeletal condition, and the availability of resources has been suggested [70,265]. Ideally,

the patient's pharmacokinetic (PK) profile is taken into account to define a truly individualized regimen that optimizes both safety and resource utilization [238]. To facilitate the adoption of PK-based dosing regimens, tools such as the Web Accessible Population Pharmacokinetics Service—Hemophilia (WAPPS-Hemo [170,252]) provide estimates of individual PK parameters from a minimal number of samples by leveraging population PK data. Despite the development of these platforms, the majority of hemophilia patients are still dosed according to total body weight, as initially proposed by Ingram in 1981 [266]. For instance, hemophilic children in Canada are started on a once-weekly regimen (50 IU kg^{-1}), then step up to either twice weekly (30 IU kg^{-1}) or every 48 h (25 IU kg^{-1}) as required; prophylaxis regimens in the Netherlands (Utrecht protocol: 15–30 IU kg^{-1} three times per week) and Sweden (Malmö protocol: 25–40 IU kg^{-1} three times per week), though proposing different intensities and targeting different levels, are based on the same principle [267].

The normalization of life expectancy of individuals with hemophilia brings new challenges to hemophilia care. Overweight and obesity rates amongst hemophiliacs now match the epidemic proportions that are seen in the general population [268]. A 2011 study conducted in Ontario found 28.8% of enrolled hemophiliacs were overweight or obese, compared to 26% of healthy controls [269]. Obesity also comes with a higher risk for hemophilic arthropathy; joint range of motion has been shown to negatively correlate with body mass index (BMI) [270]. Furthermore, the total body weight-based dosing regimen currently used in hemophilia treatment may not be appropriate for overweight and obese populations. Calculations for weight-adjusted dosing are based on the following formula:

$$\text{Dose (IU)} = \frac{\text{total body weight (kg)} \times \text{desired increase in FVIII level (\%)}}{\text{IVR}}$$

In vivo recovery (IVR) is a parameter used to describe clotting factor pharmacokinetics, and reflects the rise in factor activity (in this case, FVIII) after a dose is administered. Although it has been suggested that an individual IVR value be determined for each patient [271], typically an IVR of 2 IU dL^{-1}/IU kg^{-1} is assumed. For example, a desired increase to normal FVIII levels (100%) would lead to a 50 IU kg^{-1} dose being administered. However, the assumption that IVR equals 2 for all is not always valid. A study by Henrard et al. found that overweight patients (BMI > 29.6 $kg \cdot m^{-2}$) had a median IVR of 2.70, while underweight patients (BMI < 20.3 $kg \cdot m^{-2}$) had a median IVR of 1.60 [121].

The emerging proportion of overweight and obesity in the general population has prompted research efforts aimed at identifying pharmacokinetic differences (and the corresponding dose adjustments) in this population. The relationship between body size and clearance is well established; a 2012 systematic review of this topic found that more than half of all identified models for clearance included a covariate for body size, most commonly as a power function [272]. Obesity specifically influences several factors affecting drug disposition, including body composition, metabolism by CYP450 enzymes, and plasma protein levels [273]. The most striking differences are observed for highly lipophilic drugs, where volume of distribution changes dramatically in the obese population [274]. However, this is not the case for clotting factor concentrates. FVIII concentrates are typically confined to the vascular space, with volumes of distribution approximating plasma volume (48 $mL \cdot kg^{-1}$) [71]. Since vasculature represents a very small fraction (0.005–0.010) of adipose tissue volume [122], an excess (or scarcity) of fat does not significantly alter the volume of distribution of FVIII. As a result, overweight and obese patients are likely overdosed when dose is calculated using total body weight [275]. A similar issue has been noted for dosing of unfractionated heparin, another

compound whose volume of distribution is approximately equal to the plasma volume; obese children achieved comparable anticoagulation at a lower weight-based dose [276]. Hemophilia treatment is expensive, with annual costs in the hundreds of thousands for those on prophylaxis [9], and while prophylaxis does achieve better health outcomes, these come at a significant cost that is not automatically offset by prevention of other expenses [277]. As the clotting factor itself represents the majority of the cost of prophylaxis [141], overdosing can introduce a significant waste of resources [278]. This study will explore alternative dosing regimens that optimize both safety and resource utilization in overweight and obese hemophiliacs.

Methods

Population generation, simulation, and data analysis were all conducted in MatLab R2009.

Population Generation

The generated population of virtual individuals consists of two equal sized bins classified by BMI using the cut-offs defined by Henrard et al. [121] The first group consists of average weight subjects (BMI between 20.3 and 29.6 $kg \cdot m^{-2}$); the second group represents an overweight and obese population with BMI between 29.6 $kg \cdot m^{-2}$ and 40.0 $kg \cdot m^{-2}$. These cut-off values for BMI were found to be the strongest predictors of FVIII IVR. Each group contains 1000 simulated subjects with a uniform BMI distribution. Heights were derived from the distribution provided by the NHANES database [215]. A uniform distribution of BMI's was simulated and the total body weights were calculated as the product of BMI and the square of height.

Definitions of Weight Metrics

The following weight metrics were defined for each virtual patient from their simulated total body weight (TBW, kg), height (HT, cm) and BMI ($kg \cdot m^{-2}$):

1. Lean body weight (LBW)

$$LBW = \frac{9270 \cdot TBW}{6680 + 216 \cdot BMI}$$

2. Ideal body weight (IBW—Lorentz formula)

$$IBW = HT - 100 - \left(\frac{HT - 150}{4}\right)$$

3. Adjusted body weight (ABW)

$$ABW_{25} = IBW + 0.25 \cdot (TBW - BW)$$

$$ABW_{40} = IBW + 0.40 \cdot (TBW - BW)$$

We used the semi-mechanistic model for LBW developed by Janmahasatian et al. [279] as it has been found to better describe the full range of adult heights and weights [274]. IBW was calculated using Lorentz's formula, which takes into account the patient's height and sex but not total body weight. ABW was the first weight metric intended for use in pharmacokinetic studies; it involves adding a proportion of the excess weight above IBW [280]. This proportion is variable, ranging from 25%–50%, with 40% being used most commonly; in this study, we examined both 25% (ABW_{25}) and 40% (ABW_{40}) correction factors. Correlation plots for all

body size metrics are presented in Supplementary Figure S6 and Figure S7 for normal and overweight/obese individuals, respectively.

Population Pharmacokinetic Model

Simulations were performed using the 2-compartment structure described by Garmann et al. [185] for BAY 81-8973 (Kovaltry®), built on 183 subjects. Of the 109 patients above 18 years of age, the BMI range was 15.0–38.3 $kg \cdot m^{-2}$. The details of the model structure are presented in Table 26. For each simulated individual, PK parameters were calculated. Each virtual individual was then dosed based on various weight metrics and their PK was simulated.

Table 26. Details of the model developed by Garmann et al [185]

Parameter	Estimate	Covariate Effects[c]	BSV[d] (%CV)
Clearance (CL, dL h^{-1})	1.88	$\left(\frac{Lean\ Body\ Weight}{51.1}\right)^{0.610}$	37.0
Intercompartmental clearance (Q, dL h^{-1})	1.90		
Central volume (V_1, dL)	30.0	$\left(\frac{Lean\ Body\ Weight}{51.1}\right)^{0.950}$	11.2
Peripheral volume (V_2, dL)	6.37		
Proportional RUV[a] (%CV[b])	26.7		
Additive RUV (IU dL^{-1})	1.10		

Simulation and Assessment of Treatment Regimens

For each virtual individual, FVIII levels and individual PK parameters were simulated assuming a baseline factor level of 0.5 IU dL^{-1}. FVIII levels were simulated using time steps of 0.2 h following dosing regimens for four weeks to ensure that steady state was reached, and results from the 5th week were used in subsequent analysis steps. In a first instance, we analyzed a typical dosing strategy (20 IU kg^{-1} TBW every 48 h) to evaluate its appropriateness.

We then simulated various regimens wherein equal doses were given at regular intervals (i.e., 48 h). Each patient was dosed from 10 IU kg^{-1} for each weight metric (10 IU kg^{-1} of TBW, 10 IU kg^{-1} of LBW, etc.) up to 210 IU kg^{-1}. Initially, the dose step was 2 IU kg^{-1} for doses up to 100 IU kg^{-1} and 10 IU kg^{-1} for doses between 100 and 210 IU kg^{-1}. After reviewing the results, the dose step was reduced to 0.1 IU kg^{-1} between 20 and 30 IU kg^{-1}, as this was the range of most interest. A regimen was considered to be safe for a BMI group if 95% of the simulated population within that group had factor levels above 1 IU dL^{-1} at all times ($C_{min} \geq 1$ IU dL^{-1}). The lowest dose per weight metric that met this safety criterion was identified and considered to be the optimal regimen for that particular metric and BMI group. A secondary measure of safety was the 95th quantile for time spent below 1 IU dL^{-1}; in other words, the amount of time per week spent below trough for the 5% of the population not meeting the safety criteria. To evaluate economic differences between regimens, we calculated the mean weekly consumption on each optimal regimen to determine which dosing regimen met safety requirements while minimizing resource expenditure. This process was then repeated for a Monday-Wednesday-Friday (M-W-F) dosing schedule. For these simulations, the optimal dose for each metric (determined in the previous simulations) was administered on Monday and Wednesday, and the Friday dose was increased until the safety criterion was reached. To evaluate the importance of the earlier

assumption of 0.5 IU dL^{-1} baseline, we repeated the above simulations assuming a baseline of 0 IU dL^{-1} to observe if similar trends emerged.

Results

Simulations of the typical regimen of 20 IU kg^{-1} TBW every 48 h were completed and the results are summarized in Table 27. We then investigated the hypothesis that a TBW-based dosing regimen results in overdosing in overweight and obese patients by determining the TBW-based dose required to meet the 1 IU dL^{-1} safety criterion in 95% of these patients. At a dose of 20 IU kg^{-1} TBW, the median minimum concentration (C_{min}) throughout the week for these patients was 5.4 IU dL^{-1}; the average consumption associated with this dosing regimen was 7.25 $\times 10^3$ IU per person per week. However, this population requires only 14 IU kg^{-1} TBW to meet the 95% safety criterion, which corresponds to an average weekly consumption of 5.07 $\times 10^3$ IU per person.

Table 27. Comparison of the typical 20 IU kg^{-1} total body weight (TBW) dose and the lowest dose meeting the safety threshold (i.e., 14 IU kg^{-1} TBW) in overweight and obese patients. Results are presented as median (90% confidence interval).

Criterion	Regimen	
	20 IU kg^{-1} TBW, Q48 h	14 IU kg^{-1} TBW, Q48 h
C_{min} (IU dL^{-1})	5.4 (1.2–17.3)	3.9 (1.0–12.3)
Consumption (IU per person per week)	7260 (5730–8780)	5080 (4010–6140)

Following this initial investigation, we explored dosing regimens using alternative weight metrics. The correlation between each weight metric and BMI is shown in Figure 33. We began by administering a dose of 10 IU kg^{-1} of each weight metric on a Q48 h dosing schedule. Once steady state was reached, the percentage of patients with $C_{min} \geq 1.0$ IU dL^{-1} was calculated. If this percentage was below 95%, the dose was incrementally increased until this threshold was reached. We then calculated the mean weekly consumption associated with the minimum dose required to reach the safety criterion for each metric to assess cost-effectiveness. Since a Monday-Wednesday-Friday dosing schedule is commonly used in hemophilia A prophylaxis, we performed analogous simulations using this schedule instead of a regular 48 h interval. We used the optimal doses found in the previous study on Monday and Wednesday, and then increased the dose on Fridays to compensate for the longer interval until the safety criterion was met.

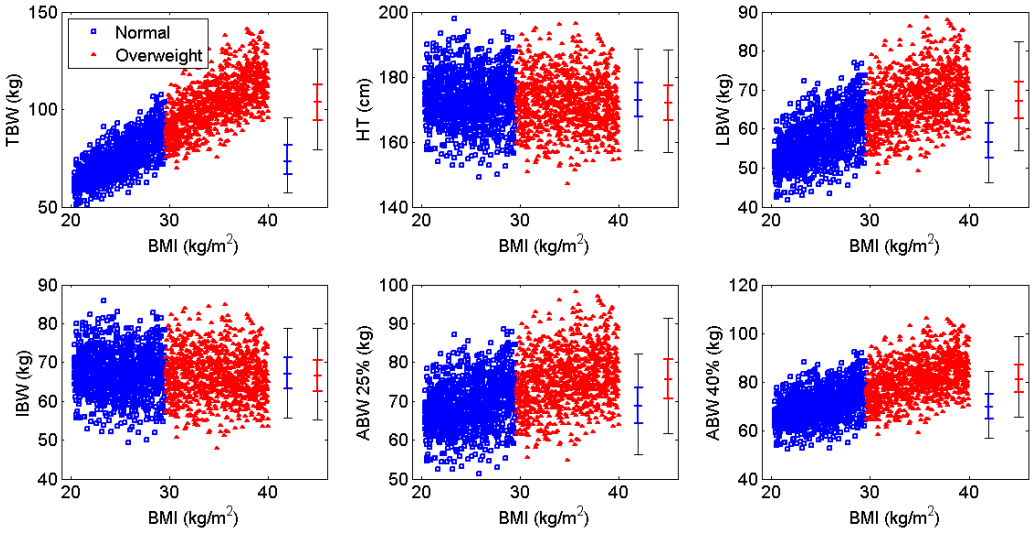


Figure 33. Correlation of body weight metrics with body mass index (BMI) for each BMI subgroup (blue = normal weight, red = overweight and obese). TBW: total body weight; HT: height; LBW: lean body weight; IBW: ideal body weight; ABW: adjusted body weight.

Table 28 and Table 29 summarize the doses per kg of each weight metric required to reach the 95% safety criterion (when infused every 48 h or Monday-Wednesday-Friday, respectively) and the associated weekly consumption in each of the BMI categories and in the merged population, assuming a baseline factor level of 0.5 IU dL^{-1}. The most appropriate regimen is the one that meets the safety requirements while consuming the least amount of factor concentrate. For patients within the normal BMI range, LBW produced the optimal regimen for both dosing schedules; for the overweight and obese cohort, an IBW-based dosing regimen was found to be most cost-effective. Furthermore, the range of mean weekly consumption across the various weight metrics was much tighter for the normal BMI subgroup (125 IU per person per week) as compared to the overweight/obese subgroup (483 IU per person per week). When the two subgroups were combined, ABW with a 25% correction factor proved to be ideal for the Q48 h regimen, with IBW a very close second with a difference of just 5 IU per person per week. Both ABW_{25} and IBW perform almost identically in terms of safety for both BMI subgroups for the Q48 h regimen (Figure 34). However, IBW performed better than all other weight metrics when a Monday-Wednesday-Friday schedule was adopted, with a difference in consumption of over 100 IU per person per week when compared to the next best metric (LBW). Nevertheless, the amount of time spent below 1 IU dL^{-1} is significantly greater when following a Monday-Wednesday-Friday regimen as compared to the Q48 h dosing schedule (Figure 35b); additionally, an extremely high Friday dose (>125 IU kg^{-1} TBW) is required to meet the 95% safety requirement, whereas a dose of 18 IU kg^{-1} TBW is successful for the Q48 h regimen (Figure 35a).

Table 28. Summary of safety and economic evaluations of different weight metrics used in a Q48 h regimen across BMI subgroups, assuming a baseline factor level of 0.5 IU dL^{-1}. Dose is the dose required to have 95% of patients with a steady state C_{min} over 1 IU dL^{-1}. Optimal regimens for each subgroup and the overall population are bolded.

	Normal		Overweight and Obese		All BMI Categories		
Metric	Dose (IU kg^{-1})	Mean Consumption (IU Per Person Per Week)	Dose (IU kg^{-1})	Mean Consumption (IU Per Person Per Week)	Dose (IU kg^{-1})	Mean Consumption (IU Per Person Per Week)	Difference in Consumption from TBW
TBW	20.0	5202	14.0	5074	18.0	5603	-
LBW	**25.6**	**5114**	21.3	5028	23.8	5186	−417
IBW	22.2	5222	**20.7**	**4828**	22.1	5176	−427
ABW_{25}	21.7	5239	20.0	5311	**20.4**	**5171**	**−432**
ABW_{40}	21.1	5173	18.0	5129	20.0	5301	−302

Table 29. Summary of safety and economic evaluations of different weight metrics used in a Monday-Wednesday-Friday regimen across BMI subgroups, assuming a baseline factor level of 0.5 IU dL^{-1}. Dose is the Friday dose required to have 90% of patients with a weekly C_{min} ≥1 IU dL^{-1}. Optimal regimens for each subgroup and the overall population are bolded.

	Normal		Overweight and Obese		All BMI Categories		
Metric	**Dose (IU kg^{-1})**	**Mean Consumption (IU Per Person Per Week)**	**Dose (IU kg^{-1})**	**Mean Consumption (IU Per Person Per Week)**	**Dose (IU kg^{-1})**	**Mean Consumption (IU Per Person Per Week)**	**Difference in Consumption from TBW**
TBW	74	8174	54	9320	62	8716	-
LBW	**94**	**8082**	82	8740	88	8442	−274
IBW	78	8213	**84**	**8543**	**80**	**8312**	**−404**
ABW_{25}	78	8195	72	8558	76	8459	−258
ABW_{40}	76	8126	68	8792	72	8481	−235

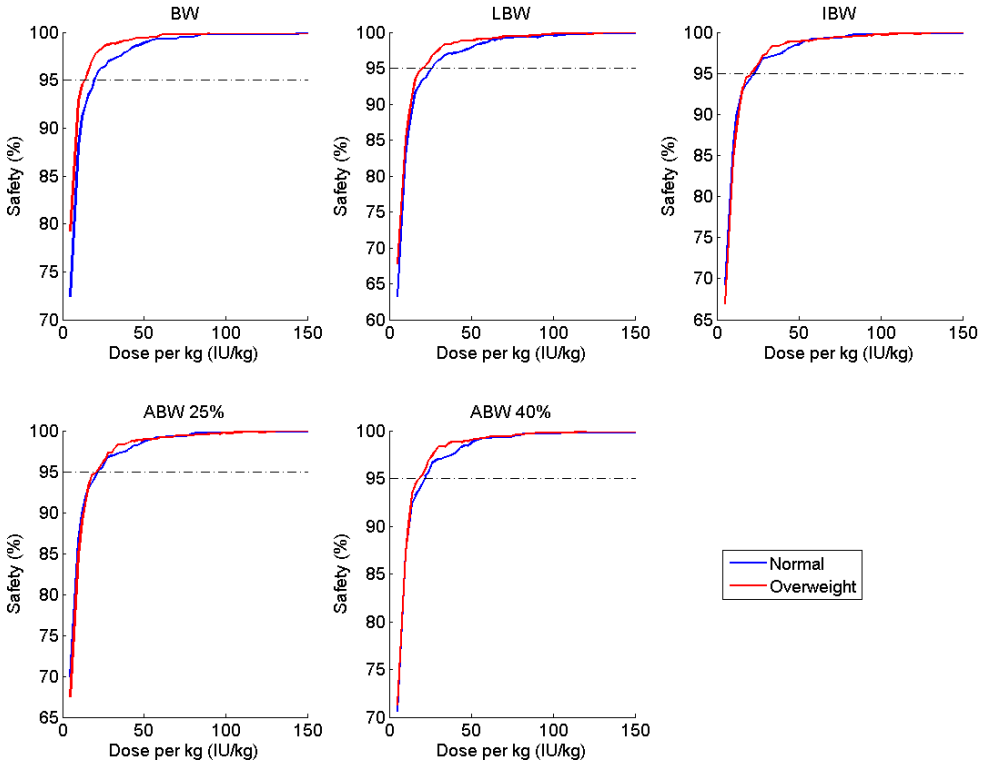


Figure 34. Percentage of patients with $C_{min} \geq 1$ IU dL^{-1} (safety) at increasing doses per kg of various weight metrics, stratified by BMI subgroup, administered at 48 h intervals.

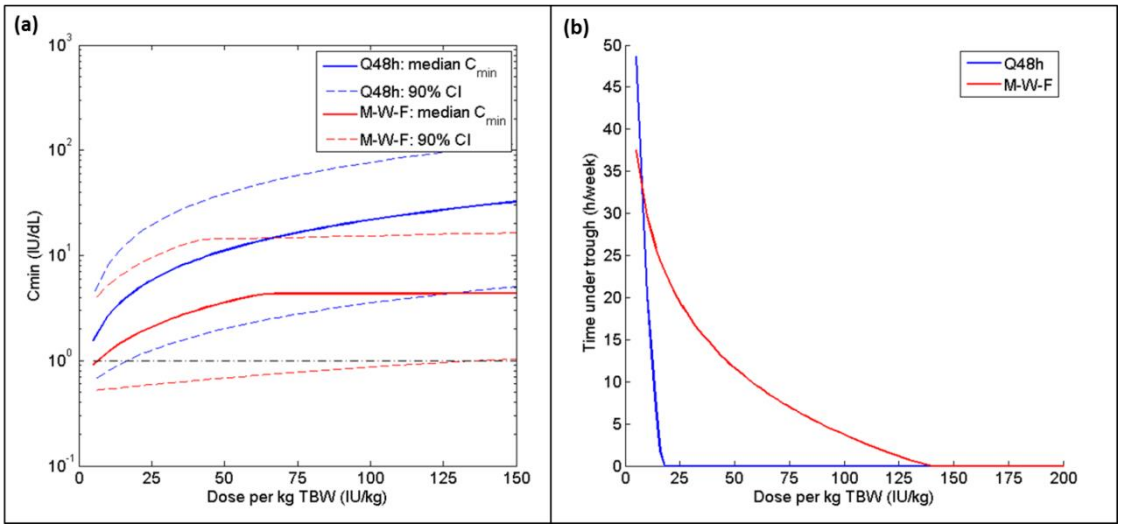


Figure 35. (a) Median and 90% confidence intervals for C_{min} and (b) 95th quantile for time spent below 1 IU dL^{-1} (hours per week) for TBW-based dosing regimen administered at different intervals for the combined group (normal + overweight/obese) for both Q48 h (blue) and Monday-Wednesday-Friday (red) dosing schedules. For the Q48 h regimen, all doses are increasing along the *X*-axis; for the Monday-Wednesday-Friday schedule, only the Friday dose is changing (Monday and Wednesday doses are fixed at 20 IU per kg TBW)

Ideal body weight continued to perform well in simulations with an assumed baseline of 0 IU dL^{-1}. The safety ratio versus dose curves are once again nearly identical for both BMI subgroups (Figure 36), although consumption was approximately doubled as compared to the Q48 h regimen.

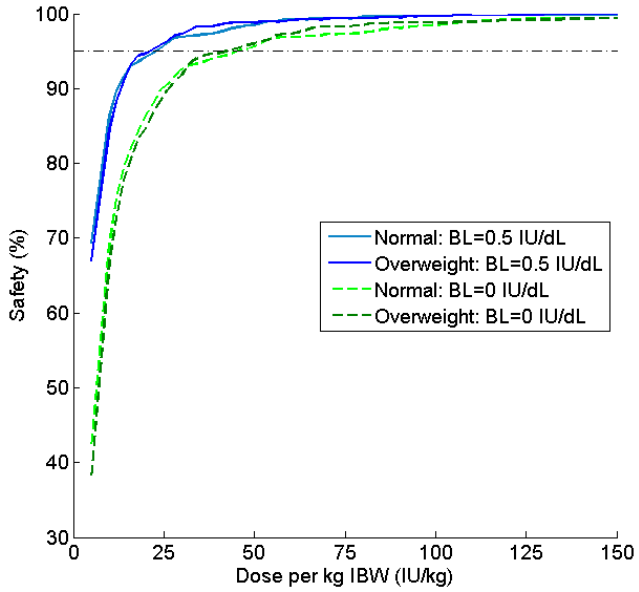


Figure 36. Comparison of safety profiles for patients simulated with baseline (BL) 0.5 IU dL^{-1} and 0 IU dL^{-1} for a Q48 h regimen. Safety (%) is the percentage of patients with $C_{min} \geq 1$ IU dL^{-1} at various doses per kg of IBW.

Discussion

We began by assessing the safety and cost-effectiveness of a typical 20 IU kg^{-1} TBW, Q48 h regimen in an overweight and obese patient population. For comparison, we determined the TBW-based dose required to meet the safety criterion. At a dose of 14 IU kg^{-1} TBW, 95% of patients had FVIII levels of at least 1 IU dL^{-1} at all times; the median C_{min} was 3.9 IU dL^{-1} and the mean consumption was just over 5000 IU per person per week. By contrast, the 20 IU kg^{-1} TBW regimen produced a median C_{min} of 5.4 IU dL^{-1} with a mean consumption of 7250 IU per person per week. Hence, the standard TBW-based dosing protocol results in over 40% higher

consumption than required in the overweight and obese population; assuming a cost of $1 US per unit of concentrate, this amounts to over $100,000 US in excess spending per person annually. From this evaluation, it is clear that TBW does not represent the optimal body weight metric to guide FVIII dosing.

Simulations using dosing regimens based on alternative weight metrics (LBW, IBW, ABW_{25}, and ABW_{40}) were carried out using the two most common dosing schedules in hemophilia A prophylaxis: a regular 48 h regimen and a Monday-Wednesday-Friday regimen. Adapting a Monday-Wednesday-Friday timetable made it extremely difficult to meet the safety requirement, regardless of which weight metric was used to define the dose. While patients are often advised to increase their FVIII dose on Friday, a simple doubling of the dose is not sufficient. A potentially harmful Friday dose of 140 IU kg^{-1} TBW was required for 95% of patients to have a $C_{min} \geq 1$ IU dL^{-1}, compared to 18 IU kg^{-1} TBW to meet this safety minimum when infused every 48 h. Furthermore, the time spent below 1 IU dL^{-1} (and, consequently, the risk of bleeding events [10]) is significantly greater when following a Monday-Wednesday-Friday regimen, even if the Friday dose is twice or three times greater than the Monday and Wednesday doses (Figure 3b). In fact, a 2010 study in which FVIII was administered three times per week found that over 80% of bleeds occurred 48–72 h post-infusion [281]. The Monday-Wednesday-Friday treatment schedule, while more convenient, is no longer considered to be optimal therapy due to this increased vulnerability to bleeds during the weekend, with alternate day dosing representing the ideal regimen [205,233].

Due to analytical limitations, it can be difficult to obtain an exact measure of a patient's baseline factor level. Many assays have a lower limit of quantification of 1 IU dL^{-1} [282,283], which is greater than endogenous levels for severe hemophilia patients. To balance both safety

and resource utilization, we ran initial simulations with an assumed baseline of 0.5 IU dL^{-1}. However, it is known that many severe hemophilia patients possess a genetic mutation such that no functional FVIII is produced endogenously. For this reason, the simulations were performed again using a baseline of 0 IU dL^{-1} to ensure similar trends were observed within this sub-population. Notably, a 95% safe ratio can be achieved in a population with no endogenous FVIII production at a reasonable dose (34 IU kg^{-1} TBW) if administered every 48 h. However, it is not possible to meet that safety threshold in this population if a Monday-Wednesday-Friday dosing schedule is employed. If the safety criteria is lowered to 90%, it can be met, but only with extremely high Friday doses (between 130 and 180 IU kg^{-1} for the various weight metrics) and associated weekly consumption (>16,000 IU per person per week); a study by Collins et al. found similarly high doses (>100 IU kg^{-1} for patients with average half-lives, and up to 400 IU kg^{-1} in extreme cases) were required to maintain FVIII levels above 1 IU dL^{-1} throughout the week when following this dosing schedule [74]. These results suggest that a regular dosing interval of 48 h offers significant advantages over the weekly Monday-Wednesday-Friday schedule in terms of both safety and cost-effectiveness.

After exploring all combinations of dosing schedule and baseline factor level, we determined that IBW-based dosing provides a safe and cost-effective regimen in the majority of scenarios, with ABW_{25} producing fairly similar results. Ideal body weight performed almost exactly the same in terms of safety between the normal and overweight groups across all of the doses and regardless of baseline, as evidenced by the closeness of the curves shown in Figures 2 and 4. Further, IBW was the most cost-effective in three out of four simulations; in the fourth, it differed by only 5 IU per person per week from the optimal regimen (ABW_{25}). If we compare the optimal regimen for a Q48 h schedule with a baseline of 0.5 IU dL^{-1} (i.e., 20.7 IU kg^{-1} IBW) to a

20 IU kg^{-1} TBW, this alterative regimen offers a savings of over 2000 IU per person per week (or nearly $110,000 US annually) for overweight and obese patients. Thus, IBW-based dosing offers a similar safety profile to the currently used TBW strategy while moderating the economic burden of clotting factor prophylaxis.

This exercise was limited by the constraints of the data. The model used herein was built on PK data from a specific brand of FVIII concentrate, although brand has not generally been found to significantly influence PK. A second limitation to the applicability of this approach is that the source data is largely from older (10+ years of age) patients, and the opinions on use of prophylaxis in adults are varied [284–286]. Obesity rates are also increasing rapidly amongst pediatric patients and similar dosing adjustments are likely appropriate in this population, but cannot be confirmed in this study. Further study of pediatric populations (and validated pediatric population PK models) is required in order to determine a dosing regimen that applies not only to all BMI's but also to all ages.

As the prevalence of obesity has risen in the general population, a number of studies have been conducted to investigate the frequency of overweight and obesity among hemophilia patients, complications such as co-morbidities and decreased quality of life, and recommendations for management strategies. Many pharmacokinetic studies exploring the relationship between excess body weight and plasma volume (and, by extension, in vivo recovery) have postulated that dosing according to body weight results in overdosing and an ineffective use of resources, suggesting instead that dosing be guided by LBW or IBW [121,273,287,288]. This study compared several weight metrics and confirmed that an IBW-based regimen is both safe and cost-effective across a range of BMI's. Ideal body weight produced slightly better results than other weight metrics because it is calculated based solely on

height; as shown in Figure 33, there is no correlation between IBW and BMI as observed with the other metrics investigated.

Although we were able to identify a weight metric that is more suitable for a variable population, the high inter-individual variability in PK handling of factor concentrates precludes the definition of a single, "one dose fits all" strategy. In order to optimize prophylaxis, regimens should be tailored to the individual PK profile. This process has been facilitated by the development of the WAPPS-Hemo service (www.wapps-hemo.org), a Canadian-based user-friendly and industry-independent platform that produces estimates of individual PK parameters through a Bayesian iterative approach. The WAPPS-Hemo service also includes a module for dosing regimen development, wherein clinicians can predict the effects of changing dose, frequency, or targeted trough for a specific patient before implementing these changes in practice. While PK-tailored dosing regimens may offer the best results, weight-based strategies are still the norm, but these can be optimized by adapting a different weight metric (i.e., IBW) to guide safe and cost-effective dosing at a population level.

Conclusions

In summary, we conducted simulations based on a previously published model of a conventional FVIII to explore the appropriateness of different weight metric-based dosing regimens for hemophilia A prophylaxis for overweight and obese patients. Regimens were required to produce a C_{min} $\geq$1 IU dL^{-1} in 95% of the population, and then the average consumption for each regimen was calculated to evaluate resource-effectiveness. From this study, we conclude that ideal body weight performs the best, maintaining safety while tempering factor consumption for overweight and obese patients.

Chapter 10: Discussion, Conclusions, and Future Directions

Discussion

The overarching objective of this thesis is to develop PopPK models for clotting factor concentrates that enable dosing regimen individualization for hemophilia patients, and to understand the clinical factors that influence the performance of such models.

The opening chapter of the thesis consists of a comprehensive review of recent pharmacokinetic studies of FVIII concentrates, including plasma-derived and recombinant, standard and extended half-life products. This exercise had two primary objectives: (i) to summarize the variability observed in FVIII concentrate PK, thereby confirming the appropriateness of a PK-tailored dosing regimen in hemophilia, and; (ii) to identify covariates that have been found to influence FVIII PK. The often noted high inter-patient variability (particularly on clearance and, consequently, half-life) was extremely apparent; not only was there considerable discrepancy between studies (SHL FVIII half-life ranged from 9.4 to 17.4 h), but within-study variability was also high (>30%) in many cases.

Age, weight, vWF level, and blood group were identified as important determinants of FVIII PK. However, the effects of age and blood group are related to vWF. Lalezari and colleagues found that vWF levels increase with age [120], hence the reduced FVIII clearance and longer half-lives observed in older hemophilia A patients. ABO blood group has also been shown to correlate with vWF level in several studies with a variety of patient populations [117,289–291], with blood group O individuals typically having approximately 25% lower plasma levels of vWF [292]. Furthermore, a 2018 study found an interaction between age and blood group with respect to vWF level; the age-related increase in vWF was more pronounced in

the non-O group than in those with O-type blood, leading to larger discrepancies later in life [117]. This proposed interconnectedness is reinforced by two studies that observed minimal blood group-related differences in vWF levels in children [117,293,294]. The details of the mechanism through which blood group and vWF levels are related remains unclear; one hypothesis proposes that O-type blood increases vWF susceptibility to cleavage by the protease ADAMTS13 [295], while another suggests that variations in antigen expression across blood groups mediates this effect [296].

Chapter 2 outlines a data analysis protocol for model development and evaluation that began the process of standardizing the model-building procedure for the WAPPS-Hemo project, which is predicated on models that can reliably produce individual PK estimates from sparse samples. This section builds on a previously published data analysis protocol [170], drawing on the WAPPS-Hemo modelling team's unique experience with PopPK models for Bayesian forecasting of clotting factor concentrate PK. The protocol was subsequently applied to build the generic SHL FVIII model (Chapter 4). This unique model is built specifically to meet the needs of the WAPPS-Hemo project; not only is the model fit for Bayesian forecasting, it is also able to predict the PK of SHL FVIII products outside of the modelling dataset. This second capability is of considerable importance, as a plethora of such products exist, but not all meet the data requirement for a brand-specific model. Since its implementation on the WAPPS-Hemo platform, the generic SHL FVIII model has been used to process over 2,000 PK requests in more than 1,240 patients; of particular note, 511 of these requests were for brands outside of the original modelling dataset.

The data analysis protocol was once again put into practice in the development of the generic SHL rFIX model described in Chapter 5. In addition to its use in the WAPPS-Hemo

project, this model was used to answer a research question stemming from an ongoing clinical trial investigating the PK of the rFIX product IXINITY in children (NCT03855280). Several PK outcomes are to be estimated following a sampling schedule based on recommendations from the European Medicines Agency [297]. However, concerns arose that participating centres may be averse or unable to collect all five of the intended samples from young patients due to pediatric sample volume limitations. To determine how omission of a sampling point would impact PK parameter estimates, a limited sampling analysis was performed using the generic SHL rFIX model, which leveraged adult and pediatric PK data from other rFIX products and adult data from IXINITY to develop a PopPK model that is valid for all ages and all available brands of rFIX. The results of this analysis highlight the robustness of Bayesian forecasting in limited sampling conditions, especially compared to the traditionally used noncompartmental methods. Further, it confirmed the sensitivity of half-life estimation to sampling duration when using NCA. The original EMA-based sampling strategy produced biased estimates of half-life (mean error: –24%) as its latest sampling time was 48 h; the addition of a 72 or 96 h sample quickly remedied this problem, without significantly altering estimates of other outcomes of interest.

In addition to developing original PopPK models using the WAPPS-Hemo database, previously published models were also employed to answer specific questions relevant to hemophilia treatment. First, the Nestorov model for Eloctate (rFVIIIFc) was used in Chapter 6 to determined limited sampling strategies for this relatively new EHL FVIII product. Sampling designs consisting of two to three timepoints were assessed not only on the accuracy of the PK parameter estimates, but also on whether the same clinical decisions regarding dose adjustment would be made compared to a rich sampling design. Distinct LSSs were determined for adult, adolescent, and pediatric patients. This highlights a need to investigate limited sampling

strategies in children for other factor concentrate products, as current ISTH guidance provides one sampling strategy per product group, regardless of age [169].

This study was undertaken while visiting St. Jude Children's Research Hospital in Memphis, Tennessee. Since their hemophilia clinic runs on a weekly basis, the hematology team at St. Jude typically only obtains patient samples at predose and peak. For this reason, factors outside of sampling time were considered in order to optimize the results of this sampling strategy, such as predose handling and study day. Through this limited sampling analysis, it was determined that when prior dosing information is taken into account and the PK study is performed 48 hours after the last dose, reasonable estimates of troughs and PK parameters can be obtained (median AE <12%) and the rate of inappropriate dose adjustments is low (<4%) for pediatric patients. Clinicians at St. Jude identified that taking the typical 72-hour predose level often resulted in a BLQ sample, and altered their protocol to collect a 48-hour predose in its place; this simulation study provides justification for this modification and quantifies the improvement seen with this new strategy. In addition, the results of the investigation into predose handling have been incorporated into the WAPPS-Hemo platform, as a description of the patient's regimen is now being collected.

The Garmann model for Kovaltry, a full-length rFVIII product, was used to explore to consequence of unmeasurable endogenous FVIII activity. This project originated while designing the Clinical Calculator component of the WAPPS-Hemo service. In this module, clinicians enter two of the following parameters to calculate the third: dose, frequency, and target trough. This amounts to scaling the infusion that was submitted for PK estimation to the selected dosing scenario. However, only exogenous factor should be scaled, as the endogenous (or baseline) level remains constant. While this may sound like a straightforward correction,

endogenous FVIII levels are often unmeasurable in severe hemophilia A patients and although the range of possible baseline values may be narrow, the consequences of mishandling this assumption can be quite significant. In Chapter 8, it is illustrated how baseline assumptions can contribute to the variation in dosing requirement to maintain the target trough of 1 IU/dL. When estimating individual PK outcomes, some parameters are more robust than others. For example, half-life differs minimally (<1 h difference) when the full range of undetectable baseline values is explored while the estimated time to reach 1 IU/dL varies by almost a full day. Although no way of calculating baseline was determined in this study, the potential error that can arise from disregarding the importance of baseline was quantified.

The final example of repurposing an existing PopPK model is in Chapter 9, where the Garmann model was again used to determine a superior body weight metric for FVIII dosing for settings in which PK-tailored dosing is difficult to implement. The suspicion that total body weight may result in suboptimal dosing regimens for some patients is based on the high *in vivo* recovery observed for overweight and obese patients. This arises from the fact that FVIII stays primarily within the plasma space and plasma volume does not necessarily increase with total body weight, leading to the hypothesis that alternative weight metrics such as lean body weight, ideal body weight, or adjusted body weight may curtail overdosing in high BMI patients while maintaining safety. While overdosing of factor products does not generally result in toxicity due to their rapid clearance, overdosing is still of concern as clotting factor concentrates are an extremely expensive therapy. When considering variable endogenous FVIII production (0.5% baseline FVIII vs. true zero) and multiple dosing schedules (Q48h vs. Monday-Wednesday-Friday), ideal body weight was found to produce the most cost-effective regimen that kept FVIII levels above 1 IU/dL for 95% of patients of all BMIs. Switching an obese patient from the label

dose of 20 IU/kg of total body weight to 20 IU/kg of ideal body weight results in a 33% cost reduction, or over $125,000 saved per year.

Finally, Chapter 7 chronicles the growth of the WAPPS-Hemo project in recent years, and demonstrates the clinical application of this particular tool for estimation of individual PK parameters to guide dosing decisions in hemophilia care. Although the potential benefits of individually tailored dosing regimens have been extolled since the early 1990s, uptake of PK-tailored dosing has traditionally been hampered by rigorous sampling requirements, the need for a washout period, and limited availability of tools for PK analysis. By examining two distinct periods since WAPPS-Hemo was launched in 2015, trends regarding both patient characteristics and sampling patterns could be elucidated. The use of WAPPS-Hemo by hemophilia treatments providers increased by more than 3-fold between the two periods investigated. More than 1,900 infusions were eligible for the analysis, with 85% corresponding to FVIII concentrates. Unsurprisingly, the usage of EHL FVIII products increased considerably during Period 2, nearly doubling from 15% to 29%. Patients also tended to be younger during the second timeframe, though still adult (median age 26 vs. 18). During both time periods, sampling times were well aligned with the windows recommended in recent ISTH guidance. However, providers continue to collect peak samples, despite no formal suggestion to do so. In terms of number of samples, there were minimal differences across the two time periods; while there was a trend towards fewer samples per infusion, a significant difference was only noted for SHL FVIII products. It was also hypothesized that increased familiarity with the PopPK approach might influence sampling strategy or patient selection. Lower use centres tended to submit infusions for younger patients, and sampled more heavily than high use centres; however, the differences between centre types narrowed in Period 2. The analysis conducted in Chapter 7 serves to quantify the

recent uptake of the WAPPS-Hemo platform for implementation of PK-tailored dosing regimens in hemophilia. For the WAPPS-Hemo network, this provides an understanding of how the service is being utilized in practice, identifies key populations for consideration during model development, and, in light of the LSA results of Chapters 5 and 6, indicates potential opportunities for user education. Furthermore, this chapter highlights the timeliness of the research contained in this thesis, as the hemophilia community has clearly embraced the PopPK approach in recent years.

Conclusions

Although several novel therapies from monoclonal antibodies to gene therapy are being explored and incorporated into hemophilia care, prophylactic use of clotting factor concentrates still dominates globally. The use of PopPK to individualize the doses of these products is relatively new to the hemophilia community, but has grown rapidly in recent years. The first formal guidance on the utilization of PopPK for dose individualization was published in 2017. The WAPPS-Hemo network has expanded enormously over the course of this project, from fewer than 25 centres in 2015 to over 400 centres at the time of this thesis, and individual PK estimates have been generated for over 6,000 patients from more than 40 countries.

The collective aim of this work is to improve the safety and efficiency of hemophilia treatment through pharmacokinetic modelling. The dissertation presents two original PopPK models, one for SHL FVIII products and one for SHL rFIX concentrates. Both models have been incorporated into the WAPPS-Hemo platform, and have been used in the estimation of over 2,100 individual PK profiles to date. A number of simulation studies were also performed to determine how clinical factors impact model performance. Limited sampling analyses were used

to identify not only the optimal timing of samples for Bayesian forecasting, but also to explore the influence of predose handling and the choice of PK study day on PK estimates, and to compare the performance of Bayesian methods against traditional noncompartmental methods in limited sampling conditions. Simulation studies were performed to demonstrate how uncertainty around endogenous factor production affects PK parameter estimation, and to propose alternative weight-based dosing strategies when PK-based tailoring is infeasible.

Future Directions

The WAPPS-Hemo project continues to grow, and recent focus has shifted from model development to tackling research questions that emerge in routine hemophilia care. The PK data repository assembled since the program was launched uniquely positions the WAPPS-Hemo team to answer questions in the hemophilia space, ranging from the practice-based (e.g. what is the prevalence of off-label dosing in hemophilia?) to the physiological (e.g. can we predict a patient's vWF level from other characteristics such as age and blood group?). The newly launched patient app (myWAPPS) may allow for the study of patient adherence and the benefits of increased patient awareness and involvement in their treatment.

As mentioned, the uptake of the PopPK approach in routine hemophilia care is ongoing. An important task will be the continued advocacy for this approach, and education to ensure the best possible results are obtained. Results from a pilot trial using WAPPS-Hemo published in 2019 reported a significant reduction in annual joint bleed rate and increased quality of life when patients were switched to PK-tailored prophylaxis [237]; further additions to the evidence base in favour of PK-tailored dosing will build provider and patient confidence in PK-based dosing.

Despite the recent progress of PopPK in this area, some patient populations remain underserved, particularly patients with inhibitors. The development of neutralizing antibodies against the exogenous clotting factor presents a significant challenge in hemophilia A, as roughly one third of severe patients will present with inhibitors at some point during treatment. Inhibitors are typically eradicated using immune tolerance induction (ITI, regular infusion of large doses of FVIII) though the specifics of this method (e.g. factor product, dosing, and use of immune modulators) remain undefined. Provided longitudinal data is available, a PopPK model built on patients with inhibitors could provide insight into the terminal phase of ITI, during which patients' half-lives begin to normalize. The time required for this half-life normalization can vary significantly between patients; a PopPK model could identify covariates responsible for this variability. The final criterion for successful ITI is a half-life of at least 6 hours; a limited sampling analysis could be used to determine the best sampling times to assess PK during ITI.

References

1. Davie EW. Biochemical and molecular aspects of the coagulation cascade. In: Thrombosis and Haemostasis. 1995. p. 1–6.

2. Davie EW, Fujikawa K, Kisiel W. The Coagulation Cascade: Initiation, Maintenance, and Regulation. *Biochemistry*. 1991;30(43):10363–70.

3. McGee MP, Li LC. Functional difference between intrinsic and extrinsic coagulation pathways. Kinetics of factor X activation on human monocytes and alveolar macrophages. *J Biol Chem*. 1991;266(13):8079–85.

4. Rand MD, Lock JB, Van't Veer C, Gaffney DP, Mann KG. Blood clotting in minimally altered whole blood. *Blood*. 1996;88(9):3432–45.

5. Brummel KE, Paradis SG, Butenas S, Mann KG. Thrombin functions during tissue factor-induced blood coagulation. *Blood*. 2002;100(1):148–52.

6. Iorio A, Stonebraker JS, Chambost H, et al. Establishing the Prevalence and Prevalence at Birth of Hemophilia in Males. *Ann Intern Med*. 2019;171(8):540.

7. Ahlberg Å. Incidence, Treatment and Prophylaxis of Arthropathy and other Musculo-Skeletal Manifestations of Haemophilia A and B. *Acta Orthop Scand*. 1965;36(sup77):3–132.

8. Nilsson IM, Berntorp E, Löfqvist T, Pettersson H. Twenty-five years' experience of prophylactic treatment in severe haemophilia A and B. *J Intern Med*. 1992;232(1):25–32.

9. Manco-Johnson MJ, Abshire TC, Shapiro AD, et al. Prophylaxis versus episodic treatment to prevent joint disease in boys with severe hemophilia. *N Engl J Med*. 2007;357(6):535–44.

10. Collins PW, Blanchette VS, Fischer K, et al. Break-through bleeding in relation to predicted factor VIII levels in patients receiving prophylactic treatment for severe hemophilia A. *J Thromb Haemost*. 2009;7(3):413–20.

11. Oldenburg J. Prophylaxis in bleeding disorders. *Thromb Res*. 2011;127:S14–7.

12. Berntorp E, Spotts G, Patrone L, Ewenstein B. Advancing personalized care in hemophilia A: ten years' experience with an advanced category antihemophilic factor prepared using a plasma/albumin-free method. *Biol Targets Ther*. 2014;8:115.

13. Fischer K, Collins PW, Ozelo MC, et al. When and how to start prophylaxis in boys with severe hemophilia without inhibitors: communication from the SSC of the ISTH. *J Thromb Haemost*. 2016;14(5):1105–9.

14. Petrini P. Identifying and overcoming barriers to prophylaxis in the management of haemophilia. *Haemophilia*. 2007;13(SUPPL. 2):16–22.

15. Carcao M, Srivastava A. Factor VIII/factor IX prophylaxis for severe hemophilia. *Semin Hematol*. 2016;53(1):3–9.

16. Carlsson M, Björkman S, Berntorp E. Multidose pharmacokinetics of factor IX: implications for dosing in prophylaxis. [Internet]. Vol. 4, Haemophilia : the official journal of the World

Federation of Hemophilia. 1998.

17. Goudemand J, Peynet J, Chambost H, et al. A cross-over pharmacokinetic study of a double viral inactivated factor IX concentrate (15 nm filtration and SD) compared to a SD factor IX concentrate. *Thromb Haemost*. 1998;80(6):919–24.

18. Björkman S, Shapiro AD, Berntorp E. Pharmacokinetics of recombinant factor IX in relation to age of the patient: implications for dosing in prophylaxis. *Haemophilia*. 2001;7(2):133–9.

19. Roth DA, Kessler CM, John Pasi K, et al. Human recombinant factor IX: Safety and efficacy studies in hemophilia B patients previously treated with plasma-derived factor IX concentrates. *Blood*. 2001;98(13):3600–6.

20. Ewenstein BM, Joist JH, Shapiro AD, et al. Pharmacokinetic analysis of plasma-derived and recombinant F IX concentrates in previously treated patients with moderate or severe hemophilia B. *Transfusion*. 2002;42(2):190–7.

21. Hoots WK, Leissinger C, Stabler S, et al. Continous intravenous infusion of a plasma-derived factor IX concentrate (Mononine®) in haemophilia B. *Haemophilia*. 2003;9(2):164–72.

22. Kisker CT, Eisberg A, Schwartz B, et al. Prophylaxis in factor IX deficiency product and patient variation. *Haemophilia*. 2003;9(3):279–84.

23. Ahnström J, Berntorp E, Lindvall K, Björkman S. A 6-year follow-up of dosing, coagulation factor levels and bleedings in relation to joint status in the prophylactic treatment of haemophilia. Vol. 10, Haemophilia. 2004. p. 689–97.

24. Ruiz-Sáez A, Hong A, Arguello A, et al. Pharmacokinetics, thrombogenicity and safety of a double viral inactivated factor IX concentrate compared with a prothrombin complex concentrate. *Haemophilia*. 2005;11(6):583–8.

25. Chang HH, Yang YL, Hung MH, Tasy W, Shen MC. Pharmacokinetic study of recombinant human factor IX in previously treated patients with hemophilia B in Taiwan. *J Formos Med Assoc*. 2007;106(4):281–7.

26. Lambert T, Recht M, Valentino LA, et al. Reformulated BeneFix®: Efficacy and safety in previously treated patients with moderately severe to severe haemophilia B. *Haemophilia*. 2007;13(3):233–43.

27. Klukowska A, Laguna P, Svirin P, Shiller E, Vdovin V. Efficacy and safety of OCTANINE F in children with haemophilia B. *Haemophilia*. 2008;14(3):531–8.

28. Aznar JA, Cabrera N, Matysiak M, et al. Pharmacokinetic study of a high-purity factor IX concentrate (Factor IX Grifols®) with a 6-month follow up in previously treated patients with severe haemophilia B. *Haemophilia*. 2009;15(6):1243–8.

29. Monahan PE, Liesner R, Sullivan ST, et al. Safety and efficacy of investigator-prescribed BeneFIX® prophylaxis in children less than 6 years of age with severe haemophilia B. *Haemophilia*. 2010;16(3):460–8.

30. Lissitchkov T, Matysiak M, Zavilska K, et al. A clinical study assessing the pharmacokinetics, efficacy and safety of AlphaNine ®, a high-purity factor IX concentrate, in patients with severe haemophilia B. *Haemophilia*. 2011;17(4):590–6.

31. Mauser-Bunschoten EP, Kleine Budde I, Lopaciuk S, et al. An ultrapure plasma-derived monoclonal antibody-purified factor IX concentrate (Nonafact®), results of phase III and IV clinical studies. *Haemophilia*. 2011;17(3):439–45.

32. Negrier C, Knobe K, Tiede A, Giangrande P, Møss J. Enhanced pharmacokinetic properties of a glycoPEGylated recombinant factor IX: A first human dose trial in patients with hemophilia B. *Blood*. 2011;118(10):2695–701.

33. Martinowitz U, Shapiro A, Quon D V., et al. Pharmacokinetic properties of IB1001, an investigational recombinant factor IX, in patients with haemophilia B: repeat pharmacokinetic evaluation and sialylation analysis. *Haemophilia*. 2012;18(6):881–7.

34. Santagostino E, Negrier C, Klamroth R, et al. Results of a phase I international clinical trial of recombinant fusion protein linking coagulation factor IX with albumin (rIX-FP) in patients with hemophilia B (PROLONG-9FP). *Hamostaseologie*. 2012;32(12):A12.

35. Serban M, Skotnicki AB, Colovic M, et al. Clinical efficacy, safety and pharmacokinetic properties of the plasma-derived factor IX concentrate Haemonine ® in previously treated patients with severe haemophilia B. *Haemophilia*. 2012;18(2):175–81.

36. Shapiro AD, Ragni M V., Valentino LA, et al. Recombinant factor IX-Fc fusion protein (rFIXFc) demonstrates safety and prolonged activity in a phase 1/2a study in hemophilia B patients. *Blood*. 2012;119(3):666–72.

37. Lissitchkov T, Matysiak M, Zavilska K, et al. Head-to-head comparison of the pharmacokinetic profiles of a high-purity factor IX concentrate (AlphaNine®) and a recombinant factor IX (BeneFIX®) in patients with severe haemophilia B. *Haemophilia*. 2013;19(5):674–8.

38. Powell JS, Pasi KJ, Ragni M V., et al. Phase 3 study of recombinant factor IX Fc fusion protein in hemophilia B. *N Engl J Med*. 2013;369(24):2313–23.

39. Alamelu J, Bevan D, Sorensen B, Rangarajan S. Pharmacokinetic and pharmacodynamic properties of plasma-derived vs. recombinant factor IX in patients with hemophilia B: A prospective crossover study. *J Thromb Haemost*. 2014;12(12):2044–8.

40. Windyga J, Lissitchkov T, Stasyshyn O, et al. Pharmacokinetics, efficacy and safety of BAX326, a novel recombinant factor IX: a prospective, controlled, multicentre phase I/III study in previously treated patients with severe (FIX level <1%) or moderately severe (FIX level ≤2%) haemophilia B. *Haemophilia*. 2014;20(1):15–24.

41. Martinowitz U, Lissitchkov T, Lubetsky A, et al. Results of a phase I/II open-label, safety and efficacy trial of coagulation factor IX (recombinant), albumin fusion protein in haemophilia B patients. *Haemophilia*. 2015;21(6):784–90.

42. Suzuki N, Takedani H, Hirakawa A, Ushijima Y, Matsushita T. The features of clearance in recombinant factor IX (BeneFIX®). *Haemophilia*. 2015;21(5):702–7.

43. Carcao M, Zak M, Abdul Karim F, et al. Nonacog beta pegol in previously treated children with hemophilia B: results from an international open-label phase 3 trial. *J Thromb Haemost*. 2016;14(8):1521–9.

44. Kenet G, Chambost H, Male C, et al. Long-acting recombinant fusion protein linking coagulation factor IX with albumin (rIX-FP) in children: Results of a phase 3 trial. *Thromb Haemost*.

2016;116(4):659–68.

45. Korth-Bradley JM, Rendo P, Smith L, Altisent C. Pharmacokinetics, Efficacy, and Safety of Nonacog Alfa in Previously Treated Patients with Moderately Severe to Severe Hemophilia B. *Clin Ther*. 2016;38(4):936–44.

46. Santagostino E, Martinowitz U, Lissitchkov T, et al. Long-acting recombinant coagulation factor IX albumin fusion protein (rIX-FP) in hemophilia B: Results of a phase 3 trial. *Blood*. 2016;127(14):1761–9.

47. Fischer K, Kulkarni R, Nolan B, et al. Recombinant factor IX Fc fusion protein in children with haemophilia B (Kids B-LONG): results from a multicentre, non-randomised phase 3 study. *Lancet Haematol*. 2017;4(2):e75–82.

48. Tiede A, Abdul-Karim F, Carcao M, et al. Pharmacokinetics of a novel extended half-life glycoPEGylated factor IX, nonacog beta pegol (N9-GP) in previously treated patients with haemophilia B: results from two phase 3 clinical trials. *Haemophilia*. 2017;23(4):547–55.

49. Collins PW, Quon DVK, Makris M, et al. Pharmacokinetics, safety and efficacy of a recombinant factor IX product, trenonacog alfa in previously treated haemophilia B patients. *Haemophilia*. 2018;24(1):104–12.

50. Björkman S, Carlsson M. The pharmacokinetics of factor VIII and factor IX: Methodology, pitfalls and applications [Internet]. Vol. 3, Haemophilia. 1997.

51. Björkman S, Berntorp E. Pharmacokinetics of coagulation factors: Clinical relevance for patients with haemophilia. *Clin Pharmacokinet*. 2001;40(11):815–32.

52. Poon M-C. Pharmacokinetics of factors IX, recombinant human activated factor VII and factor XIII. *Haemophilia*. 2006;12(s4):61–9.

53. White GC, Beebe A, Nielsen B. Recombinant factor IX. In: Thrombosis and Haemostasis. 1997. p. 261–5.

54. Poon M-C, Lillicrap D, Hensman C, Card R, Scully M-F. Recombinant factor IX recovery and inhibitor safety: a Canadian post-licensure surveillance study. *Thromb Haemost*. 2002;87(3):431–5.

55. Iorio A, Fischer K, Blanchette V, et al. Tailoring treatment of haemophilia B: accounting for the distribution and clearance of standard and extended half-life FIX concentrates. *Thromb Haemost*. 2017;117(06):1023–30.

56. Ar MC, Vaide I, Berntorp E, Björkman S. Methods for individualising factor VIII dosing in prophylaxis. *Eur J Haematol*. 2014;93(S76):16–20.

57. Björkman S. Limited blood sampling for pharmacokinetic dose tailoring of FVIII in the prophylactic treatment of haemophilia A. *Haemophilia*. 2010;16(4):597–605.

58. Holford NHG, Buclin T. Safe and effective variability-a criterion for dose individualization. *Ther Drug Monit*. 2012;34(5):565–8.

59. Björkman S. Evaluation of the TCIWorks Bayesian computer program for estimation of individual pharmacokinetics of FVIII. *Haemophilia*. 2011;17(1):e239–40.

60. BK170028: myPKFiT for ADVATE | FDA [Internet].

61. Mannucci PM, Tuddenham EGD. The Hemophilias — From Royal Genes to Gene Therapy. *N Engl J Med*. 2001;344(23):1773–9.

62. Stonebraker JS, Bolton-Maggs PHB, Soucie JM, et al. A study of variations in the reported haemophilia A prevalence around the world. *Haemophilia*. 2010;16(1):20–32.

63. Castro HE, Briceño MF, Casas CP, Rueda JD. The History and Evolution of the Clinical Effectiveness of Haemophilia Type A Treatment: A Systematic Review. *Indian J Hematol Blood Transfus*. 2014;30(1):1.

64. Nilsson IM, Hedner U, Ahlberg Å. Haemophilia Prophylaxis in Sweden. *Acta Pædiatrica*. 1976;65(2):129–35.

65. Mannucci PM. AIDS, hepatitis and hemophilia in the 1980s: memoirs from an insider. *J Thromb Haemost*. 2003;1(10):2065–9.

66. Lusher JM, Arkin S, Abildgaard CF, Schwartz RS, Group the KPUPS. Recombinant Factor VIII for the Treatment of Previously Untreated Patients with Hemophilia A -- Safety, Efficacy, and Development of Inhibitors. *N Engl J Med*. 1993;328(7):453–9.

67. Schwartz RS, Abildgaard CF, Aledort LM, et al. Human Recombinant DNA–Derived Antihemophilic Factor (Factor VIII) in the Treatment of Hemophilia A. *N Engl J Med*. 1990;323(26):1800–5.

68. Nilsson IM, Blombäck M, Ramgren O. HAEMOPHILIA IN SWEDEN: VI. Treatment of Haemophilia A with the Human Antihaemophilic Factor Preparation (Fraction I—0). *Acta Med Scand*. 1962;171(379 S):61–110.

69. den Uijl IEM, Mauser Bunschoten EP, Roosendaal G, et al. Clinical severity of haemophilia A: Does the classification of the 1950s still stand? *Haemophilia*. 2011;17(6):849–53.

70. Björkman S, Folkesson A, Jönsson S. Pharmacokinetics and dose requirements of factor VIII over the age range 3–74 years. *Eur J Clin Pharmacol*. 2009;65(10):989–98.

71. Björkman S, Oh M, Spotts G, et al. Population pharmacokinetics of recombinant factor VIII: the relationships of pharmacokinetics to age and body weight. *Blood*. 2012;119(2):612–8.

72. Morfini M. Comparative pharmacokinetic studies in haemophilia. *Haemophilia*. 2002;8(s2):30–3.

73. Wakefield J. The Bayesian Analysis of Population Pharmacokinetic Models. *J Am Stat Assoc*. 1996;91(433):62–75.

74. Collins PW, Björkman S, Fischer K, et al. Factor VIII requirement to maintain a target plasma level in the prophylactic treatment of severe hemophilia A: Influences of variance in pharmacokinetics and treatment regimens. *J Thromb Haemost*. 2010;8(2):269–75.

75. Samara E, Granneman R. Role of Population Pharmacokinetics in Drug Development. *Clin Pharmacokinet*. 1997;32(4):294–312.

76. Ette EI, Williams PJ. Population Pharmacokinetics I: Background, Concepts, and Models. *Ann Pharmacother*. 2004;38(10):1702–6.

77. Sheiner LB, Beal S, Rosenberg B, Marathe V V. Forecasting individual pharmacokinetics. *Clin Pharmacol Ther*. 1979;26(3):294–305.

78. Tod MM, Padoin C, Petitjean O. Individualising Aminoglycoside Dosage Regimens after Therapeutic Drug Monitoring. *Clin Pharmacokinet*. 2001;40(11):803–14.

79. Ting LSL, Villeneuve E, Ensom MHH. Beyond cyclosporine: a systematic review of limited sampling strategies for other immunosuppressants. *Ther Drug Monit*. 2006;28(3):419–30.

80. Brenner GM, Stevens CW, Preceeded by: Brenner GM. Pharmacology. 552 p.

81. Deitcher SR, Tuller J, Johnson JA. Intranasal DDAVP induced increases in plasma von Willebrand factor alter the pharmacokinetics of high-purity factor VIII concentrates in severe haemophilia A patients. *Haemophilia*. 1999;5(2):88–95.

82. Lee CA, Owens D, Bray G, et al. Pharmacokinetics of recombinant factor VIII (recombinate) using one-stage clotting and chromogenic factor VIII assay. *Thromb Haemost*. 1999;82(6):1644–7.

83. Saez, Bosh, Boadas, et al. Pharmacokinetics and acute tolerance of a double virus inactivated plasma derived factor VIII concentrate. *Haemophilia*. 1999;5(4):260–5.

84. Abshire TC, Brackmann HH, Scharrer I, et al. Sucrose formulated recombinant human antihemophilic factor VIII is safe and efficacious for treatment of hemophilia A in home therapy--International Kogenate-FS Study Group. *Thromb Haemost*. 2000;83(6):811–6.

85. Powell JS, Bush M, Harrison J, et al. Safety and efficacy of solvent/detergent-treated antihaemophilic factor with an added 80 oC terminal dry heat treatment in patients with haemophilia A. *Haemophilia*. 2000;6(3):140–9.

86. Courter SG, Bedrosian CL. Clinical evaluation of B-domain deleted recombinant factor VIII in previously treated patients. *Semin Hematol*. 2001;38(2 Suppl 4):44–51.

87. Courter SG, Bedrosian CL. Clinical evaluation of B-domain deleted recombinant factor VIII in previously untreated patients. *Semin Hematol*. 2001;38(2 Suppl 4):52–9.

88. Rothschild C, Scharrer I, Brackmann H-H, et al. European data of a clinical trial with a sucrose formulated recombinant factor VIII in previously treated haemophilia A patients. *Haemophilia*. 2002;8 Suppl 2:10–4.

89. Lusher JM, Lee CA, Kessler CM, Bedrosian CL. The safety and efficacy of B-domain deleted recombinant factor VIII concentrate in patients with severe haemophilia A. *Haemophilia*. 2003;9(1):38–49.

90. Morfini M, Cinotti S, Bellatreccia A, et al. A multicenter pharmacokinetic study of the B-domain deleted recombinant factor VIII concentrate using different assays and standards. *J Thromb Haemost*. 2003;1(11):2283–9.

91. Blanchette V, Shapiro AD, Liesner R, et al. Characterization of the Pharmacokinetics of rFVIII in Young Pre-School Children with Hemophilia, Including an Analysis of the Influence of Age and Body Weight. *Blood*. 2004;104(11).

92. Tarantino MD, Collins PW, Hay CRM, et al. Clinical evaluation of an advanced category antihaemophilic factor prepared using a plasma/albumin-free method: pharmacokinetics, efficacy, and safety in previously treated patients with haemophilia A1. *Haemophilia*. 2004;10(5):428–37.

93. Wolf DM, Rokicka-Milewska R, Lopaciuk S, et al. Clinical efficacy, safety and pharmacokinetic properties of the factor VIII concentrate Haemoctin SDH in previously treated patients with severe haemophilia A. *Haemophilia*. 2004;10(5):438–48.

94. Kessler CM, Gill JC, White GC, et al. B-domain deleted recombinant factor VIII preparations are bioequivalent to a monoclonal antibody purified plasma-derived factor VIII concentrate: a randomized, three-way crossover study. *Haemophilia*. 2005;11(2):84–91.

95. Di Paola J, Smith MP, Klamroth R, et al. ReFacto® and Advate®: A single-dose, randomized, two-period crossover pharmacokinetics study in subjects with haemophilia A. *Haemophilia*. 2007;13(2):124–30.

96. Powell JS, Nugent DJ, Harrison JA, et al. Safety and pharmacokinetics of a recombinant factor VIII with pegylated liposomes in severe hemophilia A. *J Thromb Haemost*. 2008;6(2):277–83.

97. Recht M, Nemes L, Matysiak M, et al. Clinical evaluation of moroctocog alfa (AF-CC), a new generation of B-domain deleted recombinant factor VIII (BDDrFVIII) for treatment of haemophilia A: Demonstration of safety, efficacy, and pharmacokinetic equivalence to full-length recombinant factor V. *Haemophilia*. 2009;15(4):869–80.

98. Santoro C, Iorio A, Ferrante F, et al. Performance of recalibrated ReFacto® laboratory standard in the measurement of FVIII plasma concentration via the chromogenic and one-stage assays after infusion of recalibrated ReFacto® (B-domain deleted recombinant factor VIII). *Haemophilia*. 2009;15(3):779–87.

99. Dmoszynska A, Hellmann A, Baglin T, et al. Pharmacokinetics of Optivate®, a high-purity concentrate of factor VIII with von Willebrand factor, in patients with severe haemophilia A. *Haemophilia*. 2011;17(2):185–90.

100. El-Ekiaby M, Goubran HA, Radosevich M, et al. Pharmacokinetic study of minipooled solvent/detergent-filtered cryoprecipitate factor VIII. *Haemophilia*. 2011;17(5):no-no.

101. Martinowitz U, Bjerre J, Brand B, et al. Bioequivalence between two serum-free recombinant factor VIII preparations (N8 and ADVATE ®) - an open-label, sequential dosing pharmacokinetic study in patients with severe haemophilia A. *Haemophilia*. 2011;17(6):854–9.

102. Valentino LA, Mamonov V, Hellmann A, et al. A randomized comparison of two prophylaxis regimens and a paired comparison of on-demand and prophylaxis treatments in hemophilia A management. *J Thromb Haemost*. 2012;10(3):359–67.

103. Jiménez-Yuste V, Lejniece S, Klamroth R, et al. The pharmacokinetics of a B-domain truncated recombinant factor VIII, turoctocog alfa (NovoEight®), in patients with hemophilia A. *J Thromb Haemost*. 2015;13(3):370–9.

104. Klukowska A, Szczepański T, Vdovin V, et al. Novel, human cell line-derived recombinant factor VIII (Human-cl rhFVIII, Nuwiq ®) in children with severe haemophilia A: efficacy, safety and pharmacokinetics. *Haemophilia*. 2016;22(2):232–9.

105. Shah A, Delesen H, Garger S, Lalezari S. Pharmacokinetic properties of BAY 81-8973, a full-length recombinant factor VIII. *Haemophilia*. 2015;21(6):766–71.

106. Powell JS, Josephson NC, Quon D, et al. Safety and prolonged activity of recombinant factor VIII Fc fusion protein in hemophilia A patients. *Blood*. 2012;119(13):3031–7.

107. Coyle TE, Reding MT, Lin JC, et al. Phase I study of BAY 94-9027, a PEGylated B-domain-deleted recombinant factor VIII with an extended half-life, in subjects with hemophilia A. *J Thromb Haemost*. 2014;12(4):488–96.

108. Mahlangu J, Powell JS, Ragni M V, et al. Phase 3 study of recombinant factor VIII Fc fusion protein in severe hemophilia A. *Blood*. 2014;123(3):317–25.

109. Tiede A, Brand B, Fischer R, et al. Enhancing the pharmacokinetic properties of recombinant factor VIII: first-in-human trial of glycoPEGylated recombinant factor VIII in patients with hemophilia A. *J Thromb Haemost*. 2013;11(4):670–8.

110. Young G, Mahlangu J, Kulkarni R, et al. Recombinant factor VIII Fc fusion protein for the prevention and treatment of bleeding in children with severe hemophilia A. *J Thromb Haemost*. 2015;13(6):967–77.

111. Björkman S, Blanchette VS, Fischer K, et al. Comparative pharmacokinetics of plasma- and albumin-free recombinant factor VIII in children and adults: The influence of blood sampling schedule on observed age-related differences and implications for dose tailoring. *J Thromb Haemost*. 2010;8(4):730–6.

112. Vlot AJ, Mauser-Bunschoten EP, Zarkova AG, et al. The half-life of infused factor VIII is shorter in hemophiliac patients with blood group O than in those with blood group A. *Thromb Haemost*. 2000;83(1):65–9.

113. Fischer K, Pendu R, van Schooten CJ, et al. Models for prediction of factor VIII half-life in severe haemophiliacs: distinct approaches for blood group O and non-O patients. *PLoS One*. 2009;4(8):e6745.

114. Kepa S, Horvath B, Reitter-Pfoertner S, et al. Parameters influencing FVIII pharmacokinetics in patients with severe and moderate haemophilia A. *Haemophilia*. 2015;21(3):343–50.

115. Hazendonk HCAM, Lock J, Mathôt RAA, et al. Perioperative treatment of hemophilia A patients: blood group O patients are at risk of bleeding complications. *J Thromb Haemost*. 2016;14(3):468–78.

116. Fijnvandraat K, Peters M, Ten Cate JW. Inter-individual variation in half-life of infused recombinant factor VIII is related to pre-infusion von Willebrand factor antigen levels. *Br J Haematol*. 1995;91(2):474–6.

117. Albánez S, Ogiwara K, Michels A, et al. Aging and ABO blood type influence von Willebrand factor and factor VIII levels through interrelated mechanisms. *J Thromb Haemost*. 2016;14(5):953–63.

118. Weiss HJ, Sussman II, Hoyer LW. Stabilization of factor VIII in plasma by the von Willebrand factor. Studies on posttransfusion and dissociated factor VIII and in patients with von Willebrand's disease. *J Clin Invest*. 1977;60(2):390–404.

119. Konkle B. von Willebrand Factor and Aging. *Semin Thromb Hemost*. 2014;40(06):640–4.

120. Lalezari S, Martinowitz U, Windyga J, et al. Correlation between endogenous VWF:Ag and PK parameters and bleeding frequency in severe haemophilia A subjects during three-times-weekly prophylaxis with rFVIII-FS. *Haemophilia*. 2014;20(1):e15-22.

121. Henrard S, Speybroeck N, Hermans C. Impact of being underweight or overweight on factor VIII

dosing in hemophilia A patients. *Haematologica*. 2013;98(9):1481–6.

122. Kawai R, Lemaire M, Steimer JL, et al. Physiologically based pharmacokinetic study on a cyclosporin derivative, SDZ IMM 125. *J Pharmacokinet Biopharm*. 1994;22(5):327–65.

123. Kitchen S, Jennings I, Makris M, et al. Factor VIII assay variability in postinfusion samples containing full length and B-domain deleted FVIII. *Haemophilia*. 2016;22(5):806–12.

124. Gruppo RA, Brown D, Wilkes MM, Navickis RJ. Comparative effectiveness of full-length and B-domain deleted factor VIII for prophylaxis - a meta-analysis. *Haemophilia*. 2003;9(3):251–60.

125. Kreuz W, Ettingshausen CE, Zyschka A, et al. Inhibitor Development in Previously Untreated Patients with Hemophilia A: A Prospective Long-Term Follow-Up Comparing Plasma-Derived and Recombinant Products. *Semin Thromb Hemost*. 2002;28(3):285–90.

126. Gouw SC, van der Bom JG, Auerswald G, et al. Recombinant versus plasma-derived factor VIII products and the development of inhibitors in previously untreated patients with severe hemophilia A: the CANAL cohort study. *Blood*. 2007;109(11):4693–7.

127. Iorio A, Halimeh S, Holzhauer S, et al. Rate of inhibitor development in previously untreated hemophilia A patients treated with plasma-derived or recombinant factor VIII concentrates: A systematic review. *J Thromb Haemost*. 2010;8(6):1256–65.

128. Harrison JF, Bloom AL, Abildgaard CF. The pharmacokinetics of recombinant factor VIII. The rFactor VIII Clinical Trial Group. *Semin Hematol*. 1991;28(2 Suppl 1):29–35; discussion 36.

129. Morfini M, Marchesini E, Paladino E, et al. Pharmacokinetics of plasma-derived vs. recombinant FVIII concentrates: a comparative study. *Haemophilia*. 2015;21(2):204–9.

130. Steele MR, Nagel K, Chan AKC. Pharmacokinetics of recombinant and plasma-derived factor VIII products in paediatric patients with severe haemophilia A. *Haemophilia*. 2014;20(1):e100–1.

131. George LA, Camire RM. Profile of efraloctocog alfa and its potential in the treatment of hemophilia A. *J Blood Med*. 2015;6:131–41.

132. Ivens IA, Baumann A, McDonald TA, et al. PEGylated therapeutic proteins for haemophilia treatment: a review for haemophilia caregivers. *Haemophilia*. 2013;19(1):11–20.

133. Opar A. The clot thickens for long-lasting drugs that stop hemophilia short. *Nat Med*. 2013;19(2):121.

134. Peters RT, Toby G, Lu Q, et al. Biochemical and functional characterization of a recombinant monomeric factor VIII-Fc fusion protein. *J Thromb Haemost*. 2013;11(1):132–41.

135. Skotnicki A, Lissitchkov TJ, Mamonov V, et al. Efficacy, safety and pharmacokinetic profiles of a plasma-derived VWF/FVIII concentrate (VONCENTO®) in subjects with haemophilia A (SWIFT-HA study). *Thromb Res*. 2016;137:119–25.

136. Schulte S. Innovative coagulation factors: albumin fusion technology and recombinant single-chain factor VIII. *Thromb Res*. 2013;131:S2–6.

137. Zollner S, Raquet E, Claar P, et al. Non-clinical pharmacokinetics and pharmacodynamics of rVIII-SingleChain, a novel recombinant single-chain factor VIII. *Thromb Res*. 2014;134(1):125–31.

138. Neelakantan S, Li S, Powell J, et al. Population pharmacokinetic analysis of long-lasting recombinant factor VIII Fc fusion protein (rFVIIIFc) in patients with severe hemophilia A. *J Thromb Haemost*. 2013;11(s2):354.

139. Miners AH, Lee CA. Setting research priorities to improve cost-effectiveness estimations of primary prophylaxis with clotting factor for people with severe haemophilia. *Haemophilia*. 2004;10(s1):58–62.

140. Miners A. Revisiting the cost-effectiveness of primary prophylaxis with clotting factor for the treatment of severe haemophilia A. *Haemophilia*. 2009;15(4):881–7.

141. Johnson KA, Zhou Z-Y. Costs of care in hemophilia and possible implications of health care reform. *Hematol Am Soc Hematol Educ Progr*. 2011;2011(1):413–8.

142. Gringeri A, Wolfsegger M, Steinitz KN, Reininger AJ. Recombinant full-length factor VIII (FVIII) and extended half-life FVIII products in prophylaxis - new insight provided by pharmacokinetic modelling. *Haemophilia*. 2015;21(3):300–6.

143. Shapiro AD, Ragni M V., Kulkarni R, et al. Recombinant factor VIII Fc fusion protein: Extended-interval dosing maintains low bleeding rates and correlates with von Willebrand factor levels. *J Thromb Haemost*. 2014;12(11):1788–800.

144. Graf L. Extended Half-Life Factor VIII and Factor IX Preparations. Vol. 45, Transfusion Medicine and Hemotherapy. S. Karger AG; 2018. p. 86–91.

145. Lenting PJ, Van Schooten CJM, Denis C V. Clearance mechanisms of von Willebrand factor and factor VIII. *J Thromb Haemost*. 2007;5(7):1353–60.

146. Pipe SW, Montgomery RR, Pratt KP, Lenting PJ, Lillicrap D. Life in the shadow of a dominant partner: The FVIII-VWF association and its clinical implications for hemophilia A. *Blood*. 2016;128(16):2007–16.

147. Klamroth R, Simpson M, von Depka-Prondzinski M, et al. Comparative pharmacokinetics of rVIII-SingleChain and octocog alfa (Advate ®) in patients with severe haemophilia A. *Haemophilia*. 2016;22(5):730–8.

148. Mullins ES, Stasyshyn O, Alvarez-Román MT, et al. Extended half-life pegylated, full-length recombinant factor VIII for prophylaxis in children with severe haemophilia A. *Haemophilia*. 2017;23(2):238–46.

149. Stasyshyn O, Djambas Khayat C, Iosava G, et al. Safety, efficacy and pharmacokinetics of rVIII-SingleChain in children with severe hemophilia A: results of a multicenter clinical trial. *J Thromb Haemost*. 2017;15(4):636–44.

150. Nogami K, Shima M, Fukutake K, et al. Efficacy and safety of full-length pegylated recombinant factor VIII with extended half-life in previously treated patients with hemophilia A: comparison of data between the general and Japanese study populations. *Int J Hematol*. 2017;106(5):704–10.

151. Liu H, Wu R, Hu P, et al. An Open-label, Single-dose, Pharmacokinetic Study of Factor VIII Activity After Administration of Moroctocog Alfa (AF-CC) in Male Chinese Patients With Hemophilia A. *Clin Ther*. 2017;39(7):1313–9.

152. Meunier S, Alamelu J, Ehrenforth S, et al. Safety and efficacy of a glycoPEGylated rFVIII (Turoctocog alpha pegol, N8-GP) in paediatric patients with severe haemophilia A. *Thromb*

Haemost. 2017;117(9):1705–13.

153. Korth-Bradley J, Rupon J, Plotka A, Charnigo R, Rendo P. Assessment of Relative Bioavailability of Moroctocog Alfa and Moroctocog Alfa (AF-CC) in Subjects With Severe Hemophilia A. *Clin Transl Sci*. 2018;11(3):283–8.

154. Rusen L, Kavakli K, Korth-Bradley J, et al. Clinical experience with moroctocog alfa (AF-CC) in younger paediatric patients with severe haemophilia A: Two open-label studies. *Haemophilia*. 2018;24(4):604–10.

155. Chen ZP, Li PJ, Li G, et al. Pharmacokinetic studies of factor VIII in Chinese boys with severe Hemophilia A: A single-center study. *Chin Med J (Engl)*. 2018;131(15):1780–5.

156. Shah A, Coyle T, Lalezari S, et al. BAY 94-9027, a PEGylated recombinant factor VIII, exhibits a prolonged half-life and higher area under the curve in patients with severe haemophilia A: Comprehensive pharmacokinetic assessment from clinical studies. *Haemophilia*. 2018;24(5):733–40.

157. Shah A, Solms A, Garmann D, et al. Improved Pharmacokinetics with BAY 81-8973 Versus Antihemophilic Factor (Recombinant) Plasma/Albumin-Free Method: A Randomized Pharmacokinetic Study in Patients with Severe Hemophilia A. *Clin Pharmacokinet*. 2017;56:1045–55.

158. Merlen C, Zourikian N, Bonnefoy A, et al. Effect of ABO blood group on haemostatic parameters in severe haemophilia A patients performing acute moderate-intensity exercise. *Blood Coagul Fibrinolysis*. 2018;29(7):626–35.

159. Megías-Vericat JE, Bonanad S, Haya S, et al. Bayesian pharmacokinetic-guided prophylaxis with recombinant factor VIII in severe or moderate haemophilia A. *Thromb Res*. 2019;174(January):151–62.

160. Ljungkvist M, Olofsson H, Funding E, Berntorp E, Zetterberg E. Coagulation factor VIII is vital for increasing global coagulation after physical exercise. *Haemophilia*. 2019;25(2):e86–93.

161. Megías-Vericat JE, Bonanad S, Haya S, et al. Cross-sectional comparative study of pharmacokinetics and efficacy between sucrose-formulated recombinant factor VIII (Kogenate®) and BAY 81-8973 (Kovaltry®) in patients with severe or moderate haemophilia A in prophylaxis. *Haemophilia*. 2019;25(3):e215–8.

162. Loomans JI, Stokhuijzen E, Peters M, Fijnvandraat K. Administration of DDAVP did not improve the pharmacokinetics of FVIII concentrate in a clinically significant manner. *J Clin Transl Res*. 2018;3(Suppl 2):351–7.

163. Shah A, Solms A, Wiegmann S, et al. Direct comparison of two extended-half-life recombinant FVIII products: a randomized, crossover pharmacokinetic study in patients with severe hemophilia A. *Ann Hematol*. 2019;2035–44.

164. Chowdary P, Carcao M, Holme PA, et al. Fixed doses of N8-GP prophylaxis maintain moderate-to-mild factor VIII levels in the majority of patients with severe hemophilia A. *Res Pract Thromb Haemost*. 2019;3(3):542–54.

165. Stonebraker JS, Bolton-Maggs PHB, Soucie JM, Walker I, Brooker M. A study of variations in the reported haemophilia B prevalence around the world. *Haemophilia*. 2012;18(3):e91–4.

166. Iorio A, Marchesini E, Marcucci M, Stobart K, Chan AK. Clotting factor concentrates given to prevent bleeding and bleeding-related complications in people with hemophilia A or B. *Cochrane Database Syst Rev*. 2011;(9):CD003429.

167. McEneny-King A, Iorio A, Foster G, Edginton AN. The use of pharmacokinetics in dose individualization of factor VIII in the treatment of hemophilia A. *Expert Opin Drug Metab Toxicol*. 2016;12(11):1313–21.

168. Lee M, Morfini M, Negrier C, Chamouard V. The pharmacokinetics of coagulation factors. *Haemophilia*. 2006;12(SUPPL. 3):1–7.

169. Iorio A, Blanchette V, Blatny J, et al. Estimating and interpreting the pharmacokinetic profiles of individual patients with hemophilia A or B using a population pharmacokinetic approach: communication from the SSC of the ISTH. *J Thromb Haemost*. 2017;15(12):2461–5.

170. McEneny-King A, Foster G, Iorio A, Edginton AN. Data Analysis Protocol for the Development and Evaluation of Population Pharmacokinetic Models for Incorporation Into the Web-Accessible Population Pharmacokinetic Service - Hemophilia (WAPPS-Hemo). *JMIR Res Protoc*. 2016;5(4):e232.

171. Chelle P, Yeung CHT, Bonanad S, et al. Routine clinical care data for population pharmacokinetic modeling: the case for Fanhdi/Alphanate in hemophilia A patients. *J Pharmacokinet Pharmacodyn*. 2019;46(5):427–38.

172. Chelle P, Yeung CHT, Croteau SE, et al. Development and Validation of a Population-Pharmacokinetic Model for Rurioctacog Alfa Pegol (Adynovate®): A Report on Behalf of the WAPPS-Hemo Investigators Ad Hoc Subgroup. *Clin Pharmacokinet*. 2019;

173. Beal SL. Ways to fit a PK model with some data below the quantification limit. *J Pharmacokinet Pharmacodyn*. 2001;28(5):481–504.

174. Mould DR, Upton RN. Basic concepts in population modeling, simulation, and model-based drug development - Part 2: Introduction to pharmacokinetic modeling methods. *CPT Pharmacometrics Syst Pharmacol*. 2013;2(4).

175. Savic RM, Karlsson MO. Importance of shrinkage in empirical bayes estimates for diagnostics: problems and solutions. *AAPS J*. 2009;11(3):558–69.

176. Karlsson MO, Savic RM. Diagnosing model diagnostics. Vol. 82, Clinical Pharmacology and Therapeutics. 2007. p. 17–20.

177. Bergstrand M, Hooker AC, Wallin JE, Karlsson MO. Prediction-Corrected Visual Predictive Checks for Diagnosing Nonlinear Mixed-Effects Models. *AAPS J*. 2011;13(2):143–51.

178. Brekkan A, Berntorp E, Jensen K, Nielsen EI, Jönsson S. Population pharmacokinetics of plasma-derived factor IX: Procedures for dose individualization. *J Thromb Haemost*. 2016;14(4):724–32.

179. McEneny-King A, Chelle P, Foster G, et al. Development and evaluation of a generic population pharmacokinetic model for standard half-life factor VIII for use in dose individualization. *J Pharmacokinet Pharmacodyn*. 2019;46(5):411–26.

180. McEneny-King A, Yeung CHT, Edginton AN, Iorio A, Croteau SE. Clinical application of Web Accessible Population Pharmacokinetic Service—Hemophilia (WAPPS-Hemo): Patterns of blood sampling and patient characteristics among clinician users. *Haemophilia*. 2020;26(1):56–63.

181. Iorio A, Edginton AN, Blanchette V, et al. Performing and interpreting individual pharmacokinetic profiles in patients with Hemophilia A or B: Rationale and general considerations. *Res Pract Thromb Haemost*. 2018;2(3):535–48.

182. Abrantes JA, Nielsen EI, Korth-Bradley J, Harnisch L, Jönsson S. Elucidation of Factor VIII Activity Pharmacokinetics: A Pooled Population Analysis in Patients With Hemophilia A Treated With Moroctocog Alfa. *Clin Pharmacol Ther*. 2017;102(6):977–88.

183. Björkman S. Population pharmacokinetics of recombinant factor VIII: the relationships of pharmacokinetics To Age and Body Weight. *Blood*. 2012;119(2):612–8.

184. Nestorov I, Neelakantan S, Ludden TM, et al. Population pharmacokinetics of recombinant factor VIII Fc fusion protein. *Clin Pharmacol Drug Dev*. 2015;4(3):163–74.

185. Garmann D, McLeay S, Shah A, et al. Population pharmacokinetic characterization of BAY 81-8973, a full-length recombinant factor VIII: lessons learned – importance of including samples with factor VIII levels below the quantitation limit. *Haemophilia*. 2017;23(4):528–37.

186. Bolon-Larger M, Bressolle F, Chevalier Y, et al. Population pharmacokinetics of continuous infusion of factor VIII in hemophilia-a patients undergoing orthopedic surgery [Internet]. Vol. 7, International Journal of Pharmacy and Pharmaceutical Sciences. IJPPS; 2015. 109–114 p.

187. Karafoulidou A, Suarez E, Anastasopoulou I, et al. Population pharmacokinetics of recombinant factor VIII:C (ReFacto) in adult HIV-negative and HIV-positive haemophilia patients. *Eur J Clin Pharmacol*. 2009;65(11):1121–30.

188. Hazendonk H, Fijnvandraat K, Lock J, et al. A population pharmacokinetic model for perioperative dosing of factor VIII in hemophilia A patients. *Haematologica*. 2016;101(10):1159–69.

189. Samor B, Michalski C, Brandin M-P, et al. A qualitative and quantitative analysis of von Willebrand factor contained in a very high-purity plasma-derived FVIII concentrate. *Vox Sang*. 2012;103(1):35–41.

190. Cafuir LA, Kempton CL. Current and emerging factor VIII replacement products for hemophilia A. *Ther Adv Hematol*. 2017;8(10):303–13.

191. van der Bom JG, Fischer K, van den Berg HM. Meta-analysis on the effectiveness of B-domain deleted factor VIII for prophylaxis [1]. *Haemophilia*. 2003;9(6):744.

192. Johnston A. The relevance of factor VIII (FVIII) pharmacokinetics to TDM and hemophilia a treatment: is B domain-deleted FVIII equivalent to full-length FVIII? *Ther Drug Monit*. 2012;34(1):110–7.

193. Pipe SW. Functional roles of the factor VIII B domain. *Haemophilia*. 2009;15(6):1187–96.

194. Al-Sallami HS, Goulding A, Grant A, et al. Prediction of Fat-Free Mass in Children. *Clin Pharmacokinet*. 2015;54(11):1169–78.

195. Turecek PL, Romeder-Finger S, Apostol C, et al. A world-wide survey and field study in clinical haemostasis laboratories to evaluate FVIII:C activity assay variability of ADYNOVATE and OBIZUR in comparison with ADVATE. *Haemophilia*. 2016;22(6):957–65.

196. Barrowcliffe TW. Standardization of FVIII & FIX assays. *Haemophilia*. 2003;9(4):397–402.

197. Sommer JM, Moore N, Mcguffie-Valentine B, et al. Comparative field study evaluating the activity of recombinant factor VIII Fc fusion protein in plasma samples at clinical haemostasis laboratories. *Haemophilia*. 2014;20(2):294–300.

198. Viuff D, Barrowcliffe TW, Saugstrup T, Ezban M, Lillicrap D. International comparative field study of N8 evaluating factor VIII assay performance. *Haemophilia*. 2011;17(4):695–702.

199. Melchiorre D, Linari S, Manetti M, et al. Clinical, instrumental, serological and histological findings suggest that hemophilia B may be less severe than hemophilia A. *Haematologica*. 2016;101(2):219–25.

200. Valentino LA, Rusen L, Elezovic I, et al. Multicentre, randomized, open-label study of on-demand treatment with two prophylaxis regimens of recombinant coagulation factor IX in haemophilia B subjects. *Haemophilia*. 2014;20(3):398–406.

201. Kavakli K, Smith L, Kuliczkowski K, et al. Once-weekly prophylactic treatment vs. on-demand treatment with nonacog alfa in patients with moderately severe to severe haemophilia B. *Haemophilia*. 2016;22(3):381–8.

202. Clausen N, Petrini P, Claeyssens-Donadel S, Gouw SC, Liesner R. Similar bleeding phenotype in young children with haemophilia A or B: a cohort study. *Haemophilia*. 2014;20(6):747–55.

203. Berntorp E. Methods of haemophilia care delivery: regular prophylaxis versus episodic treatment. *Haemophilia*. 1995;1(1 S):3–7.

204. Giangrande P, Seitz R, Behr-Gross ME, et al. Kreuth III: European consensus proposals for treatment of haemophilia with coagulation factor concentrates. *Haemophilia*. 2014;20(3):322–5.

205. Richards M, Williams M, Chalmers E, et al. A United Kingdom Haemophilia Centre Doctors' Organization guideline approved by the British Committee for Standards in Haematology: guideline on the use of prophylactic factor VIII concentrate in children and adults with severe haemophilia A. *Br J Haematol*. 2010;149(4):498–507.

206. Berntorp E, Spotts G, Patrone L, Ewenstein BM. Advancing personalized care in hemophilia A: ten years' experience with an advanced category antihemophilic factor prepared using a plasma/albumin-free method. *Biol Targets Ther*. 2014;8:115–27.

207. Fischer K, Ljung R. Primary prophylaxis in haemophilia care: Guideline update 2016. *Blood Cells, Mol Dis*. 2017;67:81–5.

208. Khayat CD. Once-weekly prophylactic dosing of recombinant factor IX improves adherence in hemophilia B. *J Blood Med*. 2016;7:275–82.

209. Longo G, Cinotti S, Filimberti E, et al. Single-dose pharmacokinetics of Factor IX evaluated by model-independent methods. *Eur J Haematol*. 1987;39(5):426–33.

210. Björkman S, Carlsson M, Berntorp E. Pharmacokinetics of factor IX in patients with haemophilia B. Methodological aspects and physiological interpretation. *Eur J Clin Pharmacol*. 1994;46(4):325–32.

211. Carlsson M, Björkman S, Berntorp E. Multidose pharmacokinetics of factor IX: implications for dosing in prophylaxis. *Haemophilia*. 1998;4(2):83–8.

212. Monroe DM, Jenny RJ, Van Cott KE, Buhay S, Saward LL. Characterization of IXINITY®

(Trenonacog Alfa), a Recombinant Factor IX with Primary Sequence Corresponding to the Threonine-148 Polymorph. *Adv Hematol*. 2016;2016:7678901.

213. Turecek PL, Abbühl B, Tangada SD, et al. Nonacog gamma, a novel recombinant factor IX with low factor IXa content for treatment and prophylaxis of bleeding episodes. Vol. 8, Expert Review of Clinical Pharmacology. Expert Reviews Ltd.; 2015. p. 163–77.

214. Björkman S. A commentary on the differences in pharmacokinetics between recombinant and plasma-derived factor IX and their implications for dosing. *Haemophilia*. 2011;17(2):179–84.

215. Fryar CD, Gu Q, Ogden CL, Flegal KM. Anthropometric reference data for children and adults: United States, 2011-2014. *Vital Heal Stat*. 2016;3(39):1–46.

216. Comets E, Zohar S. A survey of the way pharmacokinetics are reported in published phase I clinical trials, with an emphasis on oncology. *Clin Pharmacokinet*. 2009;48(6):387–95.

217. Gabrielsson J, Weiner D. Non-compartmental Analysis. In Humana Press, Totowa, NJ; 2012. p. 377–89.

218. Björkman S. Population pharmacokinetics of recombinant factor IX: Implications for dose tailoring. *Haemophilia*. 2013;19(5):753–7.

219. Suzuki A, Tomono Y, Korth-Bradley JM. Population pharmacokinetic modelling of factor IX activity after administration of recombinant factor IX in patients with haemophilia B. *Haemophilia*. 2016;22(5):e359-66.

220. Preijers T, Hazendonk HCAM, Liesner R, et al. Population pharmacokinetics of factor IX in hemophilia B patients undergoing surgery. *J Thromb Haemost*. 2018;16(11):2196–207.

221. Berntorp E. Pharmacokinetics of plasma-derived and recombinant factor IX: Using population pharmacokinetics with sparse sampling data needs further study. *Haemophilia*. 2013;19(6):805–7.

222. Cheng X, Li P, Chen Z, et al. Break-through bleeding in relation to pharmacokinetics of Factor VIII in paediatric patients with severe haemophilia A. *Haemophilia*. 2018;24(1):120–5.

223. Witkop ML, McLaughlin JM, Anderson TL, et al. Predictors of non-adherence to prescribed prophylactic clotting-factor treatment regimens among adolescent and young adults with a bleeding disorder. *Haemophilia*. 2016;22(4):e245–50.

224. Brand B, Dunn S, Kulkarni R. Challenges in the management of haemophilia on transition from adolescence to adulthood. *Eur J Haematol*. 2015;95:30–5.

225. Pasi KJ, Perry DJ, Mahlangu J, et al. Long-Term Safety and Efficacy of rFVIIIFc in Adults and Adolescents with Severe Hemophilia A: A Longitudinal Analysis of A-LONG and ASPIRE. *Blood*. 2015;126(23):1087.

226. Chowdary P, Fosbury E, Riddell A, Mathias M. Therapeutic and routine prophylactic properties of rFactor VIII Fc (efraloctocog alfa, Eloctate®) in hemophilia A. *J Blood Med*. 2016;7:187–98.

227. Bolon-Larger M, Chamouard V, Bressolle F, Boulieu R. A limited sampling strategy for estimating individual pharmacokinetic parameters of coagulation factor VIII in patients with hemophilia A. *Ther Drug Monit*. 2007;29(1):20–6.

228. Stass H. Determination of minimal sampling time points for reliable pharmacokinetic evaluation

of recombinant factor VIII - an exploratory population pharmacokinetic analysis in paediatric patients suffering from severe haemophilia. *Haemophilia*. 2006;12(s4):50–5.

229. McEneny-King A, Chelle P, Iorio A, Edginton AN. The effect of unmeasurable endogenous plasma factor activity levels on factor VIII dosing in patients with severe hemophilia A. *Thromb Res*. 2018;170.

230. Frampton JE. Efmoroctocog Alfa: A Review in Haemophilia A. *Drugs*. 2016;76(13):1281–91.

231. Gringeri A, Lundin B, Von Mackensen S, et al. A randomized clinical trial of prophylaxis in children with hemophilia A (the ESPRIT study). *J Thromb Haemost*. 2011;9(4):700–10.

232. Hartmann J, Croteau SE. 2017 Clinical trials update: Innovations in hemophilia therapy. *Am J Hematol*. 2016;91(12):1252–60.

233. Collins PW, Fischer K, Morfini M, Blanchette VS, Björkman S. Implications of coagulation factor VIII and IX pharmacokinetics in the prophylactic treatment of haemophilia. *Haemophilia*. 2011;17(1):2–10.

234. Pasca S, Milan M, Sarolo L, Zanon E. PK-driven prophylaxis versus standard prophylaxis: When a tailored treatment may be a real and achievable cost-saving approach in children with severe hemophilia A. *Thromb Res*. 2017;157:58–63.

235. Nagao A, Yeung CHT, Germini F, Suzuki T. Clinical outcomes in hemophilia A patients undergoing tailoring of prophylaxis based on population-based pharmacokinetic dosing. *Thromb Res*. 2019;173:79–84.

236. Carlsson M, Berntorp E, Björkman S, Lindvall K. Pharmacokinetic dosing in prophylactic treatment of hemophilia A. *Eur J Haematol*. 1993;51(4):247–52.

237. Stemberger M, Kallenbach F, Schmit E, et al. Impact of Adopting Population Pharmacokinetics for Tailoring Prophylaxis in Haemophilia A Patients: A Historically Controlled Observational Study. *Thromb Haemost*. 2019;119(3):368–76.

238. Carlsson M, Berntorp E, Björkman S, Lethagen S, Ljung R. Improved cost-effectiveness by pharmacokinetic dosing of factor VIII in prophylactic treatment of haemophilia A. *Haemophilia*. 1997;3(2):96–101.

239. Tegenge MA, Yang H, Forshee RA. Predicting dose sparing benefit and bleeding risk of pharmacokinetic-based personalized prophylactic dosing of factor VIII products. *Haemophilia*. 2017;23(5):705–11.

240. Fernandes S, Carvalho M, Lopes M, Araújo F. Impact of an Individualized Prophylaxis Approach on Young Adults with Severe Hemophilia. *Semin Thromb Hemost*. 2014;40:785–9.

241. Feldman BM, Pai M, Rivard G, et al. Tailored prophylaxis in severe hemophilia A: interim results from the first 5 years of the Canadian Hemophilia Primary Prophylaxis Study. *J Thromb Haemost*. 2006;4:1228–36.

242. Iorio A. Using pharmacokinetics to individualize hemophilia therapy. *Hematol Am Soc Hematol Educ Progr*. 2017;2017(1):595–604.

243. Björkman S, Collins P. Measurement of factor VIII pharmacokinetics in routine clinical practice. *J Thromb Haemost*. 2013;11(1):180–2.

244. Morfini M. Pharmacokinetic studies: International guidelines for the conduct and interpretation of such studies. *Haemophilia*. 2006;12(SUPPL. 4):6–11.

245. Croteau SE, Callaghan MU, Davis J, et al. Focusing in on use of pharmacokinetic profiles in routine hemophilia care. *Res Pract Thromb Haemost*. 2018;2(3):607–14.

246. Ragni M V., Croteau SE, Morfini M, Cnossen MH, Iorio A. Pharmacokinetics and the transition to extended half-life factor concentrates: communication from the SSC of the ISTH. *J Thromb Haemost*. 2018;16(7):1437–41.

247. Rydz N, Leggo J, Tinlin S, James P, Lillicrap D. The Canadian "National program for hemophilia mutation testing" database: A ten-year review. *Am J Hematol*. 2013;88(12):1030–4.

248. Miller CH, Benson J, Ellingsen D, et al. F8 and F9 mutations in US haemophilia patients: Correlation with history of inhibitor and race/ethnicity. *Haemophilia*. 2012;18(3):375–82.

249. Iorio A, Marchesini E, Marcucci M, Stobart K, Chan AK. Clotting factor concentrates given to prevent bleeding and bleeding-related complications in people with hemophilia A or B. *Cochrane Database Syst Rev*. 2011;

250. Collins PW, Fischer K, Morfini M, Blanchette VS, Björkman S. Implications of coagulation factor VIII and IX pharmacokinetics in the prophylactic treatment of haemophilia. Vol. 17, Haemophilia. 2011. p. 2–10.

251. Lee M, Morfini M, Schulman S, Ingerslev J. Scientific and Standardization Committee Communication: The Design and Analysis of Pharmacokinetic Studies of Coagulation Factors. 2001.

252. Iorio A, Keepanasseril A, Foster G, et al. Development of a Web-Accessible Population Pharmacokinetic Service—Hemophilia (WAPPS-Hemo): Study Protocol. *JMIR Res Protoc*. 2016;5(4):e239.

253. McEneny-King A, Chelle P, Henrard S, et al. Modeling of body weight metrics for effective and cost-efficient conventional factor VIII dosing in hemophilia a prophylaxis. *Pharmaceutics*. 2017;9(4).

254. Jiménez-Yuste V, Auerswald G, Benson G, et al. Achieving and maintaining an optimal trough level for prophylaxis in haemophilia: The past, the present and the future. Vol. 12, Blood Transfusion. SIMTI Servizi Sri; 2014. p. 314–9.

255. Ahnström J, Berntorp E, Lindvall K, Björkman S. A 6-year follow-up of dosing, coagulation factor levels and bleedings in relation to joint status in the prophylactic treatment of haemophilia. *Haemophilia*. 2004;10(6):689–97.

256. Arlt W. The approach to the adult with newly diagnosed adrenal insufficiency. *J Clin Endocrinol Metab*. 2009;94(4):1059–67.

257. Chandler, MD WL, Ferrell, MT(ASCP) C, Lee, MT(ASCP) J, Tun, MT(ASCP) T, Kha, MT(ASCP) H. Comparison of Three Methods for Measuring Factor VIII Levels in Plasma. *Am J Clin Pathol*. 2003;120(1):34–9.

258. Zhang Y, Roberts J, Tortorici M, et al. Population pharmacokinetics of recombinant coagulation factor VIII-SingleChain in patients with severe hemophilia A. *J Thromb Haemost*. 2017;15(6):1106–14.

259. Shima M, Matsumoto T, Ogiwara K. New assays for monitoring haemophilia treatment. *Haemophilia*. 2008;14(SUPPL. 3):83–92.

260. Shima M, Matsumoto T, Fukuda K, et al. The utility of activated partial thromboplastin time (aPTT) clot waveform analysis in the investigation of hemophilia A patients with very low levels of factor VIII activity (FVIII:C). *Thromb Haemost*. 2002;87(3):436–41.

261. Matsumoto T, Nogami K, Ogiwara K, Shima M. A modified thrombin generation test for investigating very low levels of factor VIII activity in hemophilia A. *Int J Hematol*. 2009;90(5):576–82.

262. Matsumoto T, Nogami K, Tabuchi Y, et al. Clot waveform analysis using CS-2000i™ distinguishes between very low and absent levels of factor VIII activity in patients with severe haemophilia A. *Haemophilia*. 2017;23(5):e427–35.

263. Gouw SC, Van Den Berg HM, Oldenburg J, et al. F8 gene mutation type and inhibitor development in patients with severe hemophilia A: Systematic review and meta-analysis. *Blood*. 2012;119(12):2922–34.

264. Loomans JI, van Velzen AS, Eckhardt CL, et al. Variation in baseline factor VIII concentration in a retrospective cohort of mild/moderate hemophilia A patients carrying identical F8 mutations. *J Thromb Haemost*. 2017;15(2):246–54.

265. Oldenburg J. Optimal treatment strategies for hemophilia: achievements and limitations of current prophylactic regimens. *Blood*. 2015;125(13):2038–44.

266. Ingram GIC. CALCULATING THE DOSE OF FACTOR VIII IN THE MANAGEMENT OF HAEMOPHILIA. *Br J Haematol*. 1981;48(2):351–4.

267. Poon M-C, Lee A. Individualized prophylaxis for optimizing hemophilia care: can we apply this to both developed and developing nations? *Thromb J*. 2016;

268. Ullman M, Zhang QC, Brown D, Grant A, Soucie JM. Association of overweight and obesity with the use of self and home-based infusion therapy among haemophilic men. *Haemophilia*. 2014;20(3):340–8.

269. Revel-Vilk S, Komvilaisak P, Blanchette VS, et al. The changing face of hepatitis in boys with haemophilia associated with increased prevalence of obesity. *Haemophilia*. 2011;17(4):689–94.

270. Young G. New challenges in hemophilia: long-term outcomes and complications. Vol. 2012, Hematology / the Education Program of the American Society of Hematology. American Society of Hematology. Education Program. 2012. p. 362–8.

271. Shapiro AD, Korth-Bradley J, Poon MC. Use of pharmacokinetics in the coagulation factor treatment of patients with haemophilia. Vol. 11, Haemophilia. 2005. p. 571–82.

272. McLeay SC, Morrish GA, Kirkpatrick CMJ, Green B. The relationship between drug clearance and body size: Systematic review and meta-analysis of the literature published from 2000 to 2007. Vol. 51, Clinical Pharmacokinetics. 2012. p. 319–30.

273. Cheymol G. Effects of obesity on pharmacokinetics: Implications for drug therapy. *Clin Pharmacokinet*. 2000;39(3):215–31.

274. Hanley MJ, Abernethy DR, Greenblatt DJ. Effect of obesity on the pharmacokinetics of drugs in

humans. Vol. 49, Clinical Pharmacokinetics. 2010. p. 71–87.

275. Tuinenburg A, Biere-Rafi S, Peters M, et al. Obesity in haemophilia patients: Effect on bleeding frequency, clotting factor concentrate usage, and haemostatic and fibrinolytic parameters. *Haemophilia*. 2013;19(5):744–52.

276. Taylor BN, Bork SJD, Kim S, Moffett BS, Yee DL. Evaluation of weight-based dosing of unfractionated heparin in obese children. *J Pediatr*. 2013;163(1):150–3.

277. Risebrough N, Oh P, Blanchette V, et al. Cost-utility analysis of Canadian tailored prophylaxis, primary prophylaxis and on-demand therapy in young children with severe haemophilia A. *Haemophilia*. 2008;14(4):743–52.

278. Majumdar S, Ostrenga A, Latzman RD, et al. Pharmacoeconomic impact of obesity in severe haemophilia children on clotting factor prophylaxis in a single institution. Vol. 17, Haemophilia. 2011. p. 717–8.

279. Janmahasatian S, Duffull SB, Ash S, et al. Quantification of lean bodyweight. *Clin Pharmacokinet*. 2005;44(10):1051–65.

280. Green B, Duffull SB. What is the best size descriptor to use for pharmacokinetic studies in the obese? Vol. 58, British Journal of Clinical Pharmacology. 2004. p. 119–33.

281. Collins P, Faradji A, Morfini M, Enriquez MM, Schwartz L. Efficacy and safety of secondary prophylactic vs. on-demand sucrose-formulated recombinant factor VIII treatment in adults with severe hemophilia A: Results from a 13-month crossover study. *J Thromb Haemost*. 2010;8(1):83–9.

282. Shapiro AD. Long-lasting recombinant factor VIII proteins for hemophilia A. Vol. 2013, Hematology / the Education Program of the American Society of Hematology. American Society of Hematology. Education Program. 2013. p. 37–43.

283. Barrowcliffe TW. Monitoring haemophilia severity and treatment: new or old laboratory tests? *Haemophilia*. 2004;10(s4):109–14.

284. Ljung R. Aspects of prophylactic treatment of hemophilia. *Thromb J*. 2016;14.

285. Jackson SC, Yang M, Minuk L, et al. Prophylaxis in older Canadian adults with hemophilia A: Lessons and more questions. *BMC Hematol*. 2015;15(1).

286. Oldenburg J, Brackmann HH. Prophylaxis in adult patients with severe haemophilia A. Vol. 134, Thrombosis Research. Elsevier Ltd; 2014. p. S33–7.

287. Graham A, Jaworski K. Pharmacokinetic analysis of anti-hemophilic factor in the obese patient. *Haemophilia*. 2014;20(2):226–9.

288. Komwilaisak P, Blanchette V. Pharmacokinetic studies of coagulation factors: Relevance of plasma and extracellular volume and body weight. *Haemophilia*. 2006;12(SUPPL. 4):33–9.

289. Kamphuisen PW, Houwing-Duistermaat JJ, van Houwelingen HC, et al. Familial clustering of factor VIII and von Willebrand factor levels. *Thromb Haemost*. 1998;79(2):323–7.

290. Miller CH, Haff E, Platt SJ, et al. Measurement of von willebrand factor activity: Relative effects of ABO blood type and race. *J Thromb Haemost*. 2003;1(10):2191–7.

291. Liu X, Chen X, Yang J, Guo R. Association of ABO blood groups with von Willebrand factor, factor VIII and ADAMTS-13 in patients with lung cancer. *Oncol Lett*. 2017;14(3):3787–94.

292. O'Donnell J, Laffan MA. The relationship between ABO histo-blood group, factor VIII and von Willebrand factor. Vol. 11, Transfusion Medicine. 2001. p. 343–51.

293. Akin M, Balkan C, Karapinar DY, Kavakli K. The influence of the ABO blood type on the distribution of von Willebrand factor in healthy children with no bleeding symptoms. *Clin Appl Thromb*. 2012;18(3):316–9.

294. Klarmann D, Eggert C, Geisen C, et al. Association of ABO(H) and i blood group system development with von Willebrand factor and Factor VIII plasma levels in children and adolescents. *Transfusion*. 2010;50(7):1571–80.

295. Franchini M, Capra F, Targher G, Montagnana M, Lippi G. Relationship between ABO blood group and von Willebrand factor levels: from biology to clinical implications. *Thromb J*. 2007;5:14.

296. O'Donnell J, Boulton FE, Manning RA, Laffan MA. Amount of H antigen expressed on circulating von Willebrand factor is modified by ABO blood group genotype and is a major determinant of plasma von Willebrand factor antigen levels. *Arterioscler Thromb Vasc Biol*. 2002;22(2):335–41.

297. Committee for Medicinal Products for Human Use, European Medicines Agency. Guideline on clinical investigation of recombinant and 4 human plasma-derived factor IX products Guideline on the clinical investigation of recombinant and 12 human plasma-derived factor IX products [Internet]. Vol. 6.3. 2018.

Appendices

Letters of Copyright Permission

Chapter 2

22 November 2019

Dear Alanna McEneny-King,

Material Requested: lanna McEneny-King, Alfonso Iorio, Gary Foster & Andrea N. Edginton (2016) The use of pharmacokinetics in dose individualization of factor VIII in the treatment of hemophilia A, Expert Opinion on Drug Metabolism & Toxicology, 12:11, 1313-1321, DOI: 10.1080/17425255.2016.1214711

Thank you for your correspondence requesting permission to reproduce your **authors accepted manuscript** from our Journal in your printed thesis and to be posted in the university's repository – University of Waterloo.

We will be pleased to grant permission on the sole condition that you acknowledge the original source of publication and insert a reference to the article on the Journals website: http://www.tandfonline.com

This is the authors accepted manuscript of an article published as the version of record in Expert Opinion on Drug Metabolism & Toxicology © 2016, republished by permission of Informa UK Limited, trading as Taylor & Francis Group, available online https://doi.org/10.1080/17425255.2016.1214711

This permission does not cover any third party copyrighted work which may appear in the material requested.

Please note that this license does not allow you to post our content on any third party websites or repositories.

This licence does not allow the use of the Publishers version/PDF (this is the version of record that is published on the publisher's website) to be posted online.

With best wishes,

Lee-Ann

Lee-Ann Anderson – Senior Permissions & Licensing Executive, Journals
Routledge, Taylor & Francis Group
3 Park Square, Milton Park, Abingdon, Oxon, OX14 4RN, UK.
Permissions Tel: (0)20 7017 7617
Permissions e-mail: permissionrequest@tandf.co.uk
Direct Tel: +44 (0)20 7017 7932
Web: www.tandfonline.com
e-mail: lee-ann.anderson@tandf.co.uk

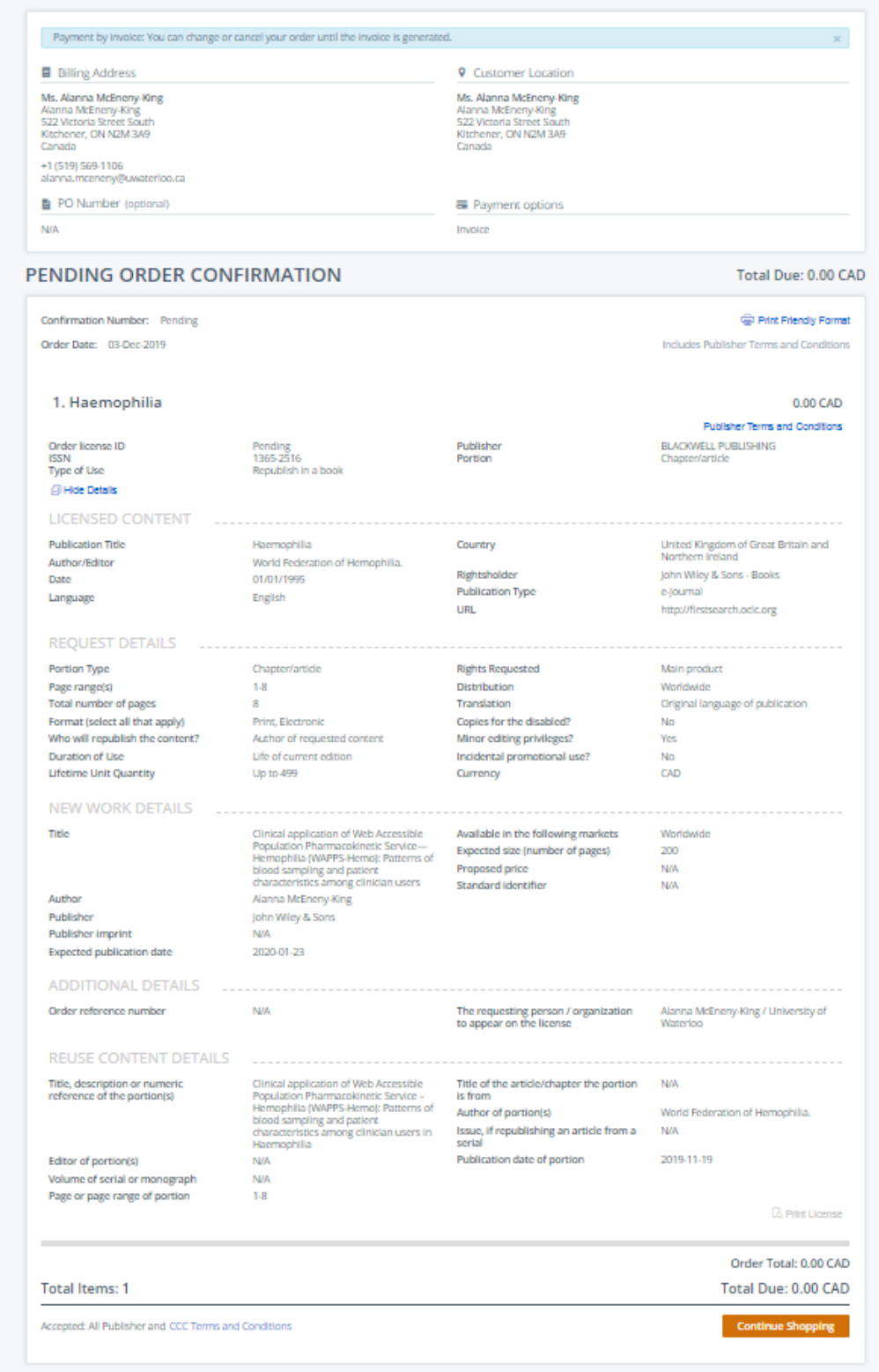
Chapter 4

SPRINGER NATURE LICENSE
TERMS AND CONDITIONS
Nov 12, 2019

This Agreement between Alanna McEneny-King -- Alanna McEneny-King ("You") and Springer Nature ("Springer Nature") consists of your license details and the terms and conditions provided by Springer Nature and Copyright Clearance Center.

License Number	4706601139076
License date	Nov 12, 2019
Licensed Content Publisher	Springer Nature
Licensed Content Publication	Journal of Pharmacokinetics and Pharmacodynamics
Licensed Content Title	Development and evaluation of a generic population pharmacokinetic model for standard half-life factor VIII for use in dose individualization
Licensed Content Author	Alanna McEneny-King, Pierre Chelle, Gary Foster et al
Licensed Content Date	Jan 1, 2019
Licensed Content Volume	46
Licensed Content Issue	5
Type of Use	Thesis/Dissertation
Requestor type	academic/university or research institute
Format	print and electronic
Portion	full article/chapter
Will you be translating?	no
Circulation/distribution	1 - 29
Author of this Springer Nature content	yes
Title	PhD Candidate
Institution name	University of Waterloo
Expected presentation date	Jan 2020
Requestor Location	Alanna McEneny-King 522 Victoria Street South Kitchener, ON N2M 3A9 Canada Attn: Alanna McEneny-King
Billing Type	Invoice
Billing Address	Alanna McEneny-King 522 Victoria Street South Kitchener, ON N2M 3A9 Canada Attn: Alanna McEneny-King
Total	0.00 CAD

Chapter 7



Payment by invoice: You can change or cancel your order until the invoice is generated.

Billing Address

Ms. Alanna McEneny-King
Alanna McEneny-King
522 Victoria Street South
Kitchener, ON N2M 3A9
Canada

+1 (519) 569-1106
alanna.mceneny@uwaterloo.ca

Customer Location

Ms. Alanna McEneny-King
Alanna McEneny-King
522 Victoria Street South
Kitchener, ON N2M 3A9
Canada

PO Number (optional)

N/A

Payment options

Invoice

PENDING ORDER CONFIRMATION Total Due: 0.00 CAD

Confirmation Number: Pending
Order Date: 03-Dec-2019

Print Friendly Format
Includes Publisher Terms and Conditions

1. Haemophilia 0.00 CAD

Publisher Terms and Conditions

Order license ID	Pending	Publisher	BLACKWELL PUBLISHING
ISSN	1365-2516	Portion	Chapter/article
Type of Use	Republish in a book		

Hide Details

LICENSED CONTENT

Publication Title	Haemophilia	Country	United Kingdom of Great Britain and Northern Ireland
Author/Editor	World Federation of Hemophilia.		
Date	01/01/1995	Rightsholder	John Wiley & Sons - Books
Language	English	Publication Type	e-journal
		URL	http://firstsearch.oclc.org

REQUEST DETAILS

Portion Type	Chapter/article	Rights Requested	Main product
Page range(s)	1-8	Distribution	Worldwide
Total number of pages	8	Translation	Original language of publication
Format (select all that apply)	Print, Electronic	Copies for the disabled?	No
Who will republish the content?	Author of requested content	Minor editing privileges?	Yes
Duration of Use	Life of current edition	Incidental promotional use?	No
Lifetime Unit Quantity	Up to 499	Currency	CAD

NEW WORK DETAILS

Title	Clinical application of Web Accessible Population Pharmacokinetic Service—Hemophilia (WAPPS-Hemo): Patterns of blood sampling and patient characteristics among clinician users	Available in the following markets	Worldwide
		Expected size (number of pages)	200
		Proposed price	N/A
		Standard identifier	N/A
Author	Alanna McEneny-King		
Publisher	John Wiley & Sons		
Publisher imprint	N/A		
Expected publication date	2020-01-23		

ADDITIONAL DETAILS

Order reference number	N/A	The requesting person / organization to appear on the license	Alanna McEneny-King / University of Waterloo

REUSE CONTENT DETAILS

Title, description or numeric reference of the portion(s)	Clinical application of Web Accessible Population Pharmacokinetic Service—Hemophilia (WAPPS-Hemo): Patterns of blood sampling and patient characteristics among clinician users in Haemophilia	Title of the article/chapter the portion is from	N/A
		Author of portion(s)	World Federation of Hemophilia.
		Issue, if republishing an article from a serial	N/A
Editor of portion(s)	N/A	Publication date of portion	2019-11-19
Volume of serial or monograph	N/A		
Page or page range of portion	1-8		

Print License

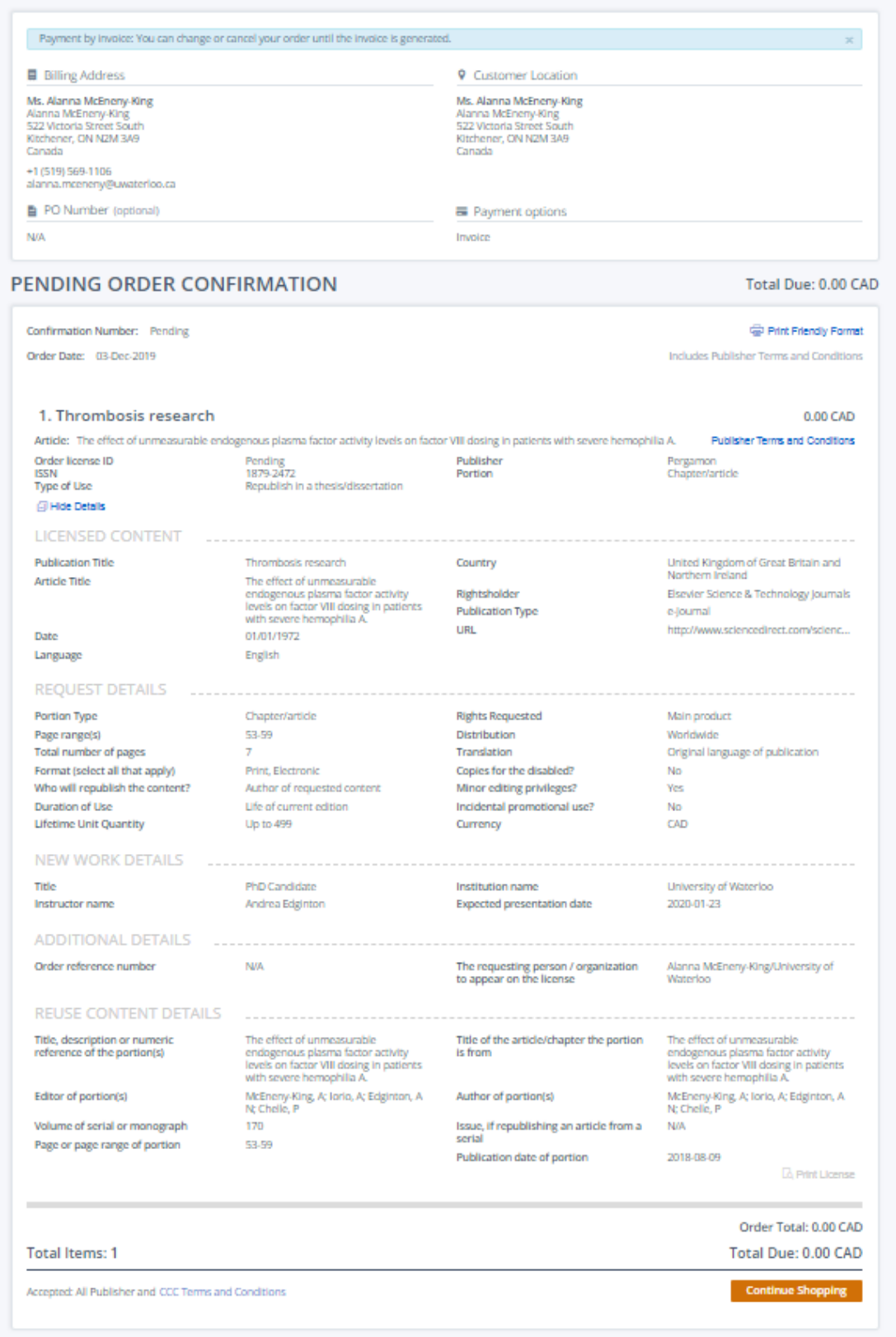
Order Total: 0.00 CAD

Total Items: 1 Total Due: 0.00 CAD

Accepted: All Publisher and CCC Terms and Conditions

Continue Shopping

Chapter 8



Payment by invoice: You can change or cancel your order until the invoice is generated.

Billing Address

Ms. Alanna McEneny-King
Alanna McEneny-King
522 Victoria Street South
Kitchener, ON N2M 3A9
Canada

+1 (519) 569-1106
alanna.mceneny@uwaterloo.ca

Customer Location

Ms. Alanna McEneny-King
Alanna McEneny-King
522 Victoria Street South
Kitchener, ON N2M 3A9
Canada

PO Number (optional)

N/A

Payment options

Invoice

PENDING ORDER CONFIRMATION

Total Due: 0.00 CAD

Confirmation Number: Pending
Order Date: 03-Dec-2019

Print Friendly Format
Includes Publisher Terms and Conditions

1. Thrombosis research

0.00 CAD

Article: The effect of unmeasurable endogenous plasma factor activity levels on factor VIII dosing in patients with severe hemophilia A. Publisher Terms and Conditions

Order license ID	Pending	Publisher	Pergamon
ISSN	1879-2472	Portion	Chapter/article
Type of Use	Republish in a thesis/dissertation		

Hide Details

LICENSED CONTENT

Publication Title	Thrombosis research	Country	United Kingdom of Great Britain and Northern Ireland
Article Title	The effect of unmeasurable endogenous plasma factor activity levels on factor VIII dosing in patients with severe hemophilia A.	Rightsholder	Elsevier Science & Technology Journals
		Publication Type	e-journal
Date	01/01/1972	URL	http://www.sciencedirect.com/scienc...
Language	English		

REQUEST DETAILS

Portion Type	Chapter/article	Rights Requested	Main product
Page range(s)	53-59	Distribution	Worldwide
Total number of pages	7	Translation	Original language of publication
Format (select all that apply)	Print, Electronic	Copies for the disabled?	No
Who will republish the content?	Author of requested content	Minor editing privileges?	Yes
Duration of Use	Life of current edition	Incidental promotional use?	No
Lifetime Unit Quantity	Up to 499	Currency	CAD

NEW WORK DETAILS

Title	PhD Candidate	Institution name	University of Waterloo
Instructor name	Andrea Edginton	Expected presentation date	2020-01-23

ADDITIONAL DETAILS

Order reference number	N/A	The requesting person / organization to appear on the license	Alanna McEneny-King/University of Waterloo

REUSE CONTENT DETAILS

Title, description or numeric reference of the portion(s)	The effect of unmeasurable endogenous plasma factor activity levels on factor VIII dosing in patients with severe hemophilia A.	Title of the article/chapter the portion is from	The effect of unmeasurable endogenous plasma factor activity levels on factor VIII dosing in patients with severe hemophilia A.
Editor of portion(s)	McEneny-King, A; Iorio, A; Edginton, A N; Chelle, P	Author of portion(s)	McEneny-King, A; Iorio, A; Edginton, A N; Chelle, P
Volume of serial or monograph	170	Issue, if republishing an article from a serial	N/A
Page or page range of portion	53-59	Publication date of portion	2018-08-09

Print License

Order Total: 0.00 CAD

Total Items: 1

Total Due: 0.00 CAD

Accepted: All Publisher and CCC Terms and Conditions

Continue Shopping

Chapter 9

Dear Alanna,

Thank you very much for your interest in said material.

All MDPI journals are Open Access and subject to the Creative Commons Attribution License (CC BY). The CC BY permits unrestricted use, distribution, and reproduction of the material in any medium, even commercially, ***provided the original work is properly cited***. You do not have to pay anything for permission.

For more information on the CC BY License, please see here:
https://creativecommons.org/licenses/by/4.0/legalcode

Best regards,

Tim Gasser

--
Tim Gasser
Communications & Marketing Assistant, MDPI
St. Alban-Anlage 66, 4052 Basel, Switzerland
www.mdpi.com

Tel. +41 61 683 77 34; Fax +41 61 302 89 18
E-mail: gasser@mdpi.com

Supplemental Information

Table S1. Categorical covariate summary among high-use centres (HUCs, top 25th percentile) and other centres (non-HUCs) for Period 1 and Period 2

	PERIOD 1		PERIOD 2	
	HUCs	**Non-HUCs**	**HUCs**	**Non-HUCs**
Number of infusions	405	63	961	502
Number of patients	359	54	849	437
Number of centres	37	28	48	110
Age (years), Mean (SD) Median [Range]	28.5 (17.6) 27 [0.5 – 77]	26.0 (19.6) 19 [0.5 – 68]	24.6 (16.9) 19 [0.5 – 79]	22.0 (17.0) 17 [0.5 – 81]
Body weight (kg), Mean (SD) Median [Range]	65.9 (25.4) 70 [11.5 – 204]	56.1 (27.9) 60 [8.8 – 114]	62.9 (27.5) 65 [10.63 – 176]	59.6 (29.4) 64 [6.9 – 179]
Infusions by Hemophilia Type				
Hemophilia A	87.8%	79.4%	84.5%	86.1%
Hemophilia B	12.2%	20.6%	15.5%	13.9%
Infusions by Product Type				
SHL FVIII	73.4%	63.5%	57.9%	53.4%
EHL FVIII	14.4%	15.9%	26.6%	32.7%
SHL FIX	2.7%	7.9%	5.3%	5.4%
EHL FIX	9.5%	12.7%	10.1%	8.5%

Table S2. Summary of number of samples per patient per infusion for each product type for Period 1 and Period 2 for high-use centres (HUCs) and other centres (non-HUCs).

CENTRE AND FACTOR TYPE	PERIOD 1	PERIOD 2	p-VALUE
HUCs			
SHL FVIII			
Mean (SD)	3.4 (1.8)	3.3 (1.5)	$p = 0.472$
Median [Range]	3 [1–9]	3 [1–10]	
EHL FVIII			
Mean (SD)	3.8 (1.6)	3.3 (1.6)	$p = 0.050$
Median [Range]	4 [1–8]	3 [1–7]	
SHL FIX			
Mean (SD)	3.0 (1.7)	3.0 (1.4)	$p = 0.972$
Median [Range]	2 [1–5]	3 [1–7]	
EHL FIX			
Mean (SD)	3.7 (1.6)	3.8 (2.1)	$p = 0.724$
Median [Range]	4 [1–8]	4 [1–8]	
Non-HUCs			
SHL FVIII			
Mean (SD)	4.4 (2.6)	3.2 (1.6)	$p = 0.007$
Median [Range]	4.5 [1–12]	3 [1–10]	
EHL FVIII			
Mean (SD)	3.0 (1.8)	4.1 (1.8)	$p = 0.082$
Median [Range]	2 [1–7]	4 [1–10]	
SHL FIX			
Mean (SD)	5.8 (0.8)	4.0 (2.2)	$p = 0.005$
Median [Range]	5.5 [1–7]	4 [1–9]	
EHL FIX			
Mean (SD)	4.3 (1.3)	4.1 (1.8)	$p = 0.776$
Median [Range]	4 [1–7]	4 [1–11]	

Table S3. Model building log

Structural Model				
Run	Structure	BSV Terms	RUV Model	OFV
1	2-compartment	None	Additive	30015
2	2-compartment	None	Proportional	35072
3	2-compartment	None	Combined	32691
4	2-compartment	CL, V_1, Q, V_2	Additive	1E+07
5	2-compartment	CL, V_1, Q, V_2	Proportional	34480
6	2-compartment	CL, V_1, Q, V_2	Combined	27517
7	2-compartment	CL, V_1	Additive	30015
8	2-compartment	CL, V_1	Proportional	26044
9	2-compartment	CL, V_1	Combined	26033
Covariate Model				
Run	Covariate Added	Form	BSV_{CL} (Δ) BSV_{V1} (Δ)	OFV (Δ)
10	Body weight (CL and V_1)	Power	44.6% (-10.4%) 34.4% (-26.1%)	25581 (-452)
11	Fat-free mass (CL and V_1)	Power	44.5% (-10.5%) 32.4% (-28.1%)	25548 (-485)
12	Age (CL and V_1)	Power	50.9% (-4.1%) 59.6% (-0.5%)	25832 (-201)
13	Age (CL and V_1)	Linear	53.2% (-1.8%) 46.7% (-13.8%)	25846 (-187)
14	Fat-free mass (CL, V_1 and V_2)	Power	44.9% (+0.4%) 33.3% (+0.9%)	25432 (-116)
15	Fat-free mass Age (CL)	Power Power	43.1% (-1.8%) 44.3% (-0.1%)	25414 (-18)
16	Fat-free mass Age (CL)	Power Linear	43.5% (-1.4%) 33.2% (-0.1%)	25413 (-19)
17	Fat-free mass Age (CL)	Power Piecewise linear	43.2% (-1.7%) 33.2% (-0.1%)	25410 (-22)
Brand Model				
Run	Brand Grouping Scheme	OFV	AIC	Number of Parameters
18	Individual	25321	25369	24
19	Based on Run 18	25325	25367	21
20	Source	25417	25417	14
21	Source and structure	25389	25389	16

Table S4. Results of 5-fold cross-validation of final SHL FVIII model

Parameter	Median Error	95th Percentile of Error
Half-Life	0.42%	1.82%
Time to 2% Activity	0.23%	1.19%
CL	0.21%	0.96%
V_1	0.33%	1.60%

Table S5. Details of sampling schemes explored and results of limited sampling analysis

Design	Sampling Times (h)	Half-Life Error (%)		CL Error (%)		V1 Error (%)		TAT2% Error (%)	
		Median	90th Percentile	Median	90th Percentile	Median	90th Percentile	Median	90th Percentile
Design 1	Pre-1-24	4.6%	17.6%	2.9%	11.7%	5.3%	15.9%	4.0%	15.5%
Design 2	Pre-1-30	3.4%	13.9%	2.6%	9.0%	5.4%	16.0%	2.9%	11.9%
Design 3	Pre-1-48	1.9%	8.1%	3.0%	9.7%	5.4%	16.0%	1.4%	6.0%
Design 4	Pre-1-54	1.6%	7.2%	3.2%	10.5%	5.4%	16.0%	1.2%	5.2%
Design 5	Pre-1-72	1.4%	16.8%	4.2%	18.0%	5.3%	15.7%	1.0%	18.7%
Design 6	Pre-3-24	5.5%	19.6%	2.9%	11.3%	7.1%	20.5%	4.5%	16.8%
Design 7	Pre-3-30	4.2%	15.5%	2.9%	9.6%	7.0%	20.1%	3.2%	13.0%
Design 8	Pre-3-48	2.3%	9.0%	3.7%	10.9%	6.7%	19.4%	1.5%	6.5%
Design 9	Pre-3-54	2.0%	7.8%	3.9%	11.7%	6.6%	19.2%	1.3%	5.5%
Design 10	Pre-3-72	1.8%	16.7%	4.8%	17.4%	6.5%	18.9%	1.0%	17.7%
Design 11	24-30-48	5.5%	16.3%	7.0%	19.7%	15.5%	40.2%	2.3%	10.0%
Design 12	24-30-54	4.9%	14.0%	6.9%	19.2%	14.3%	37.9%	1.9%	7.9%
Design 13	24-30-72	5.5%	13.9%	8.0%	20.3%	15.8%	36.2%	2.4%	7.0%
Design 14	24-48-54	6.0%	17.5%	10.2%	24.4%	19.5%	42.3%	2.1%	10.1%
Design 15	24-48-72	5.0%	12.9%	9.7%	23.5%	17.4%	38.1%	1.6%	5.9%
Design 16	30-48-54	6.6%	18.7%	11.7%	27.3%	21.8%	45.9%	2.1%	10.6%
Design 17	30-48-72	5.4%	14.0%	10.9%	26.2%	19.2%	41.4%	1.6%	6.2%
Design 18	30-54-72	5.8%	15.7%	15.3%	35.4%	24.6%	52.3%	1.3%	6.8%
Design 19	48-54-72	5.8%	15.5%	14.3%	33.2%	23.8%	49.7%	1.2%	6.7%
Design 20	24-30	12.4%	31.4%	8.3%	21.9%	24.9%	49.3%	7.7%	24.0%
Design 21	24-48	7.6%	21.0%	9.7%	23.8%	20.6%	43.7%	3.4%	13.8%
Design 22	24-54	6.6%	18.4%	9.8%	23.8%	19.6%	42.1%	2.8%	11.2%
Design 23	24-72	5.6%	14.9%	9.6%	23.6%	17.6%	39.7%	2.2%	7.5%
Design 24	30-48	7.9%	21.8%	11.2%	27.1%	23.0%	47.5%	3.3%	14.1%
Design 25	30-54	7.1%	19.4%	11.3%	27.0%	21.8%	45.6%	2.6%	11.5%
Design 26	30-72	5.8%	15.0%	10.9%	26.5%	19.4%	42.6%	2.0%	6.8%
Design 27	48-54	7.0%	19.8%	14.5%	33.6%	25.7%	53.1%	2.0%	10.7%
Design 28	48-72	5.8%	15.5%	14.6%	33.6%	23.8%	50.2%	1.4%	6.6%
Design 29	54-72	5.8%	15.7%	15.3%	35.4%	24.6%	52.35%	1.3%	6.8%
Design 30	3-24	10.4%	28.7%	4.0%	15.2%	11.4%	27.3%	8.4%	25.6%
Design 31	3-30	7.9%	23.8%	3.5%	11.5%	10.6%	26.5%	6.0%	20.1%
Design 32	3-48	4.3%	13.9%	4.1%	11.6%	9.1%	24.0%	2.6%	10.5%
Design 33	3-54	3.7%	12.2%	4.3%	12.4%	8.7%	23.3%	2.2%	9.1%
Design 34	3-72	3.6%	15.7%	5.0%	14.8%	8.2%	21.8%	2.1%	14.6%

Table S6. Median [95th percentile] absolute error on PK outcomes for limited sampling designs for the 2-year-old population.

	Design	Half-Life	CL	V_1	AUC	C_{72}	C_{96}	C_{max}	TAT2%	TAT1%
4-Sample Designs	EMA	2.8 [7.8]	3.1 [9.5]	5.2 [15.6]	3.2 [9.3]	5.4 [14.7]	5.9 [15.6]	4.9 [13.8]	3.3 [10.6]	4.5 [14.3]
	Pre-0.5-6-24	3.4 [9.9]	4.2 [11.5]	5.3 [15.4]	4.3 [11.8]	7.0 [19.8]	7.7 [22.2]	4.9 [13.7]	4.3 [13.8]	5.8 [18.5]
	Pre-0.5-6-48	3.0 [8.5]	3.7 [10.0]	5.5 [15.8]	3.6 [10.5]	6.1 [16.4]	6.4 [18.3]	5.0 [14.2]	3.6 [11.6]	4.8 [15.6]
	Pre-0.5-24-48	2.8 [8.1]	3.5 [9.8]	6.2 [17.8]	3.5 [10.0]	5.5 [14.8]	5.9 [16.0]	5.7 [16.5]	3.3 [10.4]	4.5 [14.1]
	Pre-6-24-48	3.1 [8.7]	4.1 [12.5]	9.8 [30.1]	4.1 [12.0]	5.5 [14.6]	5.9 [15.9]	9.1 [25.7]	3.4 [10.7]	4.7 [14.8]
	0.5-6-24-48	4.2 [13.0]	4.8 [16.1]	5.3 [15.7]	4.7 [13.9]	8.1 [23.5]	8.7 [25.7]	4.9 [13.8]	5.0 [18.4]	6.8 [24.8]
3-Sample Designs	Pre-0.5-6	4.1 [11.4]	5.3 [13.7]	5.5 [15.7]	5.4 [14.8]	8.9 [23.9]	9.6 [26.1]	5.0 [14.3]	5.3 [16.2]	7.0 [21.6]
	Pre-0.5-24	3.4 [10.1]	4.6 [12.7]	6.4 [17.9]	4.7 [13.3]	7.3 [20.4]	7.8 [22.1]	5.9 [16.7]	4.3 [13.8]	5.9 [18.6]
	Pre-0.5-48	3.0 [8.7]	4.1 [11.3]	6.3 [18.1]	4.1 [12.0]	5.9 [16.5]	6.5 [18.1]	6.1 [17.2]	3.6 [11.8]	4.8 [15.9]
	Pre-6-24	3.6 [10.8]	5.0 [14.1]	9.8 [29.5]	5.0 [14.1]	7.0 [19.8]	7.6 [22.0]	9.1 [25.6]	4.3 [13.6]	5.7 [18.7]
	Pre-6-48	3.2 [9.0]	4.7 [13.3]	9.7 [29.4]	4.7 [13.2]	6.1 [16.5]	6.4 [18.2]	9.2 [27.7]	3.6 [11.5]	5.0 [15.5]
	Pre-24-48	3.3 [9.5]	5.3 [14.7]	12.8 [35.3]	5.4 [14.7]	5.6 [14.9]	6.0 [16.1]	12.1 [38.8]	3.4 [10.7]	4.6 [15.0]
	0.5-6-24	6.3 [17.9]	6.9 [24.4]	5.4 [15.9]	6.8 [19.8]	12.8 [33.4]	13.7 [35.9]	4.9 [13.9]	7.7 [25.9]	10.4 [34.5]
	0.5-6-48	4.6 [15.1]	5.7 [20.0]	5.5 [15.6]	5.6 [16.8]	9.6 [28.0]	10.4 [30.4]	5.1 [14.1]	5.8 [22.7]	7.6 [30.6]
	0.5-24-48	4.2 [12.9]	5.1 [16.7]	6.3 [18.0]	5.0 [14.5]	8.5 [23.7]	8.9 [26.0]	5.8 [16.5]	5.0 [18.9]	6.8 [25.3]
	6-24-48	4.3 [13.3]	5.4 [18.0]	9.7 [29.7]	5.3 [15.4]	8.2 [23.3]	8.6 [25.9]	9.1 [25.4]	4.9 [18.7]	6.7 [25.8]
2-Sample Designs	Pre-0.5	4.0 [11.4]	5.6 [15.7]	6.5 [17.8]	5.8 [16.7]	9.0 [25.0]	9.4 [27.3]	6.2 [17.9]	5.2 [16.6]	6.9 [21.8]
	Pre-6	4.1 [12.0]	6.4 [16.2]	9.7 [29.7]	6.5 [17.4]	8.9 [24.0]	9.4 [26.0]	9.3 [27.9]	5.1 [16.5]	6.7 [21.8]
	Pre-24	3.8 [10.9]	6.0 [17.0]	12.7 [35.7]	6.1 [19.0]	7.4 [20.4]	7.7 [22.1]	11.9 [41.0]	4.2 [13.7]	5.7 [18.6]
	Pre-48	3.4 [9.4]	5.9 [15.9]	12.8 [37.2]	6.0 [17.6]	6.1 [16.9]	6.4 [18.2]	11.9 [42.7]	3.7 [11.3]	5.0 [15.5]
	0.5-6	9.7 [27.5]	12.0 [41.7]	5.4 [15.9]	11.8 [33.0]	20.6 [55.1]	21.9 [58.0]	5.1 [14.3]	12.2 [44.5]	16.4 [59.6]
	0.5-24	6.2 [18.6]	7.5 [26.6]	6.4 [17.8]	7.4 [21.2]	13.1 [34.8]	14.1 [37.1]	5.9 [16.5]	7.9 [27.2]	10.6 [37.0]
	0.5-48	4.6 [15.2]	6.4 [22.2]	6.4 [18.1]	6.4 [18.2]	10.1 [28.8]	10.7 [31.4]	6.1 [17.3]	5.8 [23.4]	8.0 [30.6]
	6-24	6.4 [18.4]	7.3 [27.2]	9.8 [29.6]	7.2 [21.5]	12.9 [33.5]	14.0 [35.7]	9.0 [25.4]	7.7 [26.4]	10.3 [35.3]
	6-48	4.7 [15.6]	6.5 [21.4]	9.6 [30.0]	6.4 [17.9]	9.7 [28.1]	10.4 [30.7]	9.0 [27.1]	5.7 [22.6]	7.7 [30.3]
	24-48	4.5 [14.1]	6.3 [19.8]	13.1 [35.6]	6.2 [17.2]	8.6 [23.9]	9.0 [26.2]	12.2 [37.3]	5.0 [19.1]	6.7 [26.2]

Table S7. Absolute error (%, as median [95th percentile]) on half-life estimates, for all permutations of study day and predose handling for each age group. To assist with interpretation, a gradient has been applied, with absolute error increasing from green (lowest) to red (highest).

	DESIGN		HALF-LIFE											
			TH				M				W			
			A		B		A		B		A		B	
ADULTS	2	Pre-1-24-48-72	2.7	[7.4]	2.0	[5.2]	2.8	[7.6]	1.8	[4.4]				
	3	Pre-1-24-48-96	2.1	[5.3]	1.5	[3.6]	2.3	[5.7]	1.4	[3.7]				
	4	Pre-1-24-72-96	1.2	[3.5]	0.8	[2.1]	1.1	[3.3]	0.7	[2.6]				
	5	Pre-1-48-72-96	0.3	[1.0]	0.3	[1.1]	0.3	[1.0]	0.4	[1.1]				
	6	Pre-1-72-96	1.3	[3.7]	0.9	[2.4]	1.3	[3.3]	0.9	[2.6]				
	7	Pre-1-48-96	2.2	[6.0]	1.5	[3.9]	2.4	[5.9]	1.5	[3.9]				
	8	Pre-1-24-96	3.1	[9.0]	1.9	[5.0]	3.3	[9.0]	2.1	[6.5]				
	9	Pre-1-48-72	2.7	[7.3]	2.0	[5.3]	2.9	[7.6]	1.8	[4.5]				
	10	Pre-1-24-72	3.5	[9.5]	2.4	[6.3]	3.8	[9.7]	2.3	[5.5]				
	11	Pre-1-24-48	5.8	[15.0]	3.2	[8.6]	5.5	[14.9]	2.8	[6.7]				
	12	Pre-1-24	10.9	[26.8]	4.2	[10.7]	10.1	[25.9]	3.9	[9.5]				
	13	Pre-1-48	6.1	[15.8]	3.3	[8.8]	6.0	[15.5]	2.9	[6.7]				
	14	Pre-1-72	3.7	[10.3]	2.5	[6.6]	4.0	[10.1]	2.4	[5.7]				
	15	Pre-1-96	3.8	[11.4]	2.0	[5.7]	3.9	[10.9]	2.4	[7.2]				
	16	Pre-1	21.1	[52.6]	4.4	[11.8]	21.4	[55.3]	4.5	[11.7]				
	17	1-24	10.7	[25.8]	10.1	[23.9]	9.6	[23.9]	9.9	[25.9]				
	18	1-48	6.2	[15.8]	6.0	[15.0]	5.9	[15.0]	6.4	[17.0]				
	19	1-72	4.4	[11.9]	3.8	[10.1]	4.2	[10.6]	4.5	[11.4]				
	20	1-96	4.3	[11.4]	4.1	[12.1]	4.1	[10.8]	4.8	[12.4]				
	21	1	20.7	[52.3]	20.0	[52.1]	21.2	[54.8]	21.5	[55.8]				
ADOLESCENTS	2	Pre-1-24-48-72	1.6	[4.9]	1.2	[3.4]	1.6	[5.0]	1.2	[3.2]	1.8	[5.3]	1.4	[4.5]
	3	Pre-1-24-48-96	2.2	[5.4]	1.5	[3.7]	2.3	[5.8]	1.8	[4.6]	2.1	[5.9]	1.6	[4.3]
	4	Pre-1-24-72-96	2.1	[6.7]	1.5	[4.7]	2.2	[6.1]	1.8	[5.9]	2.0	[6.2]	1.3	[3.5]
	5	Pre-1-48-72-96	0.8	[3.2]	0.7	[2.5]	0.8	[2.9]	0.8	[2.9]	0.7	[3.2]	0.4	[1.8]
	6	Pre-1-72-96	3.3	[11.6]	2.3	[7.5]	3.4	[12.6]	3.0	[12.1]	3.2	[10.9]	1.6	[4.7]
	7	Pre-1-48-96	2.8	[7.3]	1.9	[4.8]	2.8	[7.0]	2.3	[6.3]	2.6	[6.8]	1.9	[4.6]
	8	Pre-1-24-96	4.4	[11.7]	2.6	[6.9]	4.5	[12.2]	3.4	[9.8]	4.1	[11.2]	2.6	[6.3]
	9	Pre-1-48-72	2.2	[5.9]	1.6	[4.4]	2.2	[5.8]	1.7	[4.2]	2.2	[6.2]	1.6	[4.8]
	10	Pre-1-24-72	3.4	[8.2]	2.3	[5.8]	3.3	[8.2]	2.6	[6.6]	3.4	[8.3]	2.3	[5.8]
	11	Pre-1-24-48	3.7	[10.6]	2.3	[5.8]	3.8	[10.8]	2.6	[6.8]	4.0	[11.3]	2.8	[7.9]
	12	Pre-1-24	7.9	[20.9]	3.6	[8.8]	7.9	[20.1]	4.6	[12.1]	8.7	[21.9]	4.2	[11.3]
	13	Pre-1-48	4.5	[12.1]	2.7	[7.0]	4.6	[12.4]	3.2	[8.0]	4.7	[12.7]	3.0	[8.5]
	14	Pre-1-72	4.8	[13.0]	3.1	[8.2]	4.7	[13.8]	3.8	[12.1]	4.6	[13.1]	2.6	[6.7]
	15	Pre-1-96	7.8	[26.2]	3.8	[11.5]	7.8	[25.3]	6.1	[21.7]	7.5	[25.7]	3.0	[7.6]
	16	Pre-1	19.4	[48.3]	5.1	[12.6]	19.1	[48.6]	8.2	[27.7]	20.8	[52.4]	4.9	[12.7]
	17	1-24	7.8	[20.5]	7.6	[20.0]	7.8	[19.7]	7.8	[19.6]	8.9	[23.8]	7.8	[19.8]
	18	1-48	4.6	[12.1]	4.6	[12.2]	4.6	[11.9]	4.7	[12.5]	5.2	[13.1]	4.5	[11.9]
	19	1-72	4.8	[12.6]	4.9	[12.8]	4.8	[13.8]	5.0	[13.8]	5.4	[13.5]	4.5	[13.1]
	20	1-96	8.0	[26.2]	7.9	[27.3]	7.8	[25.4]	8.0	[25.3]	8.4	[26.5]	7.1	[24.3]
	21	1	19.1	[47.5]	18.9	[45.7]	18.9	[48.1]	19.1	[47.7]	20.3	[52.5]	19.8	[49.4]
CHILDREN	2	Pre-1-24-48-72	0.3	[1.1]	0.2	[0.9]	0.3	[1.2]	0.3	[1.1]	0.3	[1.4]	0.2	[1.0]
	3	Pre-1-24-48-96	1.0	[3.1]	0.8	[2.6]	1.0	[3.2]	0.9	[2.9]	1.0	[3.7]	0.7	[2.4]
	4	Pre-1-24-72-96	3.0	[8.4]	2.7	[7.3]	3.1	[8.7]	3.1	[8.8]	3.0	[8.2]	2.0	[5.4]
	5	Pre-1-48-72-96	5.7	[18.2]	5.5	[17.3]	5.9	[18.2]	5.8	[18.2]	5.7	[18.0]	4.0	[13.8]
	6	Pre-1-72-96	13.0	[36.4]	11.3	[34.9]	13.0	[37.5]	12.8	[37.3]	12.7	[35.9]	6.1	[18.8]
	7	Pre-1-48-96	6.2	[18.3]	5.9	[18.0]	6.4	[18.6]	6.3	[18.4]	6.3	[18.1]	4.3	[14.1]
	8	Pre-1-24-96	3.6	[10.8]	3.1	[8.9]	3.6	[10.5]	3.5	[10.4]	3.8	[11.2]	2.4	[6.8]
	9	Pre-1-48-72	5.8	[18.3]	5.5	[17.4]	5.9	[18.1]	5.9	[18.3]	5.9	[18.0]	4.1	[13.8]
	10	Pre-1-24-72	3.1	[9.0]	2.7	[7.2]	3.2	[9.0]	3.1	[8.9]	3.1	[8.8]	2.1	[5.5]
	11	Pre-1-24-48	1.0	[3.5]	0.9	[2.8]	1.1	[3.5]	1.0	[3.1]	1.1	[4.3]	0.7	[2.6]
	12	Pre-1-24	3.8	[11.8]	3.2	[8.9]	3.8	[11.7]	3.7	[10.5]	4.1	[12.7]	2.4	[6.9]
	13	Pre-1-48	6.2	[18.2]	5.9	[17.9]	6.5	[18.6]	6.4	[18.5]	6.4	[18.1]	4.4	[14.2]
	14	Pre-1-72	13.2	[35.5]	11.5	[35.3]	13.1	[37.9]	12.9	[38.5]	13.0	[36.6]	6.2	[18.7]
	15	Pre-1-96	16.5	[42.1]	13.0	[37.5]	16.9	[42.3]	16.0	[41.2]	17.0	[43.3]	6.7	[18.2]
	16	Pre-1	16.9	[39.3]	13.1	[35.6]	16.8	[40.7]	16.3	[40.3]	17.9	[42.5]	6.8	[18.2]
	17	1-24	3.8	[10.8]	3.8	[11.7]	3.8	[11.3]	3.8	[11.4]	4.1	[11.8]	4.3	[12.8]
	18	1-48	6.3	[18.2]	6.3	[18.2]	6.5	[18.6]	6.5	[18.6]	6.4	[18.4]	6.5	[18.3]
	19	1-72	13.2	[35.7]	13.2	[35.4]	13.1	[37.9]	13.0	[37.8]	13.0	[36.7]	12.7	[35.8]
	20	1-96	16.5	[42.1]	16.3	[41.1]	16.9	[42.4]	16.9	[42.0]	16.8	[43.4]	16.5	[41.7]
	21	1	16.8	[39.0]	16.7	[38.9]	16.8	[40.6]	16.8	[40.8]	17.5	[41.1]	17.3	[40.8]

Table S8. Absolute error (%, as median [95th percentile]) on C_{72} estimates, for all permutations of study day and predose handling for each age group. To assist with interpretation, a gradient has been applied, with absolute error increasing from green (lowest) to red (highest).

		Design	C_{72}											
			TH				M				W			
			A		B		A		B		A		B	
ADULTS	2	Pre-1-24-48-72	5.4	[14.3]	3.9	[9.6]	5.7	[14.2]	3.7	[8.6]				
	3	Pre-1-24-48-96	5.4	[13.9]	3.7	[9.2]	5.7	[15.0]	4	[10.8]				
	4	Pre-1-24-72-96	4	[12.0]	3	[8.4]	4	[11.3]	3.2	[9.5]				
	5	Pre-1-48-72-96	1.9	[5.5]	1.5	[4.0]	1.9	[5.2]	1.7	[4.7]				
	6	Pre-1-72-96	5.3	[15.3]	3.8	[10.2]	5	[13.6]	4.1	[10.8]				
	7	Pre-1-48-96	6.2	[17.2]	4.3	[10.8]	6.6	[18.4]	4.8	[13.2]				
	8	Pre-1-24-96	8.8	[25.9]	5.5	[15.2]	9.3	[26.5]	6.6	[21.3]				
	9	Pre-1-48-72	6.2	[15.1]	4.4	[10.2]	6.5	[15.7]	4.4	[10.2]				
	10	Pre-1-24-72	8.4	[20.8]	5.8	[14.3]	8.9	[21.9]	5.9	[14.8]				
	11	Pre-1-24-48	12.6	[30.5]	7.1	[18.0]	11.9	[29.7]	6.5	[16.0]				
	12	Pre-1-24	26.3	[64.2]	10.1	[25.4]	24.3	[59.5]	10.5	[29.1]				
	13	Pre-1-48	14.2	[35.0]	7.7	[19.3]	14	[34.6]	7.4	[17.7]				
	14	Pre-1-72	9.8	[24.1]	6.6	[15.9]	10.4	[25.3]	6.9	[17.0]				
	15	Pre-1-96	12.8	[38.4]	6.7	[19.1]	13.3	[38.1]	8.9	[25.5]				
	16	Pre-1	73.9	[279.6]	11.6	[29.5]	76.3	[276.1]	14.3	[41.1]				
	17	1-24	26.2	[66.7]	25.3	[60.7]	23.9	[59.7]	23.9	[58.7]				
	18	1-48	14	[35.3]	14.5	[36.1]	13.9	[34.2]	14.9	[37.5]				
	19	1-72	9.9	[24.1]	10.4	[26.8]	10.4	[25.4]	11.5	[27.5]				
	20	1-96	13	[38.3]	13.8	[40.9]	13.4	[38.3]	14.7	[39.0]				
	21	1	73.8	[278.5]	71.3	[279.4]	76.5	[274.8]	76.1	[277.6]				
ADOLESCENTS	2	Pre-1-24-48-72	3.9	[10.8]	2.8	[7.7]	3.9	[10.9]	3	[7.8]	4.1	[11.9]	3.3	[9.8]
	3	Pre-1-24-48-96	6.2	[15.8]	4.3	[10.8]	6.3	[16.3]	5.2	[13.6]	5.9	[15.9]	4.4	[12.2]
	4	Pre-1-24-72-96	6.9	[21.5]	5	[15.8]	7	[20.1]	6.1	[18.7]	6.6	[19.5]	4.3	[11.8]
	5	Pre-1-48-72-96	3.5	[11.2]	2.8	[9.3]	3.5	[10.7]	3.2	[10.7]	3.4	[11.4]	2.4	[7.2]
	6	Pre-1-72-96	11.5	[40.1]	8.1	[26.8]	11.6	[40.9]	10.6	[38.4]	11.2	[38.5]	5.7	[16.0]
	7	Pre-1-48-96	8.2	[20.9]	5.8	[15.3]	8.4	[21.9]	7	[20.1]	8	[20.4]	5.7	[14.3]
	8	Pre-1-24-96	13.4	[36.3]	8.1	[21.9]	13.5	[37.3]	10.6	[31.2]	12.4	[36.3]	7.7	[19.3]
	9	Pre-1-48-72	6.2	[15.5]	4.6	[12.2]	6.1	[15.4]	5.1	[12.8]	6.1	[15.0]	4.5	[11.5]
	10	Pre-1-24-72	9.6	[24.2]	6.6	[17.3]	9.4	[24.1]	7.7	[20.9]	9.5	[24.2]	6.3	[15.6]
	11	Pre-1-24-48	9.4	[25.4]	5.9	[14.8]	9.8	[25.9]	7.2	[19.0]	10	[26.2]	6.9	[18.9]
	12	Pre-1-24	21.9	[53.9]	10.5	[25.1]	22	[55.4]	13.8	[37.6]	23.3	[55.7]	11.4	[28.7]
	13	Pre-1-48	12.3	[29.7]	7.7	[18.3]	12.6	[30.4]	9.2	[24.0]	12.5	[30.7]	8.2	[21.7]
	14	Pre-1-72	15	[41.5]	10	[28.1]	14.7	[42.6]	12.6	[39.7]	14.2	[41.6]	7.7	[19.1]
	15	Pre-1-96	29.1	[117.7]	12.7	[38.2]	28.6	[104.5]	22.6	[86.5]	28.2	[115.7]	9.5	[24.4]
	16	Pre-1	72.2	[242.9]	16.1	[43.6]	71	[242.3]	30.8	[118.4]	77.4	[279.1]	14	[34.5]
	17	1-24	22	[55.9]	21.6	[53.5]	21.9	[56.9]	21.9	[53.8]	23.7	[61.2]	22.1	[52.9]
	18	1-48	12.3	[29.8]	12.8	[30.5]	12.5	[30.5]	12.9	[31.4]	12.3	[30.7]	12.9	[32.0]
	19	1-72	15	[41.5]	15.6	[42.5]	14.7	[42.7]	15.2	[42.5]	14.2	[41.7]	14.8	[42.1]
	20	1-96	29.1	[117.4]	29.6	[118.8]	28.7	[104.5]	29.2	[105.2]	28.1	[115.8]	28	[103.8]
	21	1	72.2	[242.3]	71.9	[235.4]	71	[241.9]	71.4	[236.1]	77.2	[277.9]	76.5	[279.7]
CHILDREN	2	Pre-1-24-48-72	0.7	[2.9]	0.6	[2.4]	0.7	[3.1]	0.7	[3.0]	0.8	[3.8]	0.6	[2.5]
	3	Pre-1-24-48-96	2.3	[8.3]	2	[7.2]	2.3	[8.5]	2.2	[7.7]	2.5	[9.7]	1.7	[6.4]
	4	Pre-1-24-72-96	6.5	[20.3]	5.7	[17.3]	6.7	[21.8]	6.6	[21.5]	6.3	[19.9]	4.3	[13.4]
	5	Pre-1-48-72-96	9	[23.8]	8.6	[22.2]	9.3	[24.6]	9.3	[24.6]	9.1	[24.3]	6.3	[15.6]
	6	Pre-1-72-96	24.6	[69.6]	20.7	[60.9]	24.5	[69.2]	24.2	[69.1]	23.9	[67.4]	10.2	[26.9]
	7	Pre-1-48-96	10.1	[25.2]	9.4	[24.3]	10.7	[27.1]	10.5	[26.7]	10.3	[25.7]	6.9	[16.8]
	8	Pre-1-24-96	7.9	[27.7]	6.8	[21.7]	7.9	[26.8]	7.7	[25.8]	8.5	[27.6]	5.2	[17.7]
	9	Pre-1-48-72	9.2	[23.5]	8.7	[22.1]	9.5	[24.7]	9.4	[24.6]	9.3	[24.2]	6.4	[15.9]
	10	Pre-1-24-72	6.7	[21.6]	5.8	[17.8]	6.9	[21.9]	6.8	[21.4]	6.6	[20.8]	4.4	[13.8]
	11	Pre-1-24-48	2.4	[9.3]	2.1	[7.6]	2.5	[9.3]	2.4	[8.6]	2.6	[11.3]	1.8	[7.1]
	12	Pre-1-24	8.4	[29.1]	6.9	[21.7]	8.3	[28.2]	8	[26.8]	8.9	[30.8]	5.3	[17.2]
	13	Pre-1-48	10.3	[25.5]	9.6	[24.2]	10.9	[27.4]	10.7	[26.7]	10.5	[25.9]	7	[16.9]
	14	Pre-1-72	25.2	[70.1]	21.1	[58.7]	24.7	[70.1]	24.5	[67.8]	24.7	[73.8]	10.4	[27.2]
	15	Pre-1-96	33.2	[69.2]	24.9	[70.2]	34.2	[79.1]	32.2	[78.6]	34.5	[77.5]	11.6	[29.8]
	16	Pre-1	31	[69.1]	25.4	[67.8]	30.7	[69.7]	32.7	[73.3]	33.1	[74.3]	11.8	[30.7]
	17	1-24	8.3	[28.1]	8.5	[29.0]	8.3	[28.1]	8.3	[28.2]	9.1	[29.7]	9.6	[30.6]
	18	1-48	10.3	[25.4]	10.6	[26.0]	10.9	[27.4]	11	[27.7]	10.5	[26.0]	11.2	[28.8]
	19	1-72	25.2	[70.1]	25.3	[71.6]	24.7	[70.1]	24.6	[70.5]	24.7	[73.8]	24.8	[75.4]
	20	1-96	33.1	[69.2]	33.1	[69.8]	34.2	[79.1]	34.2	[79.3]	34.4	[77.3]	34.4	[81.7]
	21	1	31	[69.1]	31.1	[71.3]	30.7	[69.7]	30.8	[69.6]	33.1	[74.1]	33.3	[76.7]

Table S9. Absolute error (%, as median [95th percentile]) on C_{96} estimates, for all permutations of study day and predose handling for each age group. To assist with interpretation, a gradient has been applied, with absolute error from green (lowest) to red (highest).

	DESIGN		C96											
			TH				M				W			
			A		B		A		B		A		B	
ADULTS	2	Pre-1-24-48-72	7	[18.5]	5.1	[12.8]	7.3	[18.9]	4.7	[11.2]				
	3	Pre-1-24-48-96	6.4	[16.1]	4.4	[10.8]	6.8	[17.7]	4.6	[12.5]				
	4	Pre-1-24-72-96	4.3	[12.3]	3.1	[8.4]	4.3	[11.7]	3.2	[8.7]				
	5	Pre-1-48-72-96	1.6	[4.7]	1.1	[3.3]	1.6	[4.4]	1.3	[3.7]				
	6	Pre-1-72-96	5.2	[14.9]	3.6	[10.0]	4.9	[13.5]	3.7	[10.7]				
	7	Pre-1-48-96	7.1	[18.1]	4.8	[11.8]	7.5	[19.8]	5.2	[13.5]				
	8	Pre-1-24-96	10	[28.1]	6.2	[16.4]	10.5	[28.4]	7.1	[20.7]				
	9	Pre-1-48-72	7.6	[19.3]	5.4	[13.1]	8	[19.8]	5.1	[11.8]				
	10	Pre-1-24-72	10.1	[24.9]	7	[16.9]	10.7	[26.4]	6.8	[16.4]				
	11	Pre-1-24-48	15.8	[38.6]	8.9	[23.5]	14.9	[37.2]	8	[19.5]				
	12	Pre-1-24	32	[77.3]	12.2	[30.2]	29.3	[69.6]	12.2	[30.8]				
	13	Pre-1-48	17.3	[42.2]	9.4	[23.5]	17	[42.6]	8.7	[20.9]				
	14	Pre-1-72	11.2	[28.2]	7.5	[18.4]	11.8	[28.4]	7.5	[18.4]				
	15	Pre-1-96	13.1	[40.5]	7	[19.2]	13.6	[38.6]	8.7	[26.5]				
	16	Pre-1	88.3	[339.2]	13.4	[33.4]	90.2	[366.0]	15	[41.9]				
	17	1-24	32.6	[85.9]	30.4	[72.2]	28.9	[71.2]	28.6	[67.7]				
	18	1-48	17.3	[43.5]	17.4	[42.7]	16.8	[42.7]	18	[45.1]				
	19	1-72	11.3	[27.6]	11.8	[29.3]	11.8	[29.1]	13.2	[31.9]				
	20	1-96	13.1	[40.6]	14.4	[41.9]	13.6	[38.6]	15.4	[40.0]				
	21	1	88.1	[336.9]	84.8	[317.7]	90.2	[365.6]	89.7	[349.1]				
ADOLESCENTS	2	Pre-1-24-48-72	4.5	[13.8]	3.2	[9.8]	4.5	[13.8]	3.4	[9.4]	4.9	[15.0]	3.9	[12.4]
	3	Pre-1-24-48-96	6.4	[16.4]	4.4	[11.0]	6.6	[16.9]	5.2	[14.0]	6.1	[17.3]	4.7	[13.6]
	4	Pre-1-24-72-96	6.1	[16.1]	4.3	[12.0]	6.2	[16.0]	5.2	[14.6]	5.7	[15.6]	3.9	[9.8]
	5	Pre-1-48-72-96	2.6	[7.2]	1.9	[5.5]	2.6	[7.1]	2.3	[6.6]	2.5	[7.1]	1.8	[4.6]
	6	Pre-1-72-96	8.5	[25.5]	5.9	[16.5]	8.7	[25.5]	7.5	[23.8]	8.4	[24.5]	4.9	[12.7]
	7	Pre-1-48-96	7.7	[20.1]	5.2	[13.2]	8	[20.0]	6.4	[17.8]	7.6	[19.7]	5.6	[14.4]
	8	Pre-1-24-96	12.7	[35.1]	7.5	[19.1]	13	[34.9]	9.8	[27.0]	11.8	[33.0]	7.7	[19.9]
	9	Pre-1-48-72	6	[15.7]	4.3	[11.1]	6	[15.5]	4.7	[11.4]	6.1	[16.8]	4.6	[13.5]
	10	Pre-1-24-72	9.4	[23.7]	6.3	[15.6]	9.2	[23.2]	7.2	[18.2]	9.3	[22.8]	6.5	[17.1]
	11	Pre-1-24-48	10.4	[30.3]	6.5	[16.6]	10.7	[30.4]	7.6	[21.3]	11.2	[31.1]	7.9	[22.8]
	12	Pre-1-24	22.8	[58.8]	10.4	[25.9]	22.9	[61.3]	13.4	[36.5]	24.4	[64.7]	12.3	[34.1]
	13	Pre-1-48	12.7	[34.0]	7.6	[18.8]	13	[34.5]	9	[23.9]	13	[35.8]	8.7	[25.8]
	14	Pre-1-72	12.5	[30.0]	8.1	[19.5]	12.3	[30.4]	9.8	[25.9]	12	[30.2]	7.4	[18.9]
	15	Pre-1-96	22.2	[68.9]	10	[26.5]	22.1	[65.3]	16.4	[56.7]	21.7	[61.6]	9.1	[24.6]
	16	Pre-1	62.9	[180.0]	13.8	[32.8]	61.8	[175.4]	23.9	[71.4]	67.9	[197.4]	14.5	[38.5]
	17	1-24	23.2	[61.2]	22.3	[57.7]	22.9	[62.9]	22.7	[60.6]	26	[72.6]	22.9	[59.8]
	18	1-48	12.7	[33.6]	13.1	[34.9]	12.9	[34.9]	13.2	[35.1]	13.2	[35.7]	13	[34.6]
	19	1-72	12.5	[30.2]	13.1	[31.6]	12.3	[30.3]	13	[31.8]	12.1	[30.4]	12.5	[32.2]
	20	1-96	22.2	[69.0]	22.9	[68.0]	22.1	[65.3]	22.8	[67.4]	21.7	[61.8]	21.5	[59.0]
	21	1	62.9	[180.0]	63.3	[183.5]	61.8	[175.2]	62.7	[182.5]	67.9	[198.6]	68	[214.1]
CHILDREN	2	Pre-1-24-48-72	0.5	[2.4]	0.4	[2.0]	0.6	[2.7]	0.5	[2.7]	0.6	[3.4]	0.5	[2.3]
	3	Pre-1-24-48-96	1.5	[6.5]	1.2	[5.4]	1.5	[7.0]	1.4	[6.4]	1.7	[7.9]	1.1	[5.3]
	4	Pre-1-24-72-96	3.4	[12.6]	2.9	[10.2]	3.5	[13.9]	3.5	[13.2]	3.4	[12.2]	2.2	[8.2]
	5	Pre-1-48-72-96	3.4	[9.3]	3.2	[8.5]	3.5	[9.5]	3.5	[9.4]	3.5	[9.6]	2.4	[6.3]
	6	Pre-1-72-96	9.8	[28.7]	8	[23.1]	9.8	[28.1]	9.7	[27.3]	9.6	[29.4]	4.2	[12.8]
	7	Pre-1-48-96	4.2	[11.7]	3.8	[10.2]	4.5	[13.2]	4.3	[12.8]	4.3	[12.3]	2.8	[7.9]
	8	Pre-1-24-96	4.6	[19.9]	3.7	[14.5]	4.5	[18.7]	4.3	[16.7]	5.1	[22.1]	3	[11.8]
	9	Pre-1-48-72	3.6	[9.8]	3.3	[8.7]	3.7	[10.1]	3.6	[9.9]	3.7	[10.6]	2.5	[6.7]
	10	Pre-1-24-72	3.6	[14.6]	3	[10.9]	3.7	[14.4]	3.6	[14.4]	3.6	[14.2]	2.3	[9.1]
	11	Pre-1-24-48	1.6	[7.0]	1.3	[6.0]	1.7	[7.5]	1.5	[6.6]	1.9	[9.8]	1.3	[6.0]
	12	Pre-1-24	5	[23.2]	3.8	[14.8]	4.8	[21.6]	4.6	[18.4]	5.5	[24.5]	3.1	[12.2]
	13	Pre-1-48	4.4	[12.7]	3.9	[10.9]	4.7	[13.7]	4.5	[13.0]	4.6	[14.0]	3	[8.3]
	14	Pre-1-72	10.2	[30.0]	8.3	[23.7]	10	[29.7]	10	[28.6]	10.1	[30.6]	4.4	[13.7]
	15	Pre-1-96	14.7	[46.1]	10.3	[31.4]	15.4	[50.2]	13.9	[44.0]	15.5	[50.8]	5.2	[17.2]
	16	Pre-1	14.3	[49.1]	10.7	[31.9]	14.1	[50.1]	14.6	[44.6]	15.8	[55.0]	5.4	[17.5]
	17	1-24	4.9	[22.3]	5.1	[23.5]	4.8	[21.6]	4.8	[21.1]	5.7	[23.6]	5.7	[24.2]
	18	1-48	4.4	[12.6]	4.6	[14.4]	4.7	[13.8]	4.8	[14.5]	4.6	[13.9]	5	[15.6]
	19	1-72	10.2	[30.1]	10.3	[29.8]	10	[29.7]	10	[29.4]	10.1	[30.5]	10.3	[30.4]
	20	1-96	14.7	[45.9]	14.7	[44.8]	15.4	[50.2]	15.4	[50.5]	15.5	[50.6]	15.6	[50.8]
	21	1	14.3	[48.9]	14.4	[48.9]	14.1	[50.1]	14.2	[49.3]	15.7	[54.5]	15.9	[55.4]

Table S10. $IDAR_{72}$ and $IDAR_{96}$ for all permutations of study day and predose handling for each age group. To assist with interpretation, a gradient has been applied, with IDAR increasing from green (lowest) to red (highest).

	DESIGN		IDAR72						IDAR96					
			TH		M		W		TH		M		W	
			A	B	A	B	A	B	A	B	A	B	A	B
ADULTS	2	Pre-1-24-48-72	0.0%	0.1%	0.1%	0.0%			0.8%	0.5%	0.7%	0.9%		
	3	Pre-1-24-48-96	0.1%	0.0%	0.3%	0.3%			2.1%	1.6%	2.4%	1.7%		
	4	Pre-1-24-72-96	0.4%	0.4%	0.3%	0.4%			1.0%	0.7%	1.6%	1.1%		
	5	Pre-1-48-72-96	0.0%	0.2%	0.0%	0.3%			0.4%	0.2%	0.4%	0.4%		
	6	Pre-1-72-96	0.9%	0.7%	0.3%	0.6%			0.9%	0.8%	1.8%	1.4%		
	7	Pre-1-48-96	0.2%	0.2%	0.3%	0.4%			2.9%	1.5%	3.0%	2.4%		
	8	Pre-1-24-96	0.5%	0.2%	0.7%	1.0%			3.4%	1.5%	3.7%	2.7%		
	9	Pre-1-48-72	0.0%	0.2%	0.1%	0.4%			1.0%	0.6%	1.0%	1.3%		
	10	Pre-1-24-72	0.6%	0.6%	0.4%	0.2%			1.6%	1.4%	2.6%	1.7%		
	11	Pre-1-24-48	0.1%	0.1%	0.3%	0.3%			2.9%	2.3%	3.1%	2.1%		
	12	Pre-1-24	0.7%	0.3%	1.2%	0.9%			6.6%	2.9%	7.2%	4.2%		
	13	Pre-1-48	0.2%	0.2%	0.3%	0.6%			4.1%	2.5%	3.5%	2.7%		
	14	Pre-1-72	0.8%	0.7%	0.7%	0.8%			1.6%	1.2%	3.1%	1.8%		
	15	Pre-1-96	1.5%	0.8%	1.8%	1.9%			4.4%	2.6%	4.9%	3.9%		
	16	Pre-1	1.5%	0.9%	1.8%	2.0%			13.1%	3.7%	13.8%	5.2%		
	17	1-24	0.5%	0.8%	1.3%	1.1%			6.5%	6.3%	7.1%	7.0%		
	18	1-48	0.2%	0.1%	0.4%	0.5%			4.0%	4.1%	3.4%	3.6%		
	19	1-72	0.8%	0.9%	0.7%	0.8%			1.7%	2.2%	3.1%	3.3%		
	20	1-96	1.5%	1.6%	1.8%	2.0%			4.4%	4.5%	4.9%	5.1%		
	21	1	1.5%	1.6%	1.8%	2.0%			13.1%	13.1%	13.8%	13.6%		
ADOLESCENTS	2	Pre-1-24-48-72	0.3%	0.5%	0.2%	0.5%	0.7%	0.2%	2.1%	1.4%	1.8%	1.7%	2.1%	1.7%
	3	Pre-1-24-48-96	1.7%	1.5%	1.9%	1.9%	1.5%	0.7%	3.7%	3.1%	3.5%	2.9%	4.0%	3.1%
	4	Pre-1-24-72-96	3.8%	3.1%	1.9%	2.4%	2.3%	1.4%	3.3%	2.0%	2.4%	1.6%	2.3%	1.0%
	5	Pre-1-48-72-96	0.9%	1.1%	1.4%	1.4%	1.4%	0.8%	1.1%	1.2%	1.0%	0.8%	1.1%	0.6%
	6	Pre-1-72-96	5.5%	4.3%	4.8%	5.1%	4.7%	1.8%	3.7%	2.5%	2.5%	1.9%	2.8%	1.9%
	7	Pre-1-48-96	2.6%	1.9%	3.6%	3.1%	2.6%	1.3%	4.5%	3.5%	4.1%	3.3%	3.8%	4.0%
	8	Pre-1-24-96	5.9%	4.0%	4.6%	4.6%	4.3%	1.4%	7.5%	4.4%	8.0%	5.1%	5.5%	4.2%
	9	Pre-1-48-72	1.1%	1.0%	1.5%	1.3%	2.0%	0.7%	2.9%	2.2%	2.3%	1.9%	2.2%	2.0%
	10	Pre-1-24-72	4.6%	3.8%	2.4%	2.3%	2.5%	1.7%	5.0%	2.9%	3.3%	3.4%	3.5%	2.0%
	11	Pre-1-24-48	1.8%	1.4%	1.7%	1.8%	1.9%	0.8%	5.8%	3.6%	5.2%	4.5%	5.2%	4.4%
	12	Pre-1-24	6.8%	4.6%	4.9%	4.7%	5.4%	1.8%	14.2%	4.5%	13.6%	6.6%	13.0%	5.8%
	13	Pre-1-48	2.4%	1.8%	3.9%	2.6%	2.9%	1.6%	6.6%	4.6%	7.3%	6.0%	5.6%	4.2%
	14	Pre-1-72	6.7%	4.2%	4.7%	4.8%	5.5%	1.9%	6.1%	3.4%	4.7%	4.3%	3.6%	2.5%
	15	Pre-1-96	12.6%	6.0%	12.8%	11.8%	13.2%	2.4%	8.1%	4.8%	10.8%	6.2%	7.6%	4.3%
	16	Pre-1	16.1%	6.1%	15.1%	13.1%	16.1%	2.8%	34.5%	5.9%	34.8%	10.0%	34.0%	6.7%
	17	1-24	6.6%	6.3%	5.0%	5.4%	5.5%	5.0%	13.0%	13.4%	13.6%	13.9%	10.7%	11.6%
	18	1-48	2.6%	2.9%	3.9%	3.5%	2.9%	3.2%	6.8%	7.2%	7.2%	7.5%	5.5%	6.2%
	19	1-72	6.7%	6.8%	4.7%	4.7%	5.5%	5.6%	5.7%	6.4%	4.6%	4.8%	3.6%	4.3%
	20	1-96	12.6%	12.6%	12.8%	13.1%	13.2%	12.2%	8.2%	9.0%	10.8%	10.0%	7.6%	7.6%
	21	1	16.2%	16.2%	15.1%	15.3%	16.0%	15.1%	34.5%	34.3%	34.6%	34.3%	33.5%	33.0%
CHILDREN	2	Pre-1-24-48-72	0.4%	0.6%	0.5%	0.4%	0.5%	0.3%	0.1%	0.1%	0.1%	0.0%	0.6%	0.2%
	3	Pre-1-24-48-96	1.2%	0.8%	1.3%	1.2%	1.5%	1.2%	0.2%	0.2%	0.2%	0.3%	0.7%	0.6%
	4	Pre-1-24-72-96	2.2%	2.1%	2.6%	2.3%	2.1%	1.5%	0.0%	0.0%	0.3%	0.3%	0.4%	0.2%
	5	Pre-1-48-72-96	1.5%	0.8%	1.1%	0.8%	1.4%	1.0%	0.3%	0.1%	0.2%	0.1%	0.3%	0.3%
	6	Pre-1-72-96	3.4%	2.8%	3.7%	3.4%	3.5%	2.4%	0.3%	0.2%	0.3%	0.4%	0.5%	0.2%
	7	Pre-1-48-96	2.2%	1.5%	1.9%	1.6%	2.5%	2.0%	0.5%	0.3%	0.3%	0.2%	0.4%	0.6%
	8	Pre-1-24-96	3.6%	3.0%	4.0%	3.2%	3.4%	2.1%	0.4%	0.2%	0.4%	0.3%	0.9%	0.8%
	9	Pre-1-48-72	1.8%	1.1%	1.1%	0.8%	1.4%	1.2%	0.3%	0.1%	0.2%	0.1%	0.5%	0.5%
	10	Pre-1-24-72	2.4%	2.1%	2.9%	2.4%	2.0%	1.3%	0.2%	0.1%	0.3%	0.5%	0.5%	0.4%
	11	Pre-1-24-48	1.1%	1.0%	1.2%	1.1%	1.7%	1.3%	0.3%	0.2%	0.1%	0.3%	1.0%	0.7%
	12	Pre-1-24	4.4%	3.2%	4.0%	3.5%	4.1%	2.1%	0.6%	0.3%	0.7%	0.4%	1.5%	0.9%
	13	Pre-1-48	2.3%	1.5%	2.1%	1.7%	2.7%	1.7%	0.7%	0.3%	0.2%	0.2%	0.6%	1.0%
	14	Pre-1-72	3.8%	3.3%	4.2%	3.8%	4.7%	2.5%	0.4%	0.1%	0.4%	0.4%	0.7%	0.6%
	15	Pre-1-96	6.8%	3.8%	8.9%	6.5%	9.6%	3.7%	0.7%	0.3%	0.6%	0.3%	1.0%	1.1%
	16	Pre-1	9.2%	4.5%	9.3%	8.1%	12.1%	4.3%	0.9%	0.5%	0.7%	0.4%	1.7%	1.0%
	17	1-24	4.5%	5.0%	3.9%	4.3%	3.3%	3.3%	0.4%	0.6%	0.7%	0.9%	1.3%	1.7%
	18	1-48	2.4%	2.2%	2.0%	2.0%	2.4%	2.4%	0.5%	0.7%	0.2%	0.2%	0.9%	0.8%
	19	1-72	3.8%	4.2%	4.2%	4.1%	4.7%	5.3%	0.3%	0.3%	0.4%	0.2%	0.7%	0.7%
	20	1-96	6.8%	7.3%	8.9%	8.6%	9.6%	9.6%	0.7%	0.7%	0.6%	0.6%	1.0%	1.2%
	21	1	9.2%	9.1%	9.3%	9.2%	12.1%	11.9%	0.9%	0.9%	0.7%	0.7%	1.7%	1.9%

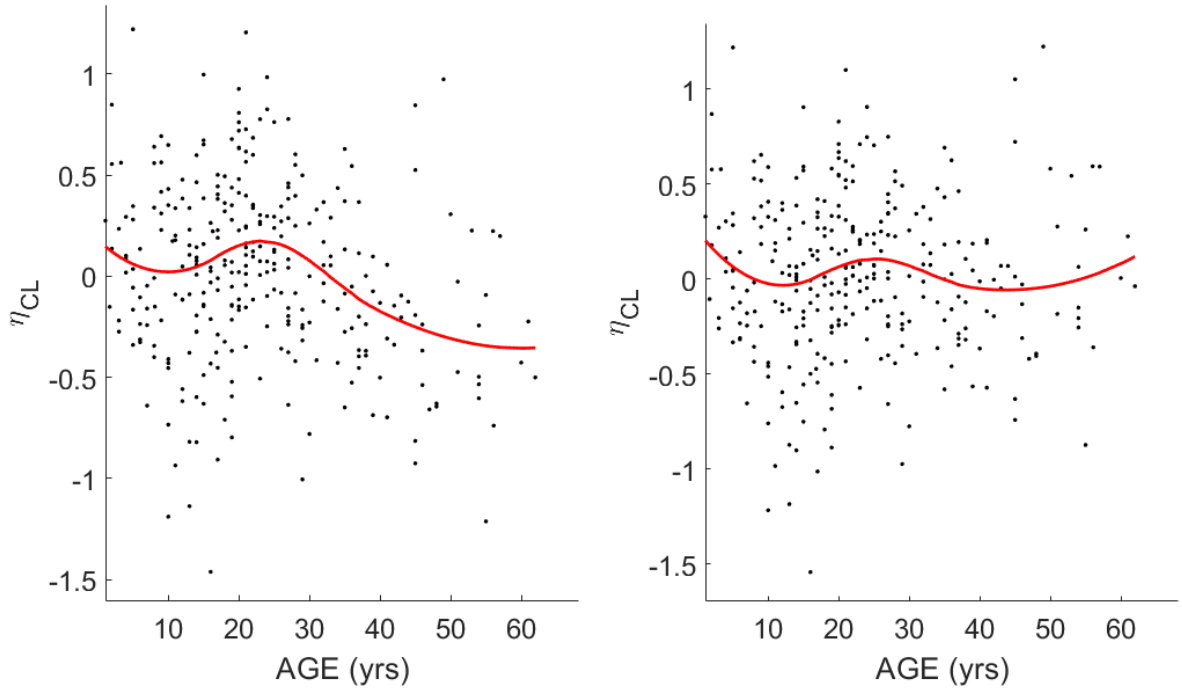


Figure S1. Scatter plots of η-values versus age following the inclusion of fat-free mass on CL, V_1 and V_2.

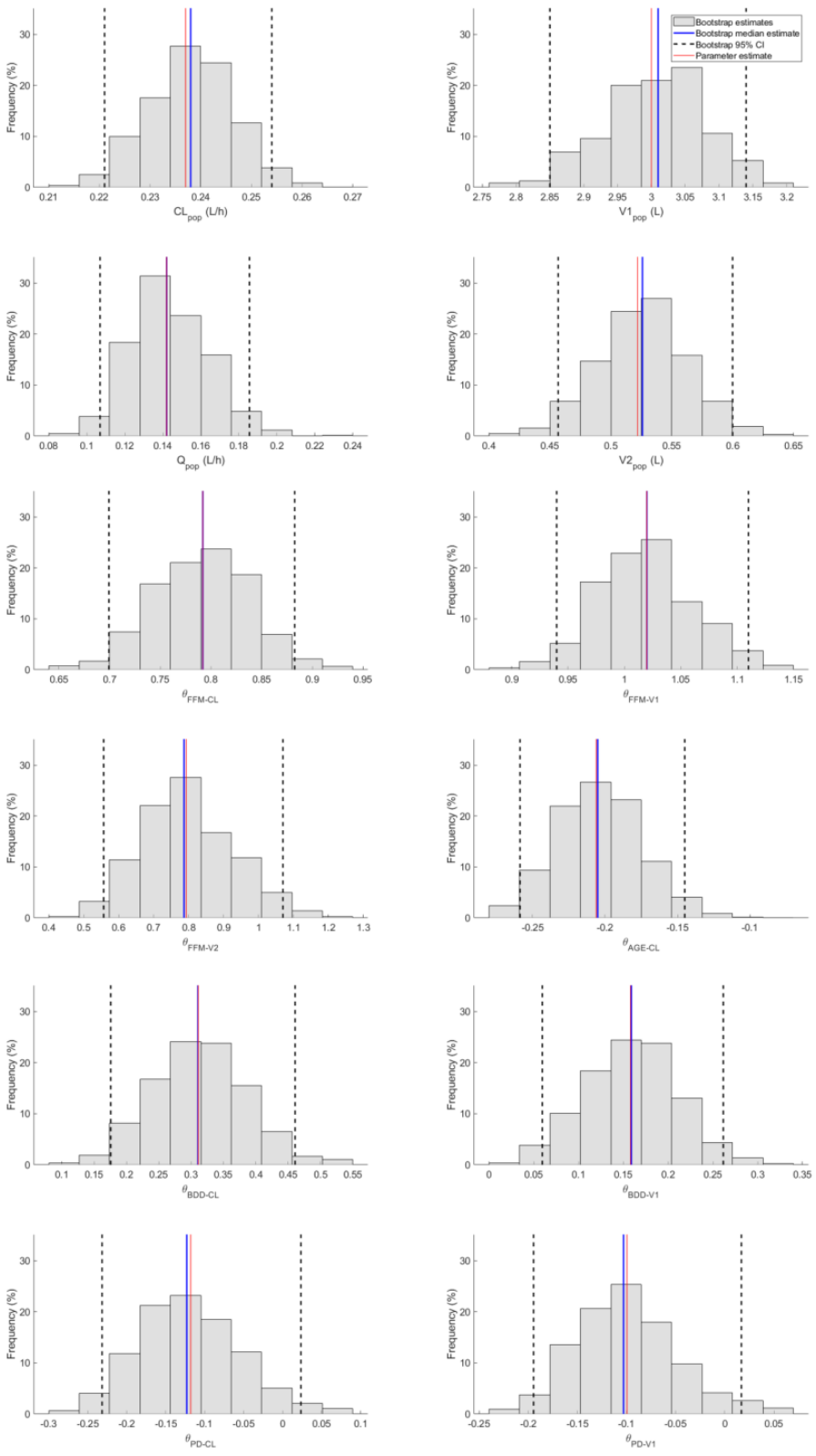


Figure S2. Histograms of parameters estimates for fixed effects from bootstrap analysis

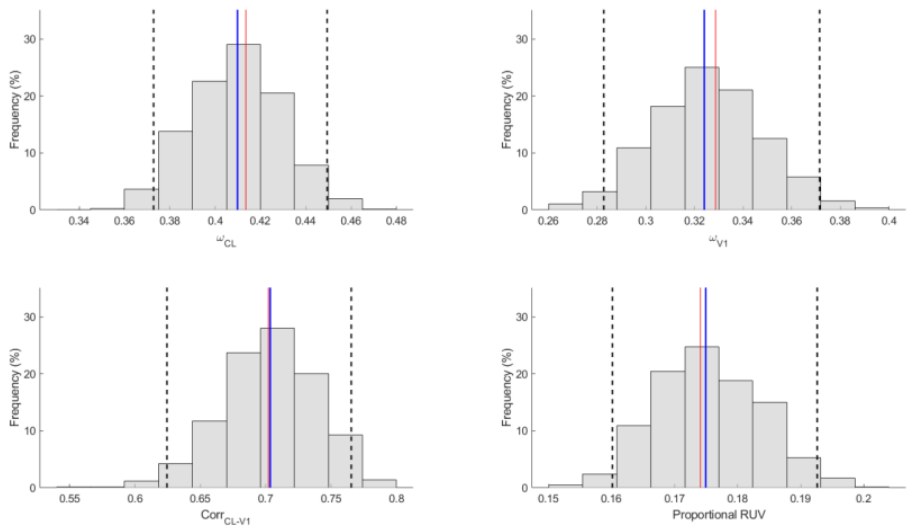


Figure S3. Histograms of parameter estimates of variability from bootstrap analysis

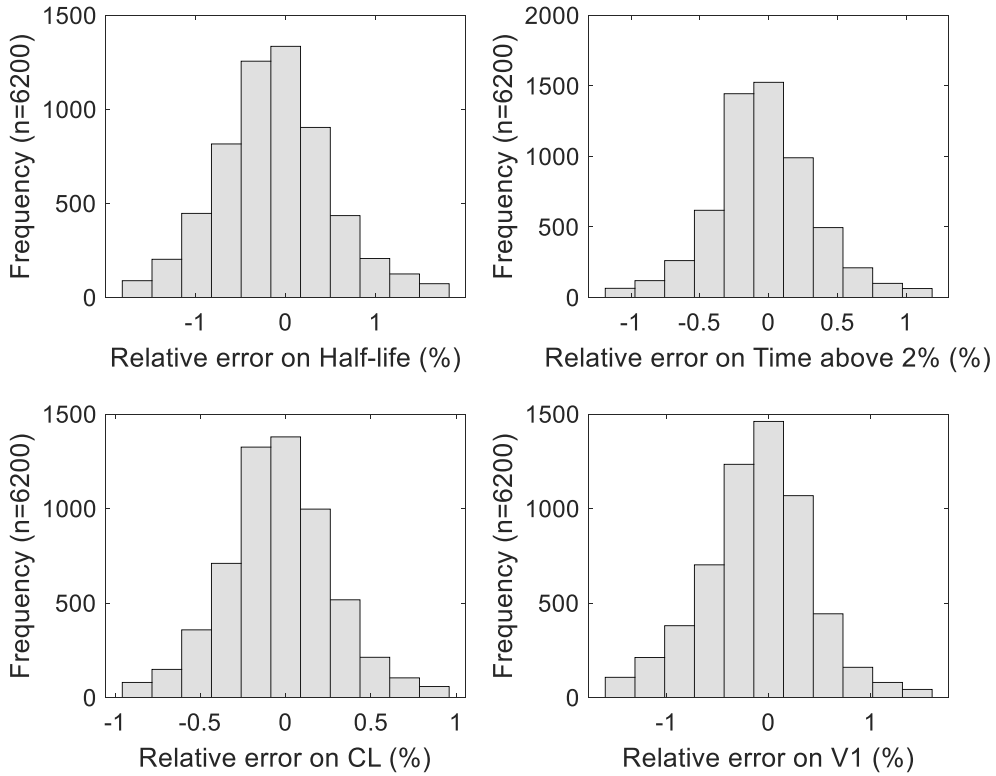


Figure S4. Histograms of prediction errors on half-life, time to 2% activity, clearance, and central volume from internal cross-validation

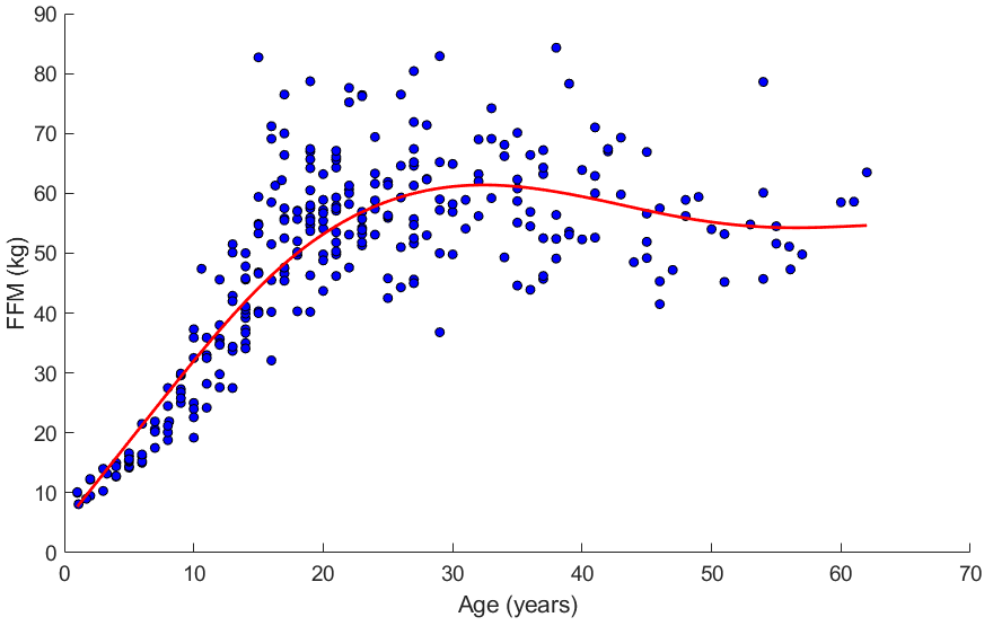


Figure S5. Correlation between FFM and age

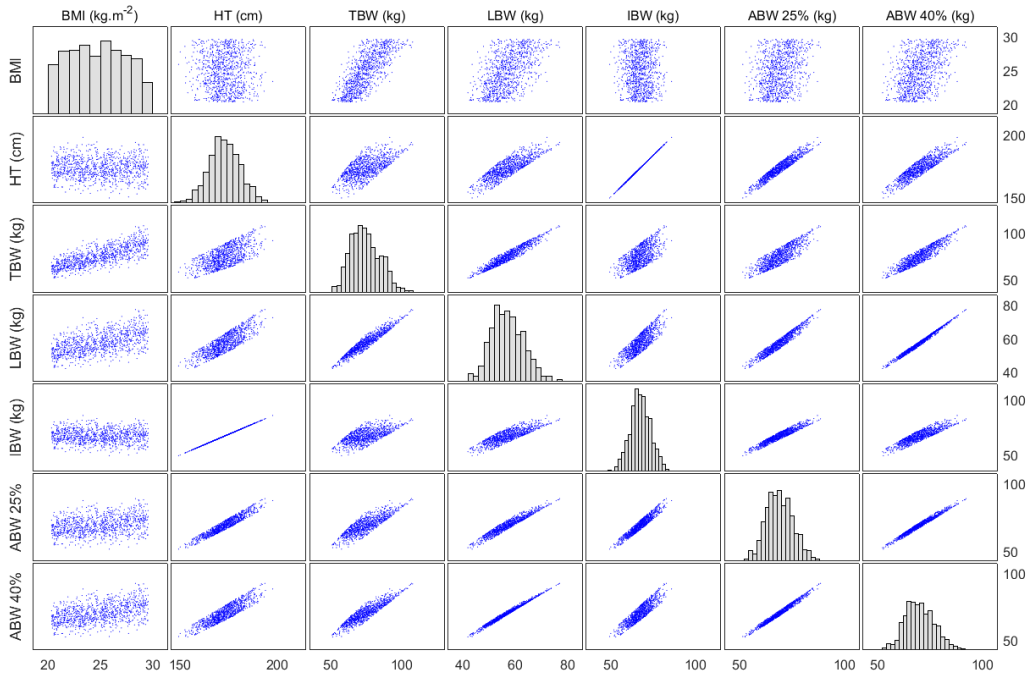


Figure S6. Correlation plots for all body size metrics used in simulations for the normal BMI subgroup. Diagonal elements contain histograms.

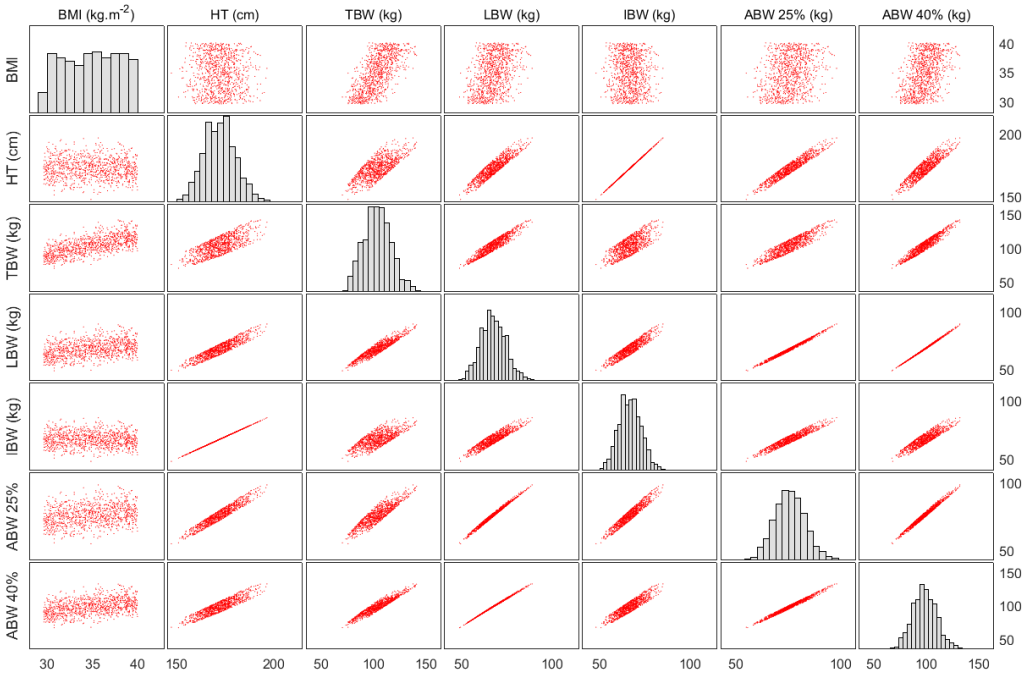


Figure S7. Correlation plots for all body size metrics used in simulations for the overweight/obese subgroup. Diagonal elements contain histograms.

List of WAPPS-Hemo Centres

Alberta Children's Hospital, Calgary, Canada
Amrita Hospital, Kochi, India
Antwerp University Hospital, Edegem, Belgium
AOU Città della Salute e della Scienza di Torino, Turin, Italy
Arthur Bloom Haemophilia centre, Cardiff, United Kingdom
Azienda Ospedaliera Pugliese-Ciaccio, Catanzaro, Italy
Azienda Ospedaliero Universitaria di Parma, Parma, Italy
BC Children's Hospital, Vancouver, Canada
Beijing Children's Hospital, Beijing, China
Belarusian Centre for Paediatric Oncology and Haematology, Minsk, Belarus
Bern University Hospital, Bern, Switzerland
Bloodworks Northwest, Seattle, United States
Calvary Mater Hospital/John Hunter Children's Hospital, Newcastle, Australia
Center for Bleeding and Clotting, Minneapolis, United States
Center for Hemorrhagic and Thrombotic Diseases, University Hospital of Udine, Udine, Italy
Center for Inherited Blood Disorders, Orange County, United States
Centre de traitement des Hémophiles Eaubonne-Montmorency, Montmorency, France
Centre de traitement des Hémophiles Hôpital, Mignot, France
Centre Hospitalier Le Mans, Le Mans, France
Centro Asistencial Regional de Hemoterapia (CARDHE), Bahia Blanca, Argentina
Centro de Hemoterapia e Hematologia do Espirito Santo, Victoria, Brazil
Centro Emofilia di Padova, Padova, Italy
Centro Emofilia e Trombosi, Bari, Italy
Centro Hospitalar de Lisboa Central, Lisbon, Portugal
Centro Médico Imbanaco, Cali, Colombia
Centro. Nacional de Hemofilia, Caracas, Venezuela
CHEO Research Institute, Ottawa , Canada
Children's Hospital Colorado Anschutz Medical Campus, Aurora, United States
Children's Hospital of Michigan, Detroit, United States
Children's Hospital, Boston, United States
Children's Hospital, Los Angeles, United States
Children's Medical University Hospital, Riga, Latvia
Children's of Minnesota, Minneapolis, United States
CHR de la Citadelle, Liège, Belgium
Christchurch Hemophilia Treatment Centre, Christchurch, New Zealand
CHRU de Besançon, Besançon, France
CHU Caen, Caen, France
CHU de Rouen, Rouen, France
CHU Sainte Justine, Montreal, Canada
CHU, University Hospital of Nancy, Nancy, France
Clinique Vasculaire et Coagulation, Angers, France
Complejo Asistencial Dr. Sótero del Río, Santiago, Chile

Complejo Hospitalario de Navarra, Pamplona, Spain
Congenital Coagulopathies Unit, Balearic Islands, Spain
CTH-Cordoba, Cordoba, Argentina
Dr. von Haunersches Kinderspital, Munich, Germany
Ege University Hospital, Izmir, Turkey
Emory University, Atlanta, United States
Erasmus MC, Sophia Children's Hospital, Rotterdam, Netherlands
Exeter and Barnstaple Haemophilia Centre, Barnstaple, United Kingdom
Farwaniya General Hospital, Al Farwaniyah, Kuwait
Fondazione IRCCS Policlinico San Matteo, Pavia, Italy
Fondazione Policlinico universitario "Agostino Gemelli", Rome, Italy
Foothills Medical Centre, Calgary, Canada
Fundación de Hemofilia de Salta, Salta, Argentina
Fundacion de la Hemofilia Rosario, Rosario, Argentina
Fundacion de la Hemofilia, Buenos Aires, Argentina
Gent University Hospital, Gent, Belgium
Gulf States Hemophilia, Houston, United States
Haematology and Haemophilia Centre Catelfranco Veneto, Catelfranco Veneto, Italy
Haemophilia Centre Copenhagen, Copenhagen, Denmark
Haemophilia Centre of Perugia, Perugia, Italy
Haemophilia Comprehensive Care Ljubljana, Ljubljana, Slovenia
Hamilton Health Sciences, Hamilton, Canada
Hämophilie-Zentrum Rhein Main GmbH, Frankfurt, Germany
Heim Pál Gyermekkórház, Budapest, Hungary
Helsinki University Hospital, Helsinki, Finland
Hematologia y oncologia del oriente SAS, Bogota, Colombia
Hematology and Oncology Department, CHU Nord, St. Etienne, France
Hemocentro Unicamp, São Paulo, Brazil
Hemofiliecentrum, UZ Leuven, Leuven, Belgium
Hemophilia Center of Western New York, Buffalo, United States
Hemophilia Center of Western Pennsylvania, Pittsburgh, United States
Hemophilia Comprehensive Care Team, Jakarta, Indonesia
Hemophilia Treatment Center of Central PA, Hershey, United States
Hemostasis and Thrombosis Center of Nevada, Las Vegas, United States
Hemostasis and Thrombosis Center Rhode Island, Rhode Island, United States
Hôpital de l'Enfant-Jésus, Quebec City, Canada
Hôpital Trousseau, CHRU de Tours, Tours, France
Hôpital Universitaire des Enfants Reine Fabiola, Huderf, Belgium
Hôpitaux Universitaires de Genève, Geneva, Switzerland
Hospital Alvaro Cunqueiro, Vigo, Spain
Hospital Clinico Universitario de Santiago, Santiago, Spain
Hospital de la Santa Creu i Sant Pau, Barcelona, Spain
Hospital de Santa Maria, Lisbon, Portugal

Hospital General Universitario de Alicante, Alicante, Spain
Hospital Humberto Notti, Mendoza, Argentina
Hospital Miguel Servet, Zaragoza, Spain
Hospital Posadas, Buenos Aires, Argentina
Hospital Regional Universitario de Málaga, Málaga, Spain
Hospital Roberto del Río, Santiago, Chile
Hospital Sant Joan de Déu, Barcelona, Spain
Hospital Teresa Herrera Materno Infantil, Coruna, Spain
Hospital Universitario Dr José Eleuterio Gonzalez, Monterrey, Mexico
Hospital Universitario La Paz, Madrid, Spain
Hospital Universitario Virgen de la Arrixaca, Murcia, Spain
Hospital University and Politechnic La Fe, Valencia, Spain
Hospital Vall d'Hebron, Barcelona, Spain
Hospital Virgen de las Nieves, Granada, Spain
Hull and East Yorkshire Hospitals NHS Trust, Hull, United Kingdom
Indiana Hemophilia and Thrombosis Center, Indianapolis, United States
Institute of Hematology and Blood Diseases Hospital Chinese Academy of Medical Science, Tianjin, China
Instituto Guatemalteco de Seguridad Social, Guatemala City, Guatemala
Intergral Solutions SD S.A.S, Bogota, Colombia
IPS Especializada, Bogota, Colombia
IWK Health Centre, Halifax, Canada
Johns Hopkins All Children's Hospital, St. Petersburg, United States
Kaohsiung Medical University Hospital, Kaohsiung, Taiwan
King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia
Kingston General Hospital, Kingston, Canada
Klinik für Kinder- und JugendmedizinUniversitätsklinikum Jena, Jena, Germany
Korea Hemophilia Foundation Seoul Clinic, Seoul, South Korea
Kuopio University Hospital, Kuopio, Finland
Kyung Hee University Hospital at Gangdong, Seoul, South Korea
Laiko General Hospital of Athens, Athens, Greece
L'hemostase de Strasbourg, Strasbourg, France
London Health Sciences Center, London, Canada
Luzerner Kantonsspital, Lucerne, Switzerland
Manitoba Health Sciences Centre, Winnipeg, Canada
Massachusetts General Hospital, Boston, United States
Maxima Medisch Centrum, Veldhoven, Netherlands
Mohács Hospital, Mohács, Hungary
Montreal Children's Hospital, Montreal, Canada
Nagoya University Hospital, Nagoya, Japan
Nanfang Hospital, Guangzhou, China
National Haemophilia Center Budapest, Budapest, Hungary
Nationwide Children's Hospital, Columbus, United States

Nemours Children's Specialty Care, Jacksonville, United States
North Dakota Hemostasis and Thrombosis Treatment Center, Fargo, United States
North Estonia Medical Center, Tallinn, Estonia
Northern Alberta Bleeding and Rare Blood Disorders Clinic - Kaye Edmonton Clinic, Edmonton, Canada
Northwest Ohio Hemophilia Treatment Center, Toledo, United States
Ogikubo Hospital, Tokyo, Japan
Oklahoma Center for Bleeding and Clotting Disorders, Oklahoma City, United States
ONCOORIENTE SAS, Villavicencio, Colombia
Oregon Health and Science University, Portland, United States
Orthopaedic Hemophilia Treatment Center, Los Angeles, United States
Ospedale S. Bortolo, Vicenza, Italy
Oulu University Hospital, Oulu, Finland
Palmetto Health Richland, Columbia, United States
Pediatric Hemophilia Center of Turin, Italy
Peking Union Medical College Hospital, Beijing , China
Phoenix Children's Hospital, Phoenix, Unites States
Policlinico di Palermo, Palermo, Italy
Policlinico Umberto I - "Sapienza" Università di Roma, Rome, Italy
Pontificia Universidad Católica de Chile, Santiago, Chile
Rady Children's Hospital, San Diego, United States
Riley Children's Health, Indianapolis, United States
Royal Adelaide Hospital, Adelaide, Australia
Royal Brisbane and Women's Hospital, Brisbane, Australia
Royal Free Hospital, London, United Kingdom
Royal London, London, United Kingdom
Ruan Rehacer IPS, Bogota, Colombia
Rush University Medical Center, Chicago, United States
Sahlgrenska University Hospital, Gothenburg, Sweden
Saskatchewan Bleeding Disorders Program, Saskatoon, Canada
Sheffield Children's Hospital, Sheffield, United Kingdom
SickKids Hospital, Toronto, Canada
Skåne University Hospital, Malmö, Sweden
South Texas Hemophilia Treatment Center, San Antonio, United States
St. George's University Hospital, London, United Kingdom
St. Joseph's Hospital - Center for Bleeding and Clotting Disorders, Tampa, United States
St. Jude Affiliate Clinic at NH Hemby Children's Hospital, Charlotte, United States
St. Jude Children's Research Hospital, Memphis, United States
St. Michael's Hospital, Toronto, Canada
St. Paul's Hospital, Vancouver, Canada
St-Luc University Hospital, Brussels, Belgium
Stollery Children's Hospital, Edmonton, Canada
Taichung Veterans General Hospital, Taichung, Taiwan
Taipei Medical University Hospital Hemophilia Center, Taipei, Taiwan

Tampere University Hospital, Tampere, Finland
The Alfred Hospital, Melbourne, Australia
The Bleeding and Clotting Disorders Institute, Peoria, United States
The Children's Hospital at Montefiore, New York, United States
The Children's Hospital of Philadelphia, Philadelphia, United States
The Children's Hospital, Zhengjiang University School of Medicine, Hangzhou, China
The Maine Hemophilia and Thrombosis Center, Scarborough, United States
The Royal Children's Hospital, Melbourne, Australia
The Women's and Childrens Hospital, Adelaide, Australia
Turku University Hospital, Turku, Finland
U.O. Pediatria Generale e Specialistica "B. Trambusti", Bari, Italy
UHHS Cleveland. University Hospitals Health System, Cleveland, United States
Universitaets - Kinderklinik Wien, Vienna, Austria
Universitätsklinikum Bonn, Bonn, Germany
University Children's Hospital Berne, Berne, Switzerland
University Children's Hospital Zurich, Zurich, Switzerland
University Hospital Bristol, Bristol, United Kingdom
University Hospital Brno, Brno, Czech Republic
University Hospital Coventry and Warwickshire, Coventry, United Kingdom
University Hospital Magdeburg, Magdeburg, Germany
University Hospital Southampton, Southampton, United Kingdom
University Hospitals of Leicester, Leicester, United Kingdom
University Medical Center Utrecht, Utrecht, Netherlands
University Medical Centre Ljubljana, Ljubljana, Slovenia
University of California San Francisco Pediatric Hemophilia Treatment Center, San Francisco, United States
University of Debrecen, Debrecen, Hungary
University of Florida Hemophilia Treatment Cente, Gainsville, United States
University of Helsinki and Children's Hospital, Helsinki, Finland
University of Iowa Children's Hospital, Iowa City, United States
University of Kentucky Hemophilia Treatment Center, Lexington, United States
University of Louisville, Louisville, United States
University of Miami Hemophilia Treatment Center, Miami, United States
University of North Carolina, Chapel Hill, United States
University of Szeged, Szeged, Hungary
University of Virginia Health System, Charlottesville, United States
University of Wisconsin Comprehensive Program for Bleeding Disorders, Madison, United States
Valley Children's Healthcare, Madera, United States
Vanderbilt University Medical Center, Nashville, United States
Vivantes Clinic in Friedrichshain, Berlin, Germany
Wake Forest University, Winston-Salem, United States
Weill Cornell Medical College, New York, United States
Zurich University Hospital, Zurich, Switzerland