Pharmacometric Approaches to Improve Dose Individualization Methods in Hemophilia A

JOÃO A. ABRANTES
Hemophilia A is a bleeding disorder caused by the lack of functional coagulation factor VIII (FVIII). The overall aim of this thesis was to improve dose individualization of FVIII replacement therapy in hemophilia A using pharmacometric approaches.

A population pharmacokinetic (PK) model of FVIII activity following the administration of moroctocog alfa was developed based on data from a large heterogeneous cohort of moderate to severe hemophilia A patients. Body weight, age, neutralizing anti-FVIII inhibitors, race, and analytical assay were found to be significant predictors of FVIII activity PK. In addition, large inter-individual variability (IIV) and inter-occasion variability (IOV) was identified highlighting the need for dose individualization.

High magnitudes of IOV are known to impair model-based therapeutic drug monitoring. Using a population PK model of FVIII activity, several approaches to handle IOV in Bayesian forecasting of individual PK parameters were assessed across a wide range of features. Considering IOV in Bayesian forecasting, but ignoring IOV in dose calculation, led to the most precise individualized doses, in particular, when sparse data was used.

The dose-exposure-response relationship of FVIII replacement therapy remains unclear. A parametric repeated time-to-categorical event (RTTCE) model was developed to characterize the relationship between the dose of octocog alfa, plasma FVIII activity, bleeding frequency and severity, and covariates, using data from clinical trials. The bleeding hazard was found to decrease throughout time and to be affected by plasma FVIII activity and number of previous bleeds. Unexplained IIV in the bleeding hazard was found to be large.

Bayesian forecasting based on the RTTCE model was used to predict the future occurrence of bleeds, and to contrast the predicted outcome using individual i) PK, ii) bleeding, and iii) PK, bleeding and covariate information, from data collected in clinical trials. The results support that individual bleed information can inform the optimization of prophylactic dosing regimens in severe hemophilia A patients.

In summary, the pharmacometric approaches presented provide a valuable quantitative framework to improve dose individualization in hemophilia A. Furthermore, enhanced dosing has the potential to reduce bleeding frequency and to lower the high costs associated to treatment.

Keywords: Bayesian forecasting, coagulation factor VIII, dose adaptation, hemophilia/haemophilia A, inter-occasion variability, NONMEM, pharmacodynamics, pharmacokinetics, pharmacometrics, therapeutic drug monitoring

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ISSN 1651-6192
urn:nbn:se:uu:diva-381218 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-381218)
Now this is not the end.
It is not even the beginning of the end.
But it is, perhaps, the end of the beginning.

Sir Winston Churchill

To my family
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


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List of Additional Papers

In addition to the appended papers, João A. Abrantes has been author of the publications listed below.


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Abbreviations

ABR annual bleeding rate
AUC\textsubscript{PR} area under the precision-recall curve
AUC\textsubscript{ROC} area under the receiver operating characteristic curve
C concentration or FVIII activity
CI confidence interval
CL clearance
cm centimeter
COV covariate
CSA chromogenic substrate assay
CV coefficient of variation
d.f. degree of freedom
dL deciliter
DNA deoxyribonucleic acid
EBE empirical Bayes estimate
FIX coagulation factor IX
FN false negative
FP false positive
FPR false positive rate
FREM full random effects modeling
FVIII coagulation factor VIII
FX coagulation factor X
FXa activated coagulation factor X
h hour(s)
HT height
IIV inter-individual variability
IMP Monte Carlo importance sampling
IMPMAP Monte Carlo importance sampling assisted by mode \textit{a posteriori}
INH inhibitor anti-coagulation factor VIII
IOV inter-occasion variability
IU international unit
\( J \) Youden’s \( J \) statistics
kDA kilodalton
kg kilogram
LBW lean body weight
<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
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<tbody>
<tr>
<td>LEOPOLD</td>
<td>long-term efficacy open-label program in severe hemophilia A disease</td>
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<tr>
<td>LLOQ</td>
<td>lower limit of quantification</td>
</tr>
<tr>
<td>MAP</td>
<td>maximum <em>a posteriori</em></td>
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<tr>
<td>mL</td>
<td>milliliter</td>
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<tr>
<td>N</td>
<td>negative</td>
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<td>OFV</td>
<td>objective function value</td>
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<td>OSA</td>
<td>one-stage clotting assay</td>
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<tr>
<td>P</td>
<td>positive</td>
</tr>
<tr>
<td>PD</td>
<td>pharmacodynamics(s)</td>
</tr>
<tr>
<td>pdFVIII</td>
<td>plasma-derived coagulation factor VIII</td>
</tr>
<tr>
<td>PE</td>
<td>prediction error</td>
</tr>
<tr>
<td>PK</td>
<td>pharmacokinetic(s)</td>
</tr>
<tr>
<td>PPV</td>
<td>positive predictive value</td>
</tr>
<tr>
<td>PR</td>
<td>precision-recall</td>
</tr>
<tr>
<td>PsN</td>
<td>Perl-speaks-NONMEM</td>
</tr>
<tr>
<td>Q</td>
<td>inter-compartmental clearance</td>
</tr>
<tr>
<td>r</td>
<td>correlation coefficient</td>
</tr>
<tr>
<td>rFVIII</td>
<td>recombinant coagulation factor VIII</td>
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<tr>
<td>ROC</td>
<td>receiver operating characteristic</td>
</tr>
<tr>
<td>RSE</td>
<td>relative standard error</td>
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<tr>
<td>RTTCE</td>
<td>repeated time-to-categorical event</td>
</tr>
<tr>
<td>RUV</td>
<td>residual unexplained variability</td>
</tr>
<tr>
<td>SIR</td>
<td>sampling importance resampling</td>
</tr>
<tr>
<td>STUD</td>
<td>study</td>
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<tr>
<td>t</td>
<td>time</td>
</tr>
<tr>
<td>TDM</td>
<td>therapeutic drug monitoring</td>
</tr>
<tr>
<td>TN</td>
<td>true negative</td>
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<tr>
<td>TNR</td>
<td>true negative rate</td>
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<tr>
<td>TP</td>
<td>true positive</td>
</tr>
<tr>
<td>TPR</td>
<td>true positive rate</td>
</tr>
<tr>
<td>TVCL</td>
<td>typical value of clearance</td>
</tr>
<tr>
<td>V1</td>
<td>central volume of distribution</td>
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<tr>
<td>V2</td>
<td>peripheral volume of distribution</td>
</tr>
<tr>
<td>VWF</td>
<td>von Willebrand factor</td>
</tr>
<tr>
<td>WT</td>
<td>body weight</td>
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<tr>
<td>α</td>
<td>alpha, significance level</td>
</tr>
<tr>
<td>ε</td>
<td>epsilon, residual</td>
</tr>
<tr>
<td>η</td>
<td>eta, individual level random effect</td>
</tr>
<tr>
<td>θ</td>
<td>theta, population fixed effect</td>
</tr>
<tr>
<td>κ</td>
<td>kappa, occasion level random effect</td>
</tr>
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</table>
1 Introduction

The treatment of hemophilia A has improved dramatically over the last 50 years. Since 1965, when the cryoprecipitate rich in coagulation factor VIII (FVIII) was found, the life expectancy of a patient with severe hemophilia A went from less than 20 years old to a practically normal life expectancy for a patient receiving prophylactic treatment. However, in 2019 this bleeding disorder is still associated with a considerable clinical and economic burden. The foundation of the modern prophylactic treatment of severe hemophilia A, through the administration of FVIII replacement therapy, is extremely costly and involves the intravenous administration of a FVIII product 2 to 3 times a week. From an efficacy standpoint, there is considerable unexplained inter-individual variability (IIV) in the pharmacokinetics (PK) and bleeding response of these exogenous compounds, which make body weight dosing for the prevention of bleeds suboptimal. These and other constraints open the door to the optimization of dosing regimens of FVIII through individualization of therapy. This thesis focuses on the development of pharmacometric approaches to improve dose individualization methods in hemophilia A.

1.1 Hemophilia A

1.1.1 A coagulation disorder

The hemostatic balance is a complex process that relies on the trade-off between natural anticoagulant and procoagulant factors. Therefore, drug therapies that affect blood coagulation must be carefully administered since a deviation from the delicate balance of coagulation factors can lead to thrombotic or hemorrhagic complications. The absence of a single protein among the more than 25 key proteins involved in the hemostatic network can lead to serious health complications such as internal bleeding, deterioration of the joints and muscle atrophy (1, 2).

One group of coagulation disorders is hemophilia. Hemophilia A is caused by a deficiency of coagulation FVIII, and hemophilia B by a deficiency of coagulation factor IX (FIX) (2). Both hemophilia A and B are hereditary recessive X-linked disorders and consequently affect mainly men. However, between 20% and 30% of the cases of hemophilia have no previously affected family
members (3). The lack of these coagulation factors results in a decreased and delayed generation of thrombin, which ultimately affects the formation of the blood clot. Hemophilia A has a global prevalence of approximately 1 in 5 000 male births and hemophilia B around 1 in 30 000 (2). The most common type of hemophilia, hemophilia A, is the focus of this thesis.

1.1.2 Diagnosis
The diagnosis of hemophilia A can be made right after birth, if the mother is a known carrier, or when bleeding symptoms occur (4). The clinical severity of patients with hemophilia A is very heterogeneous, however, in general the severity is inversely correlated with the endogenous levels of FVIII activity, i.e. the amount of functional plasma FVIII that an individual is able to produce (Table 1) (5).

Table 1. Relationship between the severity of hemophilia A and endogenous plasma FVIII activity.

<table>
<thead>
<tr>
<th>Severity</th>
<th>Endogenous plasma FVIII activity</th>
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<tbody>
<tr>
<td>Severe</td>
<td>&lt;1 IU/dL¹</td>
</tr>
<tr>
<td>Moderate²</td>
<td>1-5 IU/dL</td>
</tr>
<tr>
<td>Mild</td>
<td>5-40 IU/dL</td>
</tr>
</tbody>
</table>

¹<0.01 IU/mL or <1% of normal
²FVIII activity between 1-2 IU/dL is sometimes referred to as moderately severe.

About half of the diagnosed patients with hemophilia A have the severe form of the disease and suffer from spontaneous bleeds, which can occur into the muscles or joints (2). Patients with moderate hemophilia suffer from occasional spontaneous bleeds and prolonged bleeds with minor trauma or surgery. Patients with mild hemophilia A rarely suffer from spontaneous bleeds but may experience severe bleeds with major trauma or surgery.

1.1.3 Clinical manifestations
The clinical manifestations of hemophilia A involve bleeding. The bleeding risk varies substantially across patients and depends mainly on the severity of the disease, as mentioned previously. The first bleed in patients with severe hemophilia A occurs at an early age (6-8 months old) when the child becomes more active, and about 50% of these bleeds are into soft tissues (6). The occurrence of bleeds in joints namely in the ankle, elbow and knee, increases with age and comprises about 60% of the total number of bleeds in patients aged 18-65 years (7). Repeated bleeds in the same joint causes inflammation of the synovial tissue, resulting in increasing damage of the joint. Ultimately, repeated bleeds can lead to the development of hemophilic arthropathy, a debilitating condition characterized by chronic pain, functional impairment and reduced quality of life (8).
1.1.4 Treatment strategies
The main prevention and treatment strategy in hemophilia A patients comprises the administration of the lacking coagulation protein, coagulation FVIII, to reach adequate hemostasis. This strategy, known as FVIII replacement therapy or substitution therapy, represents the basis of the modern treatment of hemophilia A, and it is the focus of this thesis.

Other treatment strategies have been used, or start to be used, to treat hemophilia A. Desmopressin is a synthetic analog of vasopressin that increases the plasma levels of FVIII and von Willebrand factor (VWF) for patients with mild and moderate hemophilia A (9). It shows high inter-patient variability and requires the administration of a test-dose to determine the individual increase in FVIII in plasma. Emicizumab is a humanized bispecific antibody that mimics the cofactor function of FVIII by bridging activated FIX and factor X (10). It has recently been approved to prevent bleeding episodes in patients of all ages, with or without the presence of inhibitors anti-FVIII, and it is administered subcutaneously every 1-4 weeks (11, 12).

Of note, treatments for hemophilia A with mechanisms action other than replacing FVIII, such as desmopressin, emicizumab, bypassing agents, or other products in development (e.g. concizumab or fitusiran) will not be further discussed in this thesis.

1.2 Factor VIII replacement therapy
1.2.1 Prevention and treatment strategies
This thesis focuses mainly on FVIII replacement therapy given in prophylaxis to prevent the occurrence of bleeding events, however, this therapy can also be administered on-demand, after the occurrence of a bleed, or perioperatively, to prevent bleeds in the perioperative setting.

1.2.1.1 Prophylaxis
Prophylactic treatment aims at preserving the normal musculoskeletal function by avoiding bleeding and joint destruction. It is based on the observation that patients with moderate hemophilia A tend to have less spontaneous bleeds than patients with severe hemophilia A, and relies on increasing the levels of FVIII from a severe to a milder form of the disease (13, 14).

Prophylaxis starts usually at young age (<2 years), and many dosing approaches exist. Examples are the Malmö protocol (high dose prophylactic regimen) and the Utrecht protocol (low dose prophylactic regimen) with doses of 25-40 IU/kg and 15-30 IU/kg, respectively. Both involve the administration
of FVIII thrice per week (15-17). In countries with limited access to replacement therapy, doses as low as 10 IU/kg twice per week have been found to be effective (18).

1.2.1.2 On-demand
On-demand treatment is given to treat a bleed and the doses, frequency and duration of treatment depend on the type and severity of the bleeding event. The loading dose administered is usually calculated based on the average *in vivo* recovery (observed post-dose FVIII activity level corrected for baseline in IU/dL divided by dose in IU/kg), where each unit of FVIII infused per kg increases plasma FVIII activity by 2 IU/dL (19, 20).

1.2.1.3 Perioperative
In the perioperative setting, target plasma FVIII activity levels are higher than in prophylaxis, and can be achieved with continuous or intermittent infusions. To calculate the loading dose, the average *in vivo* recovery is used, as for on-demand treatment.

1.2.2 Replacement products available
Currently, there are two main types of FVIII replacement products, which are classified according to their elimination half-life: standard half-life and extended half-life products.

Standard half-life products can be either derived from plasma (plasma-derived FVIII, pdFVIII) or recombinant (rFVIII). Plasma-derived products are manufactured using human plasma, whereas rFVIII products are prepared by recombinant DNA technology (21). The selection of the type of product to administer depends on many factors, including efficacy, cost and safety. Nowadays, both types of products are considered to be exceptionally safe with respect to the transmission of pathogens, and the major topic of debate is whether one type of FVIII replacement product exhibits a higher risk of developing anti-FVIII inhibitors compared to the other (21-23). Standard half-life products are the focus of this thesis.

Extended half-life products were created to increase the half-life of FVIII, and therefore reduce the frequency of administrations while maintaining hemostatic balance. The half-life prolongation is carried out by combining a FVIII molecule with the Fc domain of human immunoglobulin G1 or with polyethylene glycol (PEGylation). The half-lives of these products are about 1.5 higher compared to standard half-life products (24, 25).
1.2.3 Pharmacokinetics of exogenous standard half-life FVIII

Factor VIII is a protein that contains 2332 amino acids (330 kDa) organized in three domains in the following order: A1-a1-A2-a2-B-a3-A3-C1-C2 (26). In healthy subjects, FVIII is presumably produced primarily in the liver, where it is also cleared by the low density lipoprotein receptor-related protein 1 (LRP1) and the low-density lipoprotein receptor (LDLR) (27). Factor VIII circulates in plasma in complex with the VWF which protects it from proteolysis and rapid clearance (CL). The FVIII-VWF complex has a high molecular weight, which results in a distribution of FVIII mainly in plasma. A reduction in VWF levels is accompanied by a reduction in FVIII levels (28). Von Willebrand factor levels are high at birth, decrease over the first year of life, and thereafter gradually increase during childhood (29, 30). In adults, VWF levels have been shown to continue to increase with age, an effect that is more pronounced in individuals with blood type non-O (31).

The PK of rFVIII and pdFVIII is usually assumed to be similar, even though small differences may exist between products (32, 33). Factor VIII activity in plasma decays bi-exponentially, with an initial half-life of about 3 hours and a terminal half-life between 11 and 16 h (32, 34). Plasma CL values are between 2.4 and 3.4 mL/h/kg and volume of distribution at steady-state between 0.043 and 0.057 L/kg (32, 34). The processes of distribution and elimination of FVIII from plasma have been described to have a large unexplained IIV (>30%CV on CL, and >10%CV on volumes) and a small to moderate variability between dose administrations (inter-occasion variability, IOV, also known as within-occasion variability; 0-15%CV on CL, and 0-10%CV on volumes) (35-37). Figure 1 shows the variability in the plasma FVIII activity levels in a median patient, and in two virtual patients, with similar demographic characteristics following the administration of dose of a FVIII product on three occasions. In addition, the prediction interval is depicted which includes 95% of the FVIII activity-time profile levels in a cohort of 500 virtual patients.

A small portion of the IIV observed in the PK of FVIII activity can be explained by differences in age, body weight, plasma levels of VWF and blood group (35, 36, 38, 39).
1.2.4 Quantification of plasma FVIII

Plasma concentrations of coagulation FVIII are difficult to measure and concentrations may not directly translate into biological activity. Therefore, FVIII is commonly measured in terms of activity using bioassays, expressed as IU, where 1 IU of FVIII approximates the activity of FVIII in 1 mL of normal plasma. The most commonly used assays for FVIII activity measurement are the one-stage activated partial thromboplastin time clotting assay (OSA) and the two-stage chromogenic substrate assay (CSA) (40). In the OSA, FVIII-deficient plasma is mixed with an equal volume of the test plasma sample with addition of phospholipids and surface activator reagents (41). Thereafter, calcium ions are added to initiate the coagulation reaction, and the activated partial thromboplastin time is recorded. The CSA comprises two stages: in the first stage, the test plasma sample is incubated with activated coagulation factor IX (FIXa), coagulation factor X (FX), thrombin (optional), calcium ions and phospholipids to generate activated FX (FXa); in the second stage, the FXa hydrolyzes a FXa-selective chromogenic substrate, and the resulting color intensity indicates the amount of FXa, which is directly proportional to the amount of FVIII in the test plasma sample (42). These two assays lead to discrepant results under some circumstances, for instance, in the presence of specific phenotypes or mutations in the F8 gene and when certain FVIII products are analyzed (such as B-domain deleted rFVIII) (40). Both assays are unable to measure FVIII activity levels below 1 IU/dL accurately.

Figure 1. Illustration of plasma FVIII activity-time profiles following an intravenous bolus administration of 2040 IU (30 IU/kg) of a FVIII product at steady-state on 3 occasions to 500 virtual patients with severe hemophilia A aged 24 years and weighing 68 kg, in the linear and semi-log scale. Simulations performed using a model developed by Björkman et al. including inter-individual variability (clearance 28%CV; central volume 17%CV) and inter-occasion variability (clearance 13%CV; central volume 10%CV) (35).
1.2.5 Limitations of treatment

The main limitations related to FVIII replacement therapy are (i) the development of inhibitors against FVIII, (ii) the high cost, and (iii) the frequent intravenous injections.

The development of circulating neutralizing antibodies against FVIII (i.e. inhibitors) is the most serious health complication associated with treatment. Inhibitors are formed in approximately 30% of patients with severe hemophilia and increase the CL of FVIII and/or decrease in vivo recovery (43). Several risk factors for inhibitor development have been identified, including hemophilia severity, age at first exposure to a FVIII product and, possibly, product type (pdFVIII vs. rFVIII) (2). When the development of inhibitors is observed, bypass FVIII agents can be administered, or patients undergo immune tolerance induction, which has an inhibitor eradication success rate between 59% (high responding inhibitors) and 86% (low responding inhibitors) (44).

Another challenge associated with FVIII replacement therapy is the cost of FVIII products. The average cost of therapy with FVIII per patient is ~200 000 EUR/year across the five most populated European Union countries (45). This represents a major economic burden and full dose-prophylaxis is often prohibitive for much of the world, which leads to adoption of low-dose prophylaxis regimens (46).

Finally, given the short half-life of standard FVIII products, patients require the administration of frequent intravenous injections in prophylaxis (3-4 times per week). This aspect is one of the main obstacles to treatment adherence (47).

1.3 Pharmacometrics

1.3.1 Basic principles

Pharmacometrics has been described as “the science of developing and applying mathematical and statistical methods to characterize, understand, and predict a drug’s PK, pharmacodynamic (PD), and biomarker outcomes behavior” (48, 49). Non-linear mixed effects modeling, or population modeling approach, is the most used modeling approach in pharmacometrics. With this technique, the estimated model describes the features of a compound for all data at a population level by estimating, in one step, fixed effects and random effects. Fixed effects are the typical model parameter estimates (e.g. CL and central volume of distribution (V1) for PK) and represent the model when individual differences are not considered. Random effects are stochastic model
parameters, estimated as variances, and may comprise three levels: i) residual unexplained variability (RUV), which includes errors of measurement (e.g. analytical assay errors or uncertain dosing/sampling times), model misspecifications and intra-individual variability; ii) variability in parameter estimates between individuals (IIV); and iii) variability in parameter estimates between occasions within the same individual (IOV). With this approach it is possible to analyze rich, sparse or unbalanced data since each individual “borrows” information from the other individuals in the data, as the same structural model is applied to all data simultaneously. For instance, the PK parameters of a 2-compartment model for subjects with just peak and trough concentrations, collected following different dosing regimens, may be calculated based on the individual observations but they are also informed by the PK behavior in the remaining patients and observations in the dataset.

The estimation of an inappropriate stochastic model can lead to biased parameter estimates. For instance, neglecting the estimation of IOV may result in the inflation of residual and/or IIV as well as biased typical parameter values (50, 51).

1.3.2 Bayesian forecasting

Bayesian forecasting relies on the estimation of the most likely individual PK or PK/PD parameters, known as empirical Bayes estimates (EBEs), based on the patient’s data (dosing regimen, covariate values, surrogate marker) and a prior population model, including fixed and random effects (52). Mathematically, the estimation of the best set of EBEs is performed through the minimization of a Maximum A Posteriori (MAP) Bayesian objective function (53):

$$
OBJ_{MAP} = \sum_{j=1}^{p} \frac{(\hat{P}_j - \bar{P}_j)^2}{\omega_{\bar{P}_j}^2} + \sum_{i=1}^{m} \frac{(\hat{C}_i - C_i)^2}{\sigma_{\hat{C}_i}^2}
$$

(eq. 1)

where $p$ is the number of parameters in the model, $\bar{P}_j$ is the typical value of the $j^{th}$ parameter in the model and $\hat{P}_j$ the estimate of the individual’s parameter, $\omega_{\bar{P}_j}^2$ is the inter-individual variance in the $j^{th}$ parameter, $m$ is the number of observations, $C_i$ is the $i^{th}$ observed concentration and $\hat{C}_i$ is the respective predicted concentration using the $\hat{P}_j$ values, and $\sigma_{\hat{C}_i}^2$ is the residual error variance.
1.4 Individualization of drug therapy

Dose individualization can occur \textit{a priori} or \textit{a posteriori}, and both approaches can take advantage of an established PK/PD relationship described in a population model.

\textit{A priori} dose individualization uses relevant prior knowledge of the patient (e.g. demographic, clinical, or genetic information) to define dosing regimens without requiring the administration of the drug. An example of \textit{a priori} dose individualization in hemophilia A is the individualization of treatment based on body weight (e.g. administration of 30 IU of FVIII product per kg). A population model can be used in \textit{a priori} dose individualization by calculating the dose required by a typical subject with certain covariate values to achieve a specific target.

\textit{A posteriori} dose individualization involves the collection of a patient-specific endpoint marker of effect after the administration of the drug (e.g. drug plasma concentration) in order to tailor the upcoming dosing regimen. This individualization approach can take advantage of a population model, but does not have to. For instance, the reduction of prophylactic FVIII doses in hemophilia A after the systematic observation that a patient does not bleed, or dose tailing based on PK parameters obtained through a non-compartmental approach are \textit{a posteriori} dose adaptation approaches that do not require a stablished population model.

Model-based therapeutic drug monitoring (TDM) is an \textit{a posteriori} dose individualization approach that uses Bayesian forecasting to estimate the individual PK/PD parameters (as introduced in section 1.3.2) and thereafter generate individualized doses to achieve a given target. Model-based TDM has demonstrated to be a valuable tool in multiple therapeutic areas (54-56), leading to an optimal balance between safety and efficacy, namely, when a drug has a narrow therapeutic margin and exhibits high IIIV compared to RUV and/or IOV (57-59). However, if the magnitude of the RUV and/or IOV is moderate to high, the individual response on the next occasion is difficult to predict, and the usefulness of model-based TDM may be limited (50, 60, 61). One of the focus of this thesis is on how different magnitudes of IOV affect model-based TDM and how IOV can be handled in dose individualization.
1.5 *A posteriori* dose individualization of FVIII replacement therapy

Bayesian forecasting based on individual PK observations has been suggested as a promising tool to tailor FVIII dosing regimens in clinical practice, particularly in patients with severe hemophilia A under prophylactic treatment (PK-based/driven/guided/tailored prophylaxis) (38, 39, 62, 63).

1.5.1 Target FVIII activity

The choice of an individualized dosing regimen (dose and dosing interval) requires the definition of a plasma FVIII activity target above which the patient’s observed trough FVIII activity should be. Traditionally, a value of 1 IU/dL is used (13, 64). However, there is increasing evidence that a one-target-fits-all strategy is not appropriate and that the plasma FVIII activity to protect patients against bleeds is highly patient-specific (e.g. it may depend on patient’s response to treatment, physical activity and state of underlying joint disease) (64-71). Recently, alternative target levels have been suggested for different groups of patients, based on the individual clinical characteristics and on the patient’s lifestyle, however, these still lack clinical validation (72).

1.5.2 Bayesian prior models

Bayesian forecasting of PK parameters can only be done reliably if the patient has similar characteristics than the group of patients used to develop the prior population PK model. The population PK of FVIII activity have been extensively studied, and numerous models are described in the literature (for instance (35-37, 73, 74)). Even though these models are usually developed based on data obtained during clinical development trials, models based on other sources of data exist, such as data obtained in the perioperative setting (74). In case a model has not been developed based on a patient-specific characteristic and setting (for instance, a morbidly obese patient in the perioperative setting), an extrapolation can be considered (74, 75).

1.5.3 Model-based TDM tools for FVIII dose individualization

Bayesian forecasting of PK parameters and subsequent proposal of optimal dosing regimens has been implemented in multiple tools with the goal of facilitating model-based TDM in PK-based prophylaxis. Two examples are the Web-Accessible Population Pharmacokinetic Service—Hemophilia (WAPPS-Hemo, McMaster University, Hamilton, Ontario, Canada, www.wapps-hemo.org), which support multiple FVIII products, or the medical device myPKFit® (Shire Pharmaceutical Holdings Ireland Limited, Dublin, Ireland, www.mypkfit.com), which support a specific product (76, 77). It should be noted that different tools may lead to different dose recommendations (78).
2 Aims

The overall aim of this thesis was to improve dose individualization of FVIII replacement therapy in hemophilia A using pharmacometric approaches.

The specific aims were:

- To investigate the population PK of FVIII activity following the administration of a B-domain deleted rFVIII product, moroctocog alfa, in patients with moderate to severe hemophilia A, including relevant parameter-covariate relationships (Paper I);
- To assess the trough FVIII activity levels following several dosing schedules for prophylaxis with moroctocog alfa in pediatric cohorts through simulations (Paper I);
- To assess proposed approaches to handle inter-occasion variability (also known as within-patient variability) in model-based TDM and how \textit{a posteriori} dosing compares with conventional body weight dosing, using a population PK model of FVIII activity (Paper II);
- To characterize the relation between the dose of a full-length rFVIII product, octocog alfa, plasma FVIII activity and bleeding patterns (bleeding frequency and severity) using an integrated model based on data from patients with severe hemophilia A (Paper III);
- To find sources of inter-individual variability of PK and bleeding patterns through the comprehensive assessment of the relationship between the integrated model parameters and patient- and study-characteristics (Paper III);
- To use the integrated model, developed in Paper III, to contrast different sources of patient information in their ability to forecast the future occurrence of bleeds in severe hemophilia A patients receiving prophylactic FVIII replacement therapy (Paper IV).
3 Methods

3.1 Factor VIII replacement products
Data of two rFVIII replacement products, moroctocog alfa and octocog alfa, were used in the pharmacometric approaches developed in this thesis.

Moroctog alfa is a B-domain deleted rFVIII product which is marketed under two names, ReFacto AF® (Wyeth Pharmaceuticals [Pfizer]) in the European Union and other regions, and Xyntha® (Wyeth Pharmaceuticals [Pfizer]) in the United States of America, China and other regions (79). The potency of these products was assigned to different analytical assays, with ReFacto AF assigned to the CSA, and Xyntha to the OSA. Both ReFacto AF and Xyntha belong to the third generation of rFVIII products, where all exogenous human- or animal-derived proteins were removed from the manufacturing process, and resulted from the enhancement of a second generation rFVIII product, ReFacto® (Wyeth Pharmaceuticals [Pfizer]). The study reported in Paper I included pooled data following the administration of Refacto (five studies), ReFacto AF (five studies) and Xyntha (four studies).

Octocog alfa (BAY 81-8973) is a full-length rFVIII product marketed under the name of Kovaltry® (Bayer HealthCare) (80). It belongs to the third generation of rFVIII products, and is the result of the improvement of the second-generation product Kogenate® FS (Bayer HealthCare). The studies reported in Paper III and IV included pooled data from three clinical trials during which Kovaltry was administered.

The population PK model applied in the methodological work carried out in Paper II was not developed based on PK data from a specific product, but from multiple FVIII compounds, including pdFVIII and rFVIII products.

3.2 Clinical data
Informed consent was obtained from all subjects participating in the clinical trials from which data were included in this thesis, and the study protocols were approved by each participating center’s ethics committee or institutional review boards.
In Paper I, dosing, PK and covariate data from patients enrolled in 13 different clinical trials receiving prophylactic or on-demand treatment with morococog alfa were included (Table 2) (81-89). The trials were conducted from 1993 to 2013, in 25 countries, and included pediatric and adult patients. Four studies involved a single intravenous administration followed by rich sampling (11-12 samples) up to 48 or 72 hours post-dose. In four other studies, rich sampling was conducted on at least one occasion and sparse sampling (1-4 samples) on one or more occasions. In the remaining five studies, only sparse sampling was applied.

In total, data from 754 patients with moderate to severe hemophilia A were included in the development of the model. Of these, 116 patients were younger than 12 years (age range 1 day-73 years old). The median patient was a 23-year-old non-Hispanic or Latino white man, weighing 69 kg. A total of 7363 plasma FVIII activity observations (pre- or post-dose) were included, of which 910 (12.4%) were below the lower limit of quantification (LLOQ) (1 or 2 IU/dL). Factor VIII activity was measured with the OSA (4 studies) or CSA (9 studies), at a central (12 studies) or local laboratory (1 study). For 312 samples, two or more FVIII activity values were available which resulted from multiple FVIII activity measurements of the same sample. Covariate information available included body weight, lean body weight (LBW), total body water, age, race, ethnicity, analytical methods (CSA/OSA, central/local laboratory), neutralizing anti-FVIII inhibitor status and titer, study, year of study start and country.

In Paper III, dosing, PK, bleeding patterns (frequency and severity) and covariate data from patients enrolled in the three Long-Term Efficacy Open-Label Program in Severe Hemophilia A Disease (LEOPOLD) clinical trials receiving prophylactic or on-demand treatment with octocog alfa were included (Table 2) (90-92). The trials LEOPOLD I and LEOPOLD II included patients aged >12 years and LEOPOLD kids included patients <12 years. In total, data from 183 patients with severe hemophilia A were included in model development, including 11 patients who contributed with dose, PK and covariate information, but not with bleeding information. Of these, 51 patients were younger than 12 years. The median patient was a 22-year-old 60-kg white male, with a VWF level of 104%, and 11 bleeds in the 12-month period before the start of the study. A total of 1535 plasma FVIII activity observations (pre or post-dose) were included, of which 254 (17%) were below the LLOQ (1.5 or 3 IU/dL). Factor VIII activity was measured with the CSA. In addition, 663 bleeds from 172 patients receiving prophylactic treatment were available and 116 patients (67% of total) had at least one bleed during the observation period (median 2, range 0-33). Covariate information available included age, body weight, body mass index, LBW, race, VWF levels, number of bleeds in
the 12 months pre-study period, previous therapy history, on-demand or prophylaxis, number of target joints for bleeds at study start, ratio of the number of bleeds in the 12 months pre-study period to the number of target joints for bleeds at study start and study.

In Paper IV, the LEOPOLD clinical trial data were included, as in Paper III, but only patients taking prophylactic treatment with octocog alfa participating in the bleeding observation period were considered. In total, data from 172 patients with severe hemophilia A were included and were divided into two groups: <12 years (LEOPOLD kids); and ≥12 years (LEOPOLD I and LEOPOLD II). A total of 51 patients younger than 12 years were included with a median observation period of 6.1 months (range 3.8-7.2), and 121 patients aged 12 years or older with a median observation period of 12.1 months (range 3.08-13.1).

Table 2. Characteristics of the patient cohorts included in the model development/evaluations (Paper I, III and IV).

<table>
<thead>
<tr>
<th>Paper</th>
<th>Trial ID</th>
<th>Clinical Phase</th>
<th>FVIII product(s)</th>
<th>No. patients</th>
<th>Severity of hemophilia A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paper I</td>
<td>B1831003</td>
<td>IV</td>
<td>Xyntha</td>
<td>12</td>
<td>severe</td>
</tr>
<tr>
<td></td>
<td>B1831004</td>
<td>IV</td>
<td>Refacto AF</td>
<td>206</td>
<td>severe</td>
</tr>
<tr>
<td></td>
<td>B1831015</td>
<td>III</td>
<td>Xyntha</td>
<td>53</td>
<td>severe, moderately severe and moderate, mild</td>
</tr>
<tr>
<td></td>
<td>B1831053</td>
<td>III</td>
<td>Refacto</td>
<td>103</td>
<td>severe, moderately severe</td>
</tr>
<tr>
<td></td>
<td>B1831054</td>
<td>III</td>
<td>Refacto</td>
<td>91</td>
<td>severe, moderately severe</td>
</tr>
<tr>
<td></td>
<td>B1831061</td>
<td>IV</td>
<td>Refacto</td>
<td>18</td>
<td>severe</td>
</tr>
<tr>
<td></td>
<td>B1831066</td>
<td>III</td>
<td>Refacto, Refacto AF</td>
<td>30</td>
<td>severe</td>
</tr>
<tr>
<td></td>
<td>B1831067</td>
<td>III</td>
<td>Refacto AF</td>
<td>109</td>
<td>severe, moderately severe</td>
</tr>
<tr>
<td></td>
<td>B1831068</td>
<td>III</td>
<td>Refacto AF</td>
<td>96</td>
<td>severe, moderately severe</td>
</tr>
<tr>
<td></td>
<td>B1831070</td>
<td>III</td>
<td>Xyntha</td>
<td>92</td>
<td>severe</td>
</tr>
<tr>
<td></td>
<td>B1831071</td>
<td>III</td>
<td>Xyntha</td>
<td>30</td>
<td>severe, moderately severe</td>
</tr>
<tr>
<td></td>
<td>B1831077</td>
<td>I</td>
<td>Refacto AF</td>
<td>16</td>
<td>severe, moderately severe</td>
</tr>
<tr>
<td></td>
<td>B1831090</td>
<td>I</td>
<td>Refacto</td>
<td>18</td>
<td>severe, moderately severe, moderate</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>874 (754 unique)</td>
<td>severe, moderately severe, moderate</td>
</tr>
</tbody>
</table>

| Paper III¹ and IV | | | | | |
| LEOPOLD I | I/III | Kovaltry | 62 | severe |
| LEOPOLD II | II/III | Kovaltry | 59 | severe |
| LEOPOLD kids | II/III | Kovaltry | 51 | severe |
| Total | | | | | 172 | severe |

¹11 additional patients not mentioned in the table contributed with dose, PK and covariate information for model development and covariate assessment in Paper III. FVIII: factor VIII.
3.3 Model development and qualification

3.3.1 Population PK models

**Paper I** and **III** included the development of population PK models of FVIII activity.

In **Paper I**, alternative disposition models to describe the PK of FVIII activity were assessed. Since different moroctocog alfa products were aligned to different assays, the bioavailability of Xyntha (OSA calibrated) relative to ReFacto products (CSA calibrated) was set to 1.38 (93). The PK of FVIII activity was assumed to be equivalent following administration of ReFacto and ReFacto AF (86).

The patient population included severe, mild and moderate hemophilia A patients. Therefore, the individual endogenous level of FVIII activity was estimated (94). The individual model predictions resulted from the sum of three components: individual endogenous FVIII activity, FVIII activity supplied by the administration of exogenous FVIII product (morcoclog alfa), and residual FVIII activity coming from previous unknown doses of moroctocog alfa (*Figure 2*). The estimation of the latter component was included when patients had unexpected high observed FVIII activity levels before the administration of the very first dose recorded, defined as a FVIII activity observation above the criterion of acceptance of the degree of hemophilia A severity activity for each study; this portion of FVIII activity was set to decay according to the individual estimated first-order elimination (95).

*Figure 2.* Individual model-predicted plasma FVIII activity in an illustrative subject with an unexpected high pre-dose FVIII activity observation (96).

In **Paper III**, the population PK model development was based on the PK model published by Garmann et al. using the same longitudinal FVIII activity data (37). This was a two-compartment model with the influence of LBW on CL and V1. The model assumptions were comprehensively assessed and the model was updated as necessary.
3.3.1.1 Inter-individual and inter-occasion variability
In general, the structural model parameters were assumed to be log-normally distributed and IIV and IOV were modelled according to Karlsson et al. (50), as exemplified below for CL:

$$CL_{ik} = \theta \cdot \left( \frac{WT_i}{WT_{med}} \right)^{\eta} \cdot e^{\eta_{CL_i} + \kappa_{CL_{ik}}} \quad (eq. \ 2)$$

where $CL_{ik}$ represents the value of clearance for the $i^{th}$ patient on occasion $k$, $\theta$ is the estimated typical value of clearance for a patient with median body weight $WT_{med}$, $WT_i$ is the patient’s body weight, $\eta_{CL_i}$ is a normally distributed random effect with mean zero and estimated variance $\omega^2$ ($\eta_{CL_i} \sim N(0, \omega^2)$) and $\kappa_{CL_{ik}}$ is a normally distributed random effect with mean zero and an estimated variance $\pi^2$ ($\kappa_{CL_{ik}} \sim N(0, \pi^2)$). An occasion was defined as each separate dose event with FVIII activity observations associated to it (pre- or post-dose).

If a given random effect was found to follow a distribution other than a normal distribution (e.g. bimodal or skewed distributions), other distributions were explored.

3.3.1.2 Residual variability
Residual variability (RUV) was parameterized using additive, proportional, or a slope-intercept model, as exemplified below for the slope-intercept model:

$$C_{ij} = \hat{C}_{ij} \cdot (1 + \epsilon_{pij}) + \epsilon_{aij} \quad (eq. \ 3)$$

where $C_{ij}$ is the $j^{th}$ measured FVIII activity in the $i^{th}$ individual and $\hat{C}_{ij}$ is the $j^{th}$ model predicted FVIII activity in the $i^{th}$ individual. $\epsilon_{pij}$ and $\epsilon_{aij}$ are random effects describing the discrepancy between the individual prediction and the observation in a proportional or additive manner, respectively. Each random effect is assumed to be independently normally distributed with a mean of zero and estimated variances of $\sigma_{pij}^2$ and $\sigma_{aij}^2$ (e.g. $\epsilon_{pij} \sim N(0, \sigma_{pij}^2)$).

In Paper I, multiple FVIII activity measurements were available from the same sample, which allowed the estimation of a replicate-specific error as described by Karlsson et al. in addition to the RUV (97). In Paper III among the model assumptions tested was the inclusion of IIV in the RUV (98).

3.3.1.3 Data below the lower limit of quantification
In Paper I, data below the LLOQ were included in the model development when the actual measured values were available, or otherwise, were handled using the M5 method by imputing the LLOQ/2 (99). In Papers II, III and IV
data below the LLOQ were handled using the M3 method, where the observations below the LLOQ were treated as censored observations and the likelihood that these observations were indeed below the LLOQ was estimated (99, 100).

3.3.2 Repeated time-to-categorical event model

In Paper III, a model was developed to describe the time to the occurrence of bleeds and respective severity, which was integrated with the population PK model of octocog alfa described in section 3.3.1.

The time to the occurrence of bleeds was modelled using repeated time-to-event modeling, which is an extension of parametric time-to-event survival analysis using non-linear mixed effects modeling (101, 102). The probability density of each bleed and the probability of having a mild, moderate or severe bleed were estimated based on the observed time of bleeding and severity score (mild, moderate, severe) using repeated time-to-categorical event (RTTCE) modeling, which is a combination of parametric survival analysis and a proportional odds model for ordered categorical data (101-105).

The bleeding hazard, i.e. the instantaneous potential to have a bleed per unit of time, was estimated using exponential, Weibull and Gompertz functions. At the end of the bleeding observation period all individuals were assumed to have a right-censored observation. An IIV term was assessed in the overall bleeding hazard and in the logit transformation of the severity probability. In addition, the time-dependency between consecutive bleeds was assessed with a Markov hazard rate.

3.4 Parameter-covariate assessment

In Paper I, several steps were taken to evaluate the influence of covariates. First, the influence of body size on the disposition parameters was assessed by testing total body weight, LBW and total body water, using fixed allometric theory-based exponents (0.75 for CL and Q, and 1 for V1 and V2, respectively) (106), or allowing the estimation of the exponents. Second, covariates expected to affect FVIII activity disposition were tested, namely, the analytical method to influence the relative bioavailability and RUV, and the individual time-varying inhibitor status and titer, as well as age, to influence CL. Third, age, race and ethnicity were assessed in parameters with IIV using stepwise covariate modeling (forward selection significance level (α) =0.01, backward elimination α=0.005) (107). In addition, the influence of the study, year
of study start and country were explored graphically. Finally, a final backwards deletion step was carried out ($\alpha=0.005$) and clinical relevance was assessed.

In the integrated model developed in Paper III, the relation between the time-varying individual plasma FVIII activity predicted from the population PK model and the bleeding hazard was explored using a linear, an exponential and an inhibitory maximum effect model. The PK and RTTCE models were estimated simultaneously, including the time-varying FVIII effect, and thereafter covariates were integrated in the model using full random effects modeling (FREM) (108, 109). This methodology allows the characterization of the relationship between all model parameters and all covariates in a single step. Covariates were included as observations in the model, and their distributions were modelled as random effects (Figure 3). The interaction between model parameters and covariates was carried out at a random effects level in a full covariance matrix. Impactful parameter-covariate relationships were identified based on the correlation coefficient ($r$), precision of the effects sizes and scientific plausibility.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{model_covariance}
\caption{Representation of the covariance structure of the model using full random effects modeling for parameter-covariate characterization in Paper III. The shaded area represents the interaction between the random effects of model parameters and covariates. PK: pharmacokinetic; RTTCE: repeated time-to-categorical event.}
\end{figure}

### 3.4.1 Missing covariate values

In Paper I, missing height information was derived from the height-body weight relationship estimated from the height-body weight pairs available in the remaining cohort of patients (110):

$$HT_i = 51.6 + \frac{136 \cdot WT_i^{2.04}}{22.4 WT_i^{2.04}} + \eta_i$$  \hspace{1cm} (eq. 4)

where $HT_i$ is the height (cm) for a given subject with body weight $WT_i$ (kg) and an individual variability parameter $\eta_i \sim N (0, 47.8)$ (standard deviation 6.91 cm).
In **Paper III**, missing covariate data were handled implicitly by the FREM approach.

### 3.4.2 Unlikely clinical relevance

In **Paper I**, the selection of covariate was assessed using statistical and unlikely clinical relevance criteria (111). Covariates included in the model were assessed in terms of unlikely clinical relevance specified *a priori* as follows: a dichotomous covariate was dropped if the change in the typical parameter value was <10% from the most common category; a continuous covariate was dropped if the difference from the typical parameter value at the 50th percentile of the covariate distribution was <5% at the 5th and 95th percentiles of the covariate distribution.

### 3.5 Model discrimination and evaluation

Model selection was based on scientific plausibility, changes in objective function value (OFV; -2·log-likelihood), goodness-of-fit plots and precision of parameter estimates. In addition, the EBEs and diagnostic plots were used if these values were found reliable based on magnitude of the eta- and epsilon-shrinkage (112, 113). For competing hierarchical models, the likelihood ratio test was used where the addition of one parameter (1 degree of freedom, \(d.f.\)) was considered statistically significant with an OFV decrease of at least 6.64 points (\(\alpha=0.01\)).

In **Paper I** and **III**, key population PK models were qualified using prediction-corrected visual predictive checks (114). In **Paper III**, the RTTCE model was qualified with visual predictive checks of the Kaplan-Meier curves stratified by bleed number and severity, and using the kernel-based visual hazard comparison tool (115).

The precision of the parameter estimates (standard error and confidence intervals (CI)) was obtained from the covariate step in NONMEM using the sandwich matrix (**Paper I**), or the R matrix following an evaluation step using the Monte-Carlo importance sampling (IMP) estimation method (**Paper III**). In addition, the sampling importance resampling (SIR) procedure was used in **Paper I** (116).

### 3.6 Simulation of dosing regimens

In **Paper I**, several prophylactic dosing regimens with morococog alfa were assessed in multiple pediatric cohorts (0 to <2 years; 2 to <6 years; 6 to <12...
years; and 12 to <17 years), based on simulations of 1000 individuals uniformly sampled over the age range, on a month resolution level, following a fixed dose of Xyntha. For each pediatric cohort, body weight was sampled from the National Health and Nutrition Examination Survey data from years 1999–2010, available at the Centers for Disease Control and Prevention (117).

3.7 Handling inter-occasion variability in model-based TDM

In Paper II, different approaches to handle IOV (also known as within-patient variability) in model-based TDM were assessed in a simulation-based study.

Factor VIII activity data at 4, 24 and 48 h post-dose following the administration of 30 IU/kg on 4 occasions and the respective individual PK parameters were simulated for 1000 virtual severe hemophilia A patients based on the model reported by Björkman et al. (35). These data were considered to be the true data, i.e., the true PK individual parameters and the FVIII activity levels as observed in a real-world TDM setting. Thereafter, EBEs were estimated using Bayesian forecasting based on the FVIII activity levels obtained from different occasions. These EBEs were then used to calculate the dose to be administered on the next occasion (doseTDM) to reach a FVIII activity target of 1 IU/dL at 48 hours post-dose. This procedure was repeated using the Bayesian forecasting and dose calculation approaches described in the following section 3.7.1. Finally, doseTDM was compared with the dose calculated based on the true parameters simulated initially for the forecasted occasion (dosetrue) through the calculation of the relative prediction error (PE) as follows:

$$\text{PE}_i \% = \left( \frac{\text{dose}_{TDM} - \text{dose}_{true}}{\text{dose}_{true}} \right) \times 100$$  \hspace{1cm} (eq. 5)

where PEi is the prediction error in the ith simulated subject, doseTDM is the TDM individualized dose for the next occasion and dosetrue is the true dose required on the forecasted occasion. Accuracy was quantified by the 50th percentile of the PEi, and precision by the extreme 2.5th, 10th, 25th, 75th, 90th and 97.5th percentiles.

The procedure described above was repeated for alternative scenarios while varying: the number of occasions to produce the EBEs (1-3 occasions); the number of observations within an occasion (1-3 FVIII activity observations post-dose); and the IOV magnitude on CL and V1 (0%-50%CV).
In addition, the results of the model-based TDM approaches were compared with conventional body weight-based dosing. For the latter, the dose was reduced by applying a factor of 0.3 (9 IU/kg) so that the generated median FVIII activity values were close to the defined target of 1 IU/dL.

3.7.1 Model-based TDM approaches assessed

Five model-based TDM approaches were tested (118, 119), which involved two steps. Firstly, the EBEs were estimated using Bayesian forecasting. Secondly, a new set of parameters was derived from the EBEs and was used to calculate the individualized dose. A description of these two steps for the five approaches tested is exemplified for CL in Table 3, and it is represented in Figure 4.

Table 3. Model-based TDM approaches used in Bayesian forecasting and dose calculation in Paper II. Exemplified for clearance.

<table>
<thead>
<tr>
<th>Approach</th>
<th>Model to estimate the EBEs</th>
<th>Parameter to calculate the individualized dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>IIV+IOV</td>
<td>$CL_{ij} = TVCL \cdot e^{\eta_i + \kappa_{ij}}$</td>
<td>$CL_{ij \text{TDM}} = CL_{ij}$</td>
</tr>
<tr>
<td></td>
<td>$\eta_i \sim N(0, \omega^2)$, $\kappa_{ij} \sim N(0, \pi^2)$</td>
<td></td>
</tr>
<tr>
<td>IIV+IOV – IV EBE</td>
<td>$CL_{ij} = TVCL \cdot e^{\eta_i + \kappa_{ij}}$</td>
<td>$CL_{ij \text{TDM}} = TVCL \cdot e^{\eta_i}$</td>
</tr>
<tr>
<td>for dose</td>
<td>$\eta_i \sim N(0, \omega^2)$, $\kappa_{ij} \sim N(0, \pi^2)$</td>
<td>$\eta_i \sim N(0, \omega^2)$</td>
</tr>
<tr>
<td>IIV – IOV ignored</td>
<td>$CL_{ij} = TVCL \cdot e^{\eta_i}$</td>
<td>$CL_{ij \text{TDM}} = CL_{ij}$</td>
</tr>
<tr>
<td></td>
<td>$\eta_i \sim N(0, \omega^2)$, $\pi^2$ set to 0</td>
<td></td>
</tr>
<tr>
<td>IIV – IOV &amp; IIV</td>
<td>$CL_{ij} = TVCL \cdot e^{\eta_i}$</td>
<td>$CL_{ij \text{TDM}} = CL_{ij}$</td>
</tr>
<tr>
<td>summed</td>
<td>$\eta_i \sim N(0, \omega^2+\pi^2)$, $\pi^2$ set to 0</td>
<td></td>
</tr>
<tr>
<td>IIV – re-estimated</td>
<td>$CL_{ij} = TVCL \cdot e^{\eta_i}$</td>
<td>$CL_{ij \text{TDM}} = CL_{ij}$</td>
</tr>
<tr>
<td></td>
<td>$\eta_i \sim N(0, \omega^2)$, $\omega^2$ from a model re-estimated with $\pi^2$ set to 0</td>
<td></td>
</tr>
</tbody>
</table>

$CL_{ij}$, empirical Bayes estimate of clearance for the $i$th subject at the $j$th occasion; $CL_{ij \text{TDM}}$, individual parameter used to calculate the individualized dose; EBE: empirical Bayes estimate; IIV: inter-individual variability; IOV: inter-occasion variability; TVCL: typical value of clearance, calculated as $TVCL = 222 \cdot \left(\frac{WT}{68}\right)^{0.75} \cdot (1 - 0.00696 \cdot (age - 24))$; WT: body weight; $\eta_i$ and $\kappa_{ij}$ were individual IIV and IOV terms independently estimated and normally distributed with mean zero and variances $\omega^2$ and $\pi^2$, respectively.
3.8 Comparison of sources of patient information to forecast bleeds

In Paper IV, the PK-RTTCE model described in Paper III was used to contrast different sources of patient information in their ability to forecast the future occurrence of bleeds. This study was based on Bayesian estimations and on a subset of the data used to develop the PK-RTTCE model, as described in section 3.2.

3.8.1 Bayesian forecasting of bleeds

The EBEs related to PK and bleeding hazard parameters were obtained using Bayesian forecasting based on the PK-RTTCE model and varying the observation time and the nature of the observations included. For each patient, a new set of EBEs was estimated from the start of the study until the end of each consecutive 24-hour period. In the end of the study, each patient had $n_i$ sets of EBEs, with $n_i$ equal to the number of days that the $i^{th}$ patient participated in the study.
3.8.1.1 Information scenarios
Bayesian estimation was repeated including the following three information scenarios:

- **PK**: only plasma FVIII activity observations were included in the estimation of the EBEs related to PK, and other EBEs were set to zero.
- **Bleed**: Only the times of bleeds were included in the estimation of the EBEs related to the bleeding hazard, and other EBEs were set to zero.
- **All**: Plasma FVIII activity observations, bleeds and all covariate data were included in the estimation of the EBEs.

All scenarios included the original dosing information and observed LBW as required by the PK component of the model. The time-varying FVIII activity effect on the hazard was predicted based on the individual PK parameters (PK and All) or typical PK parameters (Bleed).

3.8.1.2 Bleeding probabilistic forecast
Based on each individual random effect related to the bleeding hazard ($\eta_{ih}(t)$), the probability of having a bleed in the upcoming 24 hours was calculated as:

$$
P(t_{bleeding}) = 1 - S(t + 24) = 1 - e^{-\int_{t}^{t+24} h_i(t) dt} \quad (eq. 6)
$$

where $S(t)$ is the probability of not having a bleed between $t$ and $t+24$ calculated based on the time-varying bleeding hazard $h_i(t)$, and $t$ is the end of the observation period for Bayesian forecasting.

Dosing information registered during the upcoming 24-hour period (forecasting period) were taken into account to the calculation of $h_i(t)$, however, doses taken between the occurrence of an observed bleed and the end of the respective 24-hour period were considered to be on-demand doses, which were not included in the calculation of the probabilistic forecast.

The forecasted number of bleeds over the total study time was approximated by the sum of all $P_i(bleeding)$ values calculated over the whole study period.

3.8.1.3 Varying the Bayesian forecasting period
In the default case, Bayesian forecasting was based on observations from the start of the study up to the end of each 24-hour period. However, to learn about the trade-off between longer observation periods and the most up-to-date information, the influence of varying the observation period in Bayesian forecasting was assessed by considering only the individual information observed in the past 15 days, 1, 2, 3 or 6 months, using the information scenario **Bleed**.
3.8.2 Predictive performance assessment

The comparison between the $P_i$(bleeding) obtained using the three information scenarios was carried out using separation plots, receiver operating characteristics (ROC) and precision-recall (PR) analyses.

3.8.2.1 Separation plots

A separation plot is a visual method introduced by Greenhill et al. in the field of political science that compares the ability to consistently match high-probability predictions with the actual occurrence of events (120). These diagnostic plots have the advantage of not requiring the definition of probability thresholds to distinguish between the prediction of events and no-events.

The creation of separation plots involved two steps. Firstly, the values of $P_i$(bleeding) generated throughout the study for all patients (i.e. $n_i$ · total number of individuals) were sorted in ascending order. Secondly, these values were represented graphically and $P_i$(bleeding) values associated to days when bleeds occurred were highlighted in a different color ( ). More colored values located on the right-hand side of the plot, i.e. events that are associated with a higher relative $P_i$(bleeding), denote a better predictive performance (e.g. denotes a better predictive performance than ).

3.8.2.2 Receiver operating characteristic analyses

The ROC curve is a tool to visualize, compare and select classifiers based on their predictive performance (121, 122). It provides a visual summary of the trade-off between true positives (TP) and false positives (FP) as the probability threshold (in this case, $P_i$(bleeding)) varies. The TP rate (TPR; y-axis of the ROC curve) is given by the correctly predicted positives divided by all of the actual positives, and the FP rate (FPR; x-axis of the ROC curve) is given by the incorrectly predicted negatives divided by all of the actual negatives, as shown in the confusion matrix in Figure 5.

The area under the ROC curve (AUC$_{ROC}$), calculated with the trapezoidal rule, was used to compare the different information scenarios (123). A classifier equivalent to random chance has an AUC$_{ROC}$ of 0.5 and corresponds to a curve that overlaps the diagonal line of the ROC plot, the random-classifier baseline. A perfect classifier has an AUC$_{ROC}$ of 1 and corresponds to an accuracy of 100%, whereas an AUC$_{ROC}$ lower 0.5 indicates that the FPR in greater than the TPR in average and the classifier is worse than random chance.
### Observed outcome

<table>
<thead>
<tr>
<th>Predicted outcome</th>
<th>Observed outcome</th>
<th>Positive (P) bleed</th>
<th>Negative (N) no-bleed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleed</td>
<td>True positive (TP)</td>
<td>False positive (FP)</td>
<td></td>
</tr>
<tr>
<td>No bleed</td>
<td>False negative (FN)</td>
<td>True negative (TN)</td>
<td></td>
</tr>
</tbody>
</table>

TP rate, sensitivity or recall

\[
TPR = \frac{TP}{P} \quad (eq. 7)
\]

FP rate

\[
FPR = \frac{FP}{N} = 1 - \text{specificity} \quad (eq. 8)
\]

TN rate or specificity

\[
TNR = \frac{TN}{N} \quad (eq. 9)
\]

Positive predictive value or precision

\[
PPV = \frac{TP}{TP+FP} \quad (eq. 10)
\]

**Figure 5.** Confusion matrix (or contingency table) and the derivation of summary indices.

The optimal threshold corresponds to the value of \( P_i \) (bleeding) that is optimal for discrimination between bleeds and no-bleeds (optimal trade-off between TP and FP) and was determined by the Youden’s J statistics (124). It corresponds to the maximum vertical distance between the ROC curve and the random-classifier baseline and is calculated as:

\[
J = \text{sensitivity} + \text{specificity} - 1 \quad (eq. 11)
\]

The greater the \( J \), the better the classification, and the threshold corresponding to the highest \( J \) was chosen as optimal. The CI of the ROC-related summary indices were calculated based on a stratified bootstrap (2000 replicates).

The results of the ROC curves do not depend on the class distribution, which means that if the proportion of positive to negative outcomes changes, the ROC curves will not be affected (121, 125). This property can lead to misleading conclusions about the reliability of the performance of a given classifier if, for instance, the occurrence of positive outcomes, bleeds, is rare.

#### 3.8.2.3 Precision-recall analyses

Precision-recall analyses are based on the same principles as ROC analyses but consider precision, the correctly predicted positives divided by all predicted positives, instead of the FPR (126). In contrast to the ROC curve, PR curves are sensitive to the class distribution and were performed to complement the results of the ROC analyses (125).

A point in the ROC curve has a corresponding point in the PR curve, and a PR curve shows precision (y-axis) against the TPR (x-axis), which in the PR con-
text is frequently called recall. A precision-recall curve above another one denotes a better performance. The random-classifier baseline in the PR space is determined by \( y = \frac{P}{P+N} \), and in case the class distribution is balanced, the random-classifier baseline is \( y = 0.5 \). The calculation of the AUC of the PR curve (AUC\(_{PR} \)) was carried out using non-linear interpolation, as suggested by Davis and Goadrich (126).

### 3.9 Software

All models were developed using non-linear mixed effects modeling in NONMEM, version 7.3 or higher (127). The estimation methods included the first-order conditional estimation with eta-epsilon interaction (FOCE interaction) and the Monte Carlo importance sampling assisted by mode \( a \ posteriori \) (IMPMAP) estimation method. Bayesian forecasting was performed in NONMEM. Perl-speaks-NONMEM (PsN, version 4.6.0 or higher) was used to assist model building and evaluation (128-130).

Data management and checkout, graphical and statistical analyses were carried out in R (version 3.2.2 or higher), including the R packages Xpose4, dplyr, ggplot2, pROC and PRROC (128, 131-135).
4 Results

4.1 Pharmacokinetic models for FVIII activity

4.1.1 Moroctocog alfa

The disposition of FVIII activity following the administration of moroctocog alfa (Paper I) was well described by a 2-compartment model with first-order elimination, parameterized in terms of clearances (CL and inter-compartmental clearance (Q)) and volumes of distribution (V1 and V2). Covariate effects related to body size, age, inhibitors, race and analytical assay were identified. The model was found to describe the central trend of the data and the variability well (Figure 6). The final PK parameter estimates, including the relevant covariate effects were:

\[
\text{CL}_i (\text{dL/h}) = 2.76 \cdot \left( \frac{\text{WT}_i}{70} \right)^{0.75} \cdot \text{age effect} \cdot (1 + 1.66 \cdot \text{INH}_i) \\
\cdot (1 - 0.347 \cdot \text{STUD}) \cdot e^{\eta_{\text{CL}_i} + \kappa_{\text{CL}_i}} \\
\text{age effect} = \begin{cases} 
1.13 + 0.149 \cdot (\text{AGE}_i - 1) & \text{if age } \leq 1 \text{ year old} \\
1 - 0.00678 \cdot (\text{AGE}_i - 20) & \text{if age } > 1 \text{ year old}
\end{cases} \\
\text{(eq. 12)}
\]

\[
\text{V1}_i (\text{L}) = 2.45 \cdot \left( \frac{\text{WT}_i}{70} \right)^{0.812} \\
\text{(eq. 13)}
\]

\[
\text{Q}_i (\text{dL/h}) = 25.1 \cdot \left( \frac{\text{WT}_i}{70} \right)^{0.75} \\
\text{(eq. 14)}
\]

\[
\text{V2}_i (\text{L}) = 0.923 \cdot \left( \frac{\text{WT}_i}{70} \right)^{0.812} \cdot (1 + 0.884 \cdot \text{RACE}) \cdot e^{\kappa_{\text{V2}_i}} \\
\text{(eq. 15)}
\]

where CL$_i$, V1$_i$, Q$_i$ and V2$_i$ are the $i^{th}$ patient CL, V1, Q and V2, respectively, WT$_i$ is the individual body weight, INH is equal to 1 for inhibitors positive (otherwise = 0), STUD is equal to 1 for study B1831090 (otherwise = 0), AGE$_i$ is the individual age (in years), RACE is equal to 1 for black race (otherwise = 0), $\eta_{\text{CL}_i}$ is the individual random effect accounting for the individual deviation from the typical value of CL with estimated IIV variability (%CV) 30.5%CV, $\kappa_{\text{CL}_i}$ and $\kappa_{\text{V2}_i}$ are the individual occasion-specific random effects accounting for the deviation from the typical values of CL and V2 with estimated IOV 34.7%CV and 41.0%CV. In addition, an IIV term of 13.0%CV was estimated in the relative bioavailability.
Figure 6. Population prediction-corrected visual predictive checks stratified by study (96). Dots represent the prediction-corrected FVIII activity observations, and the solid and dashed red lines the median and the 2.5th and 97.5th percentiles of the observed prediction-corrected data. The shaded areas represent the 95% confidence interval for the corresponding percentiles based on the prediction-corrected simulated data.
All parameters were estimated with adequate precision (SIR RSE <33.0%), with only the age effect up to the age 1 year on CL being a little imprecise (SIR RSE 60.1%, 95%CI 0.0116-0.352).

Two endogenous FVIII activity levels were estimated, 0.474 IU/dL (corresponding to severe hemophilia A) and 1.59 IU/dL (moderately severe), where 80.3% of the patients (or 11.0% if belonging to studies B1831015 or B1831053) were found to belong to the severe group. Inter-individual variability in the estimated endogenous levels was 7.19%CV. In addition, the estimated residual activity from previous doses not recorded was 2.84 IU/dL.

Factor VIII activity was found to be systematically 39.0% lower when measured by the OSA (14.6% lower measured by the OSA in local laboratories) and to be associated with a 40.3% higher residual error than when measured by the CSA. The proportional RUV was 19.2%CV and the proportional replicate error component was 10.4%CV.

4.1.1.1 Covariate effects
The consequences of the identified covariate effects as well as IIV and IOV variability are depicted in Figure 7.

Figure 7. Effect of covariates on the factor VIII (FVIII) activity vs. time for patients with severe hemophilia A, with varying characteristics following the administration of 3,500 IU of Xyntha overlaid with the 95% prediction interval of FVIII activity for the typical subject, taking inter-individual and inter-occasion variability into account (96).
4.1.1.2 Simulation of dosing regimens in pediatric patients

The model developed for morococog alfa was used to simulate individual trough FVIII activity levels, across a range of doses and pediatric age cohorts (Figure 8).

Figure 8. Simulated trough factor VIII activity levels following administration of morococog alfa using three dosing regimens in four pediatric cohorts across a wide range of doses (10-100 IU/kg). Median trough FVIII activity values above 1 IU/dL are represented with circles with white fill (96). PI: prediction interval.

To obtain activity levels of 1 IU/dL in 50% of the patients requires dosing every other day. Due to the large variability in PK, the administration of a dose of 100 IU/kg every other day results in a minimum of 20% of the adolescents and 40% of the youngest children not reaching a FVIII activity trough above 1 IU/dL.
4.1.2 Octocog alfa

In Paper III, the previously developed model for octocog alfa using a two-compartment PK model, with the influence of LBW on CL and V1 was found to describe the PK data well. Compared with the original model, IIV on the additive (1.52 IU/dL) and proportional (20.3%CV) RUV (63.1%CV) was added to the model since it was found to improve the model fit ($\Delta$OFV=-199) and parameter precision substantially.

All parameters were estimated with adequate precision (relative standard error (RSE) <23.0%). The final PK model parameters were:

\[
\text{CL}_i (\text{dL/h}) = 1.93 \cdot \left( \frac{\text{LBW}_i}{51} \right)^{0.65} \cdot e^{\eta_{\text{CL}_i}} \quad (\text{eq. 16})
\]

\[
\text{V1}_i (\text{dL}) = 30.3 \cdot \left( \frac{\text{LBW}_i}{51} \right)^{0.96} \cdot e^{\eta_{\text{V1}_i}} \quad (\text{eq. 17})
\]

\[
\text{Q}_i (\text{dL/h}) = 1.69 \quad (\text{eq. 18})
\]

\[
\text{V2}_i (\text{dL}) = 6.29 \quad (\text{eq. 19})
\]

\[
\frac{dA(1)}{dt} = -A(1) \cdot \frac{\text{CL}_i}{\text{V1}_i} - A(1) \cdot \frac{\text{Q}_i}{\text{V1}_i} + A(2) \cdot \frac{\text{Q}_i}{\text{V2}_i} \quad (\text{eq. 20})
\]

\[
\frac{dA(2)}{dt} = A(1) \cdot \frac{\text{Q}_i}{\text{V1}_i} - A(2) \cdot \frac{\text{Q}_i}{\text{V2}_i} \quad (\text{eq. 21})
\]

\[
\text{FVIII}_i(t) (\text{IU/dL}) = \frac{A(1)}{\text{V1}_i} \quad (\text{eq. 22})
\]

where CL$_i$, V1$_i$, Q$_i$ and V2$_i$ are the $i$th patient CL, V1, Q and V2, respectively, LBW$_i$ is the individual lean body weight at study start, $\eta_{\text{CL}_i}$ and $\eta_{\text{V1}_i}$ are the individual random effects accounting for the individual deviation from the typical values of CL and V1, with estimated IIV variability (%CV) 30.2 and 15.1, respectively; A(1) and A(2) are the amounts of the individual predicted FVIII activity in the central and peripheral compartments, and FVIII$_i(t)$ represents the time-varying FVIII activity.

The relationship between the PK parameters and covariates using a FREM approach will be further described in section 4.3.
4.2 Handling inter-occasion variability in model-based TDM

The large magnitude of IOV identified in the PK parameters of FVIII activity in Paper I was the rationale for the work carried out in Paper II, where the performance of different approaches to handle IOV in model-based TDM was studied.

The consequences of the different approaches introduced in section 3.7.1 while predicting the individualized doses using the population PK model developed by Björkman et al. (35) are represented in Figure 9.

![Figure 9. Percentiles of the relative prediction error for the different model-based TDM approaches tested when aiming at a plasma FVIII activity target of 1 IU/dL at 48 h post-dose on the next occasion (136). The information content represents the number of occasions used to generate the empirical Bayes estimates (1, 2 or 3 occasions). The results of using conventional body weight-based dosing are represented in the approach Weight. EBE: empirical Bayes estimate; IIV: inter-individual variability; IOV: inter-occasion variability.](image)

The median PE was around zero regardless of the approach used to handle IOV or the information content included, suggesting that on a population level the individualized doses were generated with low bias. However, the precision varied substantially across approaches (P97 179-268%, i.e. the individualized doses were at least 179-268% larger than the true dose on the next occasion for 2.5% of the patients). The approach IIV+IOV – IIV EBE for dose was found to lead to the most precise individualized doses, in particular when only data from only one or two occasions were available. Conventional dosing
based on body weight was found to perform substantially worse than any of the model-based TDM approaches tested.

The impact of varying magnitudes of IOV on CL and on V1 was also studied (Figure 10). On a population level the approaches including IOV in the estimation of the EBEs (IIV+IOV, IIV+IOV – IIV EBE for dose) led to unbiased individualized doses, but the remaining model-based TDM approaches showed a trend towards under-prediction of the plasma FVIII target when the magnitude of IOV on CL was large (≥30%) and data from more than one occasion were used.

Figure 10. Median, 2.5th and 97.5th percentiles of the relative prediction error for the different model-based TDM approaches tested when aiming at a plasma FVIII activity target of 1 IU/dL at 48 h post-dose on the next occasion, while varying inter-occasion variability on CL and on V (136). The information content represents the number of occasions used to generate the empirical Bayes estimates (1, 2 or 3 occasions). The results of using conventional body weight-based dosing are represented in the approach Weight. CL: clearance; EBE: empirical Bayes estimate; IIV: inter-individual variability; IOV: inter-occasion variability; V: central volume of distribution.

On a patient level, the approach IIV+IOV – IIV EBE for dose performed best, regardless of the magnitude of IOV in the data, and IIV+IOV performed worst. When the magnitude of IOV was high, conventional body weight dosing was shown to be better than some of the model-based TDM approaches assessed.
In addition, the advantages of using **IIV+IOV – IIV EBE for dose** over the remaining model-based TDM approaches was verified when varying the number of samples within an occasion, and when comparing the FVIII activity resulting from the individualized doses directly with the target (data not shown).

### 4.3 Repeated time-to-categorical event model for occurrence of bleeds and bleeding severity

A Gompertz hazard function with a bleeding hazard that decreases over time was found to describe the time to bleed data well. The effect of the individual predicted plasma FVIII activity throughout the time of study was best described with an inhibitory maximum effect model, assuming full inhibition for high FVIII activity levels. The individual bleeding hazard \( h_i \) for patients under prophylactic treatment with octocog alfa was given by:

\[
h_i(t) = 2.96 \cdot e^{-0.566 \cdot (t - 1)} \cdot \left(1 - \frac{\text{FVIII}_i(t)}{10.2}\right) \cdot e^{\eta h_{i(t)}}
\]  

(eq. 23)

where \( h_i(t) \) is the \( i \)th patient bleeding hazard at time \( t \), 2.96 year\(^{-1} \) is the bleeding hazard at 1 year after the study started assuming a plasma FVII activity of 0 IU/dL under prophylactic treatment, -0.566 is the shape factor of the Gompertz distribution, FVIII\(_i\)(t) is the individual PK model-predicted FVIII activity at time \( t \), 10.2 IU/dL is the FVIII activity resulting in half-maximum inhibition of the hazard, and \( \eta h_{i(t)} \) is the random effect that describes the difference between the typical and the individual bleeding hazard with estimated IIV variability 136 %CV.

A time-dependency between consecutive bleeds could not be identified \((P>0.05)\). The estimated probability of having a bleed of mild, moderate or severe severity was 39.6, 55.7 and 4.72%, respectively.

The model described the repeated time-to-bleed and severity data well, as shown in **Figure 11**. The estimated correlations between the model parameters and the covariates are presented in **Figure 12**.
Figure 11. Observed Kaplan-Meier curves representing the percentage of bleed-free patients vs. time after the start of the study with the 95% confidence interval of the model-predicted KM curves (shaded areas), stratified by bleed number and severity. The vertical lines represent that a patient was censored.

Figure 12. Estimated associations between PK (CL, V1, PK residual error, bleeding hazard and bleeding severity) and bleeding hazard model parameters and covariates. 1unexplained inter-individual variability on the residual error; 2VWF: von Willebrand factor; 3number of bleeds in the 12-month pre-study period; 4number of target joints for bleedings at study start; 5ratio of the number of bleeds in the 12 months pre-study period to the number of target joints for bleedings at study start.
The strongest relationships between the model parameters and covariates were found to be VWF on CL (correlation coefficient $r=-0.54$) and number of bleeds in the 12-month period pre-study on the bleeding hazard ($r=+0.45$). In addition, race Black and race Asian decreasing and increasing CL ($r=-0.25$, $r=+0.17$), respectively, and age and VWF being positively correlated to the severity of bleeding ($r=+0.23$, $r=+0.28$) were potentially clinically significant effects. In addition, correlations were identified between model parameters (e.g. CL and V1, $r=+0.45$) and between covariates (e.g. treatment history and number of bleeds pre-study period, $r=-0.71$).

The addition of covariates to the model resulted in a decrease in the unexplained IIV from 136%CV to 111%CV. Based on simulations, the administration of a dose of octocog alfa to typical virtual patients every two days leading to a FVIII activity trough of 1 IU/dL resulted in at least 2.73 bleeds in 50% of the patients, and at least 11.2 bleeds in 10% of the patients at the end of one year of treatment. The discrepancy in the number of simulated bleeds between patients is given by the large unexplained IIV in the bleeding hazard, which characterizes the variability in the bleeding phenotype.

### 4.4 Comparison of sources of patient information to forecast bleeds

The PK-RTTCE model described in sections 4.1.2 and 4.3 was used in Bayesian forecasting of bleeding events. The values of the individual random effect related to the bleeding hazard (i.e. EBEs $\eta^{h(t)}$) estimated through Bayesian forecasting at the end of each 24-hour period throughout the study, using the 3 information scenarios described in section 3.8.1.1, are shown in Figure 13.

**Figure 13.** Values of $\eta^{h(t)}$ updated at the end of each 24-hour period throughout the study estimated based on the individual observations of PK, bleeds, and all information (including PK, bleeds and covariates) available up to the day of forecast.
When PK information was included in Bayesian forecasting, $\eta_i^{h(t)}$ was set to zero, even though it is recognized that a small amount of information could be gained from the covariance between the PK parameters and the bleeding hazard. When bleeds were included in Bayesian forecasting, $\eta_i^{h(t)}$ started from zero at the end of day one (i.e. the typical bleeding hazard was assumed) and increased over time, if a bleed occurred, or decreased in case a bleed did not occur. When all information was included, values of $\eta_i^{h(t)}$ were much more variable since they were informed by PK, bleeds and covariate observations.

The number of observed and predicted bleeds when using Bayesian forecasting based on the different information scenarios is represented in Table 4.

Table 4. Number of observed and predicted bleeds during the study for each information scenario and age group.

<table>
<thead>
<tr>
<th>Age group</th>
<th>Observed bleeds</th>
<th>Predicted bleeds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PK</td>
</tr>
<tr>
<td>&lt;12 years</td>
<td>101</td>
<td>66</td>
</tr>
<tr>
<td>≥12 years</td>
<td>530</td>
<td>218</td>
</tr>
</tbody>
</table>

The individual forecasted probability of having a bleed in the upcoming 24-hour period ($P_i(bleeding)$) was higher for bleed-days, than for no-bleed days, for the approaches Bleed and All compared with PK (Figure 14).

![Figure 14](image)

*Figure 14.* Separation plots for the probabilistic forecast of bleeding in the upcoming 24-hour period ($P_i(bleeding)$). Each vertical bar represents the forecast for one day for each subject with light grey representing no-bleed days, and dark grey representing bleed days. Dark grey bars were emphasized since bleed days were rare compared with no-bleed days. The solid black line represents the values of $P_i(bleeding)$ associated with each vertical bar, and the vertical dashed lines represent the 25th and 50th percentiles of the bleed days data.

The ROC curves, showing the TPR against the FPR at all values of $P_i(bleeding)$, are shown in Figure 15.
Figure 15. Receiver operating characteristic curves describing the ability to forecast bleeds based on PK, bleed and all information (including PK, bleeds and covariates). The dashed line represents a random-classifier baseline.

The predictive performance given by the information scenarios *Bleed* and *All* were found to be superior to *PK*, with higher true positive rates and lower false positive rates for all $P_i$(bleeding) cut-off values. For the group <12 years, the AUC$_{ROC}$ was 0.67 (95% CI: 0.61-0.72) for *PK*, 0.74 (0.69-0.79) for *Bleed* and 0.77 (0.73-0.81) for *All*. For the group $\geq$12 years, the difference between information scenarios was larger, with an AUC$_{ROC}$ of 0.67 (95% CI: 0.65-0.69) for *PK*, 0.78 (0.76-0.80) for *Bleed*, and 0.79 (0.77-0.81) for *All*. These results were confirmed with the precision-recall analysis, with *Bleed* and *All* found to be superior to *PK*. For the group <12 years, the AUC$_{PR}$ was 0.023 for *PK*, 0.030 for *Bleed* and 0.033 for *All*, respectively, and 0.011 for a random classifier; for the group $\geq$12 years, it was 0.028 for *PK*, 0.064 for *Bleed*, 0.064 for *All*, and 0.012 for a random classifier.

In patients with a low bleeding risk (did not bleed during the study (<12 years, N=23 patients; $\geq$12 years, N=33)), bleeds were over-predicted and longer observation periods improved the bleeding forecast only slightly. In patients with a moderate risk (1-2 bleeds (group <12 years, N=11) or 1-4 bleeds ($\geq$12 years, N=49)), the forecast was unbiased and an observation period longer than 60 days did not substantially improve the precision of the forecast. Finally, in patients with high bleeding risk ($\geq$3 bleeds (group <12 years, N=17) or $\geq$5 bleeds ($\geq$12 years, N=39)), bleeds were under-predicted and longer periods in particular, 60-90 days, were associated with an improved forecast of bleeds.
5 Discussion

This thesis describes the development of pharmacometric approaches to improve dose individualization methods of FVIII replacement therapy in hemophilia A. It includes the characterization of the population PK of two FVIII replacement products as well as the dose-exposure-response relationship of one FVIII product through the development of the integrated PK-RTTCE model. In addition, the PK-RTTCE model is used to forecast the occurrence of bleeds, which provides the foundation for the development of a dosing strategy not only considering the individual PK but also the individual bleeding phenotype. In addition, several approaches to handle IOV in model-based therapeutic drug monitoring are assessed, and one approach is suggested as being best to incorporate IOV in dose individualization.

Prophylactic treatment in severe hemophilia A is usually individualized using an *a priori* dosing strategy, based on each patient’s body weight. However, multiple known and unknown factors affect the PK of FVIII replacement therapy as well as the bleeding phenotype. For this reason, the administration of the same dose to different patients with an identical body weight may lead to a considerably different disposition of FVIII in plasma and a variable protection against bleeds (35, 137, 138).

In this thesis, several patient and study characteristics were found to affect the PK of FVIII activity. Of these, neutralizing anti-FVIII inhibitors was the most impactful covariate detected, associated with more than doubling the value of CL. The remaining covariate effects were found to have a limited impact on PK, compared to the moderate to large magnitude of unexplained inter- and IOV. In the simulations in pediatric cohorts carried out in Paper I, an *a priori* dose individualization strategy using age in addition to body weight information was found to be beneficial to the median patient but trough FVIII activity levels did not reach 1 IU/dL in all patients within the range of doses studied (10-100 IU/kg). Whereas unexplained IIV is commonly identified in population PK models of FVIII activity (~30% on CL, 0-20% on volumes), the identification of IOV is more uncommon, with some studies not estimating this type of variability, and other studies reporting a non-significant or low magnitude of IOV, which can be due to the availability of only sparse data within an occasion or just a few patients with data at several occasions (35, 36). Neglecting the estimation of IOV may result in the inflation of IIV, which
may lead to a false impression of the value of model-based TDM (50). The magnitude of IOV found in Paper I was particularly large (35% CL; 41% V2). Several factors may have contributed to this finding, including not only physiological differences in a patient throughout time (e.g. fluctuations in CL due to changes in VWF levels with aging, or unknown onset of inhibitors), but also study features (e.g. uncertain dosing times, uncertain or inadequate sampling times, or differences due to the analytical assays between occasions).

Several alternatives to body weight dosing have been suggested to individualize FVIII replacement therapy, including a posteriori dose individualization strategies, of which PK-guided methods using model-based TDM arises as a promising approach (63, 139, 140). In Paper II, a body weight-based dosing strategy, where doses were corrected so that the plasma FVIII activity in the median patient corresponded to the selected target in plasma, was compared with model-based TDM approaches. At an individual level, body weight-based dosing was found to lead to more inaccurate doses than any of the model-based TDM approaches, which was particularly noticeable when information from several occasions was used in model-based TDM. In the past years, model-based TDM based on individual PK samples has been encouraged to individualize dosing regimens of FVIII replacement therapy in prophylaxis (139, 140). Recent studies comparing body weight-based prophylactic dosing regimens with PK-based prophylaxis suggest that PK-based prophylaxis leads to a similar or lower FVIII consumption and a trend towards a reduced ABR (141-144). In a study involving 36 patients with severe hemophilia A, the annual consumption of FVIII was approximately the same between standard prophylaxis and PK-based prophylaxis (145). However, in PK-based prophylaxis, FVIII product was better distributed across patients (FVIII amount reduced in 18 patients, and increased in 14 patients), with 16 patients (46%) having a lower ABR (mean reduction -2.2) and 8 patients (23%) having an increased ABR (mean increase 1.3). Another study, involving 21 patients with severe and moderate hemophilia A found a similar consumption of FVIII and a similar ABR, but identified a tendency to fewer spontaneous bleeds for PK-based prophylaxis (143). A retrospective study including 6 children concluded that PK-based prophylaxis was associated with a decrease in the number of bleeds, from 7 to 2 bleeds during the same period, and a total saving in FVIII of €8 986 per patient per year (142). Finally, Iannazo et al. in a simulation study based on a pharmacoeconomic model found potential for a reduction in the occurrence of joint bleeds using PK-driven prophylaxis (annual joint bleed rate from 1.01 to 0.845) and a slight reduction of doses (0.360 IU/kg), resulting in a potential total saving of €5 197 per patient per year. Previous studies involving PK-based prophylaxis often define a single FVIII activity target for the individualization of dosing regimens (1 IU/dL), which relies on the assumption that the same plasma FVIII activity level protects different patients against the occurrence of bleeds.
In this thesis, the longitudinal dose-exposure-response relationship following the administration of a rFVIII product is described through the development of the integrated PK-RTTCE model. The bleeding hazard was found to decrease over time of the study, which is probably due to a normalization of the clotting system or a better adherence to treatment. The number of bleeds in the 12-month period before the study started was found to be the main predictor of the occurrence of bleeds. However, after the addition of all covariates to the model, including the time-varying protective effect of FVIII levels in plasma, the unexplained variability in the bleeding hazard was still found to be large (111%CV). This finding agrees with the clinical observation that the extent to which patients are protected against bleeds with a certain plasma FVIII activity level varies substantially between patients (65-68, 72). Data such as type of FVIII gene mutation, or level of physical activity, could help to explain part of this variability, but the main cause of such high variability in the bleeding phenotype is still unclear (69-71). Even though the sources of variability are unknown, the integrated PK-RTTCE model allows the estimation of each individual’s bleeding hazard, which is a numerical representation of the bleeding phenotype.

Individual bleed information was found to be the preferable source of patient information when the integrated PK-RTTCE model was used to forecast the occurrence of future bleeds. This result suggests that bleed information is more informative than when using the PK information available in the LEO-POLD studies to characterize the inter-patient bleeding variability, and that bleeds can add value to the optimization of prophylactic dosing regimens. A treatment strategy based on the bleeding phenotype is not a new idea, and the Canadian Hemophilia Prophylaxis Study was a forerunner with tailored prophylaxis based on bleeding frequency (frequency-escalated prophylaxis) (146, 147). In total, this study enrolled 56 children, between 1997 and 2007, and had a median follow-up period of 10.2 years (148). The dosing protocol consisted of the initial administration of 50 IU/kg of FVIII once-weekly, and the frequency and dose of FVIII administration was increased in case unacceptable bleeding occurred. The final results, reported in 2018, refer a median usage of 3 600 IU/kg per year, which is substantially lower than the 6 000 IU/kg per year used in a full-dose alternate day prophylactic dosing regimen (148, 149). In addition, the study reported very good health outcomes. Even though these results are promising, the escalation criteria included only the occurrence of bleeds, neglecting other factors such as individual differences in PK or physical activity, which resulted in joint damage in some patients. Furthermore, only patients aged 1-2.5 years with normal joints were enrolled, which limits the extrapolation to patients other than pediatric patients initiating primary prophylaxis. The model-based approach suggested in this thesis may overcome these limitations by integrating the different sources of individual information throughout time, namely, PK, bleeds and covariates,
which may lead to more cost-effective individualized doses. The first steps for the conception of such approach were taken in Paper IV, with the creation of a framework that allows the estimation of the EBEs related to PK and bleeding hazard (output I in Figure 16). Further study is required to optimize this approach, namely, to define criteria for dose adaptation in each patient (output II in Figure 16).

Figure 16. Steps comprising a PK- and bleed-based individualization of dosing regimens, based on Bayesian forecasting of PK and bleeding hazard parameters. PK: pharmacokinetic; RTTCE: repeated time-to-categorical event.

The suggested PK- and bleed-based approach could be implemented in a user-friendly computer app, where the patient with hemophilia A could register data related to treatment as well as the occurrence of bleeds. Using the iterative procedure described in Figure 16, the patient would visualize the time-varying risk of having a bleed, and the app would generate an alert if the probability of bleeding was above a certain value for a long period, requesting the patient to consult the treating physician.

Finally, this thesis also had a methodological component with general application in pharmacometrics. Different approaches to handle IOV in model-based TDM were contrasted for the first-time, using a PK model of FVIII activity as example. An approach using the unperturbed model during Bayesian forecasting, but not including the random effect related to IOV in the dose
calculation was found to forecast the most accurate and precise individualized doses. Given the wide range of model features explored and that the studied example is a commonly used two-compartment model structure, these findings are most likely applicable to other therapeutic areas. Consequently, this conclusion can be of high interest not only to modeling and simulation experts responsible for developing models to be used in model-based TDM, but also to software developers, who may want to implement such approach in TDM software packages, and to experts with the responsibility of forecasting individualized doses.
6 Conclusions

In this thesis, pharmacometric approaches to improve dose individualization methods with focus on FVIII replacement therapy in hemophilia A have been developed.

Specific findings in Paper I are:
- A population PK model of FVIII activity following administration of morococog alfa was developed based on data from a heterogeneous cohort, including 13 clinical trials, comprising 754 patients with moderate to severe hemophilia A, aged 1 day-73 years. This is currently the largest group of patients used to develop a population PK model following the administration of a FVIII replacement product. Covariate effects related to body weight, age, neutralizing anti-FVIII inhibitors, race, and analytical assay were found to be significant predictors of FVIII activity PK. The PK of morococog alfa was shown to have high inter-individual and IOV.

- Through simulations, younger pediatric patients were found to require higher body weight-adjusted doses than adolescents to achieve the same target FVIII activity. In patients younger than 17 years old, the administration of morococog alfa every other day seems to be required to observe a trough FVIII activity value of at least 1 IU/dL in the median patient.

Specific findings in Paper II are:
- Several approaches to handle IOV (also known as within-patient variability) during Bayesian forecasting were assessed and found to lead to substantially different individualized doses, particularly when sparse data is used. When the population model used in model-based TDM includes IOV, IOV should be included during Bayesian forecasting, but the portion of unexplained variability related to IOV should be excluded from the individual parameters used to calculate the future dose.

Specific findings in Paper III:
- An integrated PK-RTTCE model was developed to describe the relation between the dose of octocog alfa, plasma FVIII activity, bleeding patterns (frequency and severity) and covariates in patients with severe hemophilia A based on data from three clinical trials.
The bleeding hazard, a numerical translation of the bleeding phenotype, was found to decrease over time of the studies and to be affected by plasma FVIII activity and the number of bleeds in the previous 12 months. In addition, the bleeding hazard was associated with large IIIV.

Specific findings in **Paper IV**:
- The integrated PK-RTTCE model developed in **Paper III** was used to forecast the efficacy endpoint of interest, i.e. the occurrence of bleeds. Past bleeding information was found to be a main component driving the forecast of future bleeds, and thus an important factor to integrate in the optimization of dosing regimens.
7 Future perspectives

Even though other treatment options for hemophilia A have been emerging, involving alternative targets in the coagulation cascade or gene therapy, FVIII replacement therapy remains the treatment of choice given its efficacy, safety and familiarity among patients. Therefore, until a cure for hemophilia A is found, or in resource-limited settings, FVIII replacement therapy is expected to remain as a mainstay of treatment and the improvement of its cost-effectiveness is crucial.

Many practical matters, as well as opportunities, related to PK and/or bleed-based optimization of dosing regimens deserve to be further studied, in particular, how the PK-RTTCE model presented in this thesis can be used for dose adaptation in a real-world scenario. Some technical components need optimization, such as, the threshold to trigger dose adaptation potentially accounting also for the cost of FVIII products, or the assessment of the use of covariates for patients with past bleed information missing. In addition, the model can be further expanded to consider the PK of other FVIII products such as the novel extended half-life FVIII products. Finally, the impact on clinical and economic outcomes associated with suggested dose adaptation using the PK-RTTCE model require further assessment, preferably, through a prospective clinical study.

In the field of variability and model-based TDM, the work presented in this thesis can be further expanded to residual unexplained variability. How different residual error models, magnitudes of residual error, or down-weighting of old samples influence Bayesian forecasting is an open topic and deserve to be further explored.
8 Acknowledgements

The work presented in this thesis was carried out at the Department of Pharmaceutical Biosciences, Faculty of Pharmacy at Uppsala University, Sweden.

I am grateful for the financial support from Pfizer and Bayer. In addition, I would like to express my gratitude to Apotekarsocieteten (Apotekare Gunnar Hylténs stiftelse, Elisabeth och Alfred Ahlqvists stiftelse, IFs stiftelse), and Smålands nation (Anna Maria Lundins stipendiefond) for awarding me travel grants to attend countless high-quality courses and present my research at international conferences.

There is a long list of people who, directly or indirectly, have contributed to this thesis and encouraged me during my time as a PhD student. In particular, I would like to thank:

My supervisors Mats Karlsson and Elisabet Nielsen. Mats, for adopting me as a PhD student. Thank you for your enthusiasm and brilliant input on the projects. It was a tremendous privilege to work with you and to be part of your research group. Elisabet, for your invaluable guidance, patience, and support. Thank you for being so easy to communicate with and for being there for me when you felt I needed it the most. Thank you so much for your thorough exceptional scientific input on all projects.

My co-supervisor Siv Jönsson, for believing in me and for providing me with extraordinary support and encouragement. Your willingness to help was a constant from the first day we started working together. Thank you for giving me a strong foundation in pharmacometrics and for teaching me how to become not just a better scientist but a better person also.

My co-authors. From Pfizer, Joan Korth-Bradley and Lutz Harnisch, for bringing your clinical expertise and ideas to the project and for the great discussions on the moroctocog alfa project. From Bayer, Dirk Garmann and Alexander Solms, for always being available for a good discussion and for your invaluable input on the octocog alfa projects.

My half-time committee members Maria Kjellsson, Mia Wadelius and Johan Wallin, for your valuable feedback at an important stage of my PhD studies.
Sebastian Ueckert, for the expert graphing assistance in Paper I, and Gunnar Yngman, for the skillful assistance during the implementation of the full random effects modelling approach in Paper III.

I would also like to acknowledge: Astrid Oosten and colleagues from the Erasmus MC Cancer Institute, for the fruitful collaboration that led to the papers on fentanyl and morphine pharmacokinetics; and Nicolas Frey, for providing me with the opportunity to do an internship at F. Hoffmann-La Roche, in Basel, and for the extensive guidance during that period.

Margareta Hammarlund-Udenaes, for providing an excellent work environment and for your support with the AAPS student chapter.

I am thankful to all my colleagues in the pharmacometrics (PM) research group at Uppsala University.

Lena Friberg, Andrew Hooker, Maria Kjellsson, Elodie Plan, Martin Bergstrand, Joakim Nyberg, Sebastian Ueckert, Ulrika Simonsson, for your scientific input during meetings and seminars. In addition, Joakim for your support with repeated time-to-event and equation-related matters.

Rikard Nordgren, Svetlana Freiberga, and previously Kajsa Harling, for developing and being always willing to help with PsN.

All the teachers in pharmacokinetics and pharmacotherapy, in particular, Jörgen Bengtsson for providing me with the opportunity to teach.

Marina, Karin, Ulrica, Magnus, Jerker and Tobias, for administrative and IT support. Agneta, the barista, for your help.

Members of the TDM group of the A3:3 inner appendix, for sharing your experiences in the field of dose individualization, including Kajsa, Brenda, Felix, Maria and Olof. Also in TDM, Ron Keizer for sharing your thoughts and know-how in model-based TDM.

Lénaïg, for being such a supportive colleague and friend, and for always having something nice to say. Sreenath, for having my back, at work and in life in general. Philippe, for encouraging me to keep my shoulders strong and for your scientific input. One day we will build a snowman. Gopi, for many bike rides while still being half asleep, late dinners at Max and enlightening conversations. Moustafa and Ari, for cheering for me in the front row in Montreux. Henrik, for your help with difficult topics at work, and for introducing me to the bean game. Yasunori, for being such a relaxed travel buddy in Portugal, and for being so humble and helpful. Benjamin, for your precious help every time I got stuck with R. Emilie, for the fun moments in India. Also, thank you both for welcoming me so warmly in Basel, and for the awesome
birthday cake. **Robin**, for being a fantastic office mate. **Gunnar**, for everything you taught me. **Anders T**, for showing that not all Danes are like the guy at the Copenhagen airport. **Gustaf**, for the amazing acting skills. **Chen-yan**, for being such a helpful neighbor (at work and at home) and for the events we organized together. **Shijun**, for being so genuine (I swear I did not see your shrimp in Sils-Maria either). **Viktor**, for your pragmatism. **Jesmin**, for looking at the bright side of things. **Ida**, for the thought-provoking questions during presentations. **Marina S**, for showing me clinical applications of pharmacometrics. **Estelle**, for being a helpful teaching buddy. **Yevgen**, for the nice conversations over fika.

All the present and past postdocs and researchers. **Xiaomei**, for always checking in with a smile. **Eva**, for being the safe car driver in Kiruna. **Carolina**, for bringing a Latin-American mindset to the group. **Iris**, for offering help multiple times, and **Jill**, for matching your mugs with your dresses. **Tomás**, for speaking some Portuguese; **Rory**, for all the breaks; and **Felix**, for the gazpacho.

I would also like to thank other previous group members. **Chayan**, for the tutorial in R many years ago, trips and parties. **Camille**, for the fun moments in Portugal, and for your wise thoughts on career options. Thank you also **Brendan, Salim, Margreke, Elke, Julia K, Anne-Gaëlle, Ana K, Anders K, Chunli, Winn, Elin S, Thomas D, David K, Anna L, Siti, Steve C, Yu-ancheng, Ronald, Waqas, Åsa, Eric S, Oskar A, Oskar C** for having contributed to a nice work environment.

My master student **Easin** for the hard work and allow me to practice my supervision skills, and other students who I had the pleasure to meet, **Alan, Maddie, Suzanne, Flavia, Marian, Vincent, Johanna, Linda, Kia, Sara, Budi** and **Lina**.

I would like to thank my colleagues in the translational pharmacokinetics-pharmacodynamics (tPKPD) group at Uppsala University. **Erik**, for the nice trip to Kiruna and for the late-night politics discussions. **Irena**, for always saying hi with a smiling. **Nebojša**, for the most stunning interpretation of *My Heart Will Go* that the world will ever see. **Yang**, for always showing up first.

Outside the department there are many people who contributed indirectly to the completion of this thesis. I am very grateful to:

**Daniel, the first**, and **Daniel el Salvador Giraldo**, por me fazerem sentir em casa na Suécia e pelas noites de conversas e cartas. **Emma**, for that special birthday cake. **Mr. Tobias**, for always being there. **Patrik**, pelos truques mágicos. **Thomas Chauvin**, for that day when you came to rescue me with your van. **Mário**, por arranjar sempre forma de ajudar no chapter.

**Thor** and **Margareta**, for introducing me to meditation and giving me guidance that helped me staying focused during hectic periods. **Maria**, for introducing me to Lindy hop. **Lani**, for the trips and parties. **Marta**, pelo apoio nos Stockholm International Toastmasters. **Juliane**, for the visit in Basel on such a short notice. **Fredrik**, for giving the best spinning classes at the gym.

From Portugal, à **Joana Sousa**, por me ter apoiado na ideia de vir até à Suécia e ter estado a meu lado no início desta experiência. Às minhas colegas dos tempos de doutoramento em Coimbra, nomeadamente, à **Joana B, Daniela G** e **Ana S**, pela vossa amizade e pelo que aprendi com vocês. Igualmente, à **Susana S, Edna, Carla V, Miguel O, Toni e Valente**.

Aos meus amigos de Coimbra, nomeadamente, ao **FarRafa, Xicozorro e Patrícia R, Gonçalo e Renata, Maria Inês, Ana Rita, Salete, Diana e Nuno R, Rui de Guimarães e Sónia, Marisa, Adelino, Pedro S**, e tantos outros, pela vossa amizade. E, em especial, à **Cátia Coura**, pelas conversas ao telefone quando as coisas não correram tão bem.

À **Dra. Ana Melo**, por me ter dado uma base forte para chegar até aqui.

À minha família, nomeadamente, à minha mãe **Luísa**, irmão **Miguel** e ao **Sr. Vitor**, pelo vosso apoio à distância durante este período. À **Sra. Dna Ercília**, pela companhia nas viagens e aniversários.

Finally, I would like to express my immeasurable gratitude to **Janneke**, for your continued support and encouragement during the last part of these PhD studies. You experienced all the ups and downs of my research at home, and gave me invaluable advice on working more efficiently and keeping the focus on the full picture until I could see light at the end of the tunnel. The best is yet to come, together.

The last four and a half years in Sweden were incredible. I am sad to see this period coming to an end, but I am extremely excited about the future. A future in which I hope to meet all of you again.

João
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Acta Universitatis Upsaliensis

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