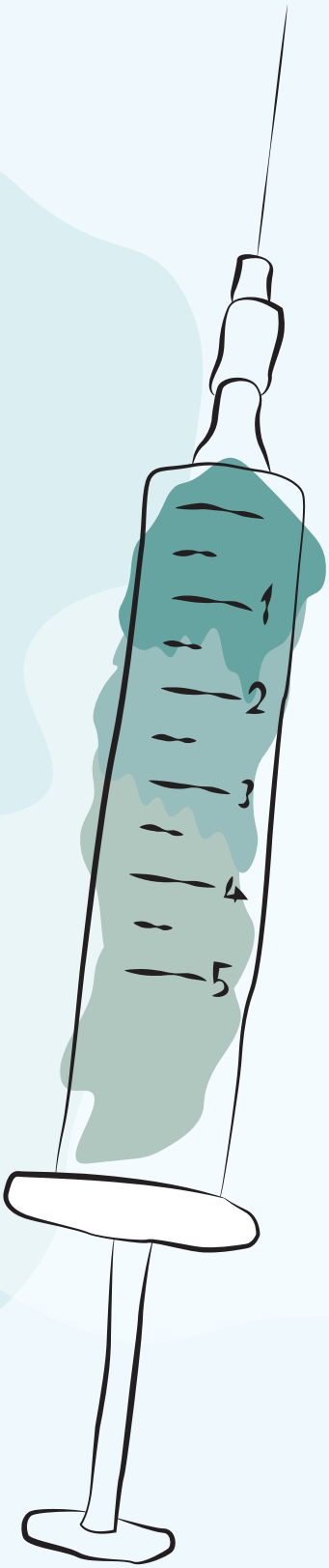


Personalizing Factor Replacement Therapy in Hemophilia

Iris van Moort

Personalizing Factor Replacement Therapy in Hemophilia

Iris van Moort

ISBN: **978-94-6361-470-2**
Lay-out and printing: **Optima Grafische Communicatie**
Cover: **Optima Grafische Communicatie**

Printing of this thesis was kindly supported by: Stichting Haemophilia, Bayer, CSL Behring, Sobi and Menapia BV.

Personalizing Factor Replacement Therapy in Hemophilia

Personaliseren van suppletietherapie met stollingsfactor concentraat in hemofilie patiënten

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus

Prof.dr. R.C.M.E. Engels

en volgens besluit van het College voor Promoties.
De openbare verdediging zal plaatsvinden op

Dinsdag 17 november 2020 om 15.30 uur

door

Iris van Moort
geboren te Wolphaartsdijk

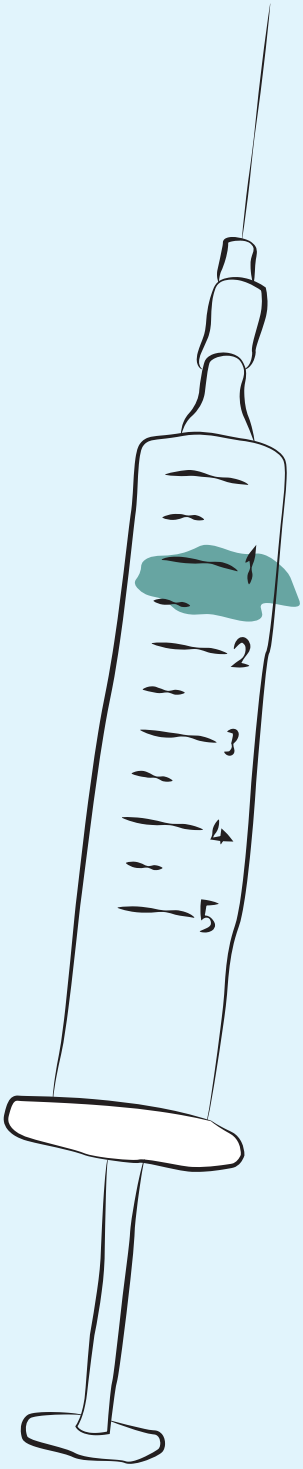
Erasmus University Rotterdam

Erasmus

PROMOTIECOMMISSIE:

Promotoren:	prof. dr. F.W.G. Leebeek prof. dr. R.A.A. Mathôt
Overige leden:	prof. dr. M.P.M. de Maat prof. dr. S.N. de Wildt prof. dr. J. Voorberg
Copromotor:	dr. M.H. Cnossen

TABLE OF CONTENTS



1

General introduction and outline of thesis

"I understand pharmacokinetics, as you have explained it, completely. In daily life you want me in a perfectly tailored suit and when I am playing sports in baggy sweat pants, so I have extra factor concentrate on board to prevent me from bleeding". Hemophilia patient, 2015

This quote perfectly summarizes the main message and hypotheses studied in this thesis. Medication can be more optimally tailored according to each patient's characteristics and necessities during varying circumstances. Personalization of treatment can improve quality of care and therefore quality of life and may potentially lead to societal benefits through cost reduction of treatment.

Hemophilia

Hemophilia patients suffer from a (partial) deficiency of either coagulation factor VIII (FVIII) or coagulation factor IX (FIX), caused by respectively *F8* or *F9* gene mutations. As both genes are located on the X chromosome, mainly males are affected. Females are carriers of these bleeding disorders and therefore generally not or only mildly affected. Disease severity is classified according to residual plasma FVIII or FIX levels. Patients with mild hemophilia have FVIII or FIX levels between 0.05-0.40 IU/mL, leading to bleeding only after trauma or during surgery. Patients with moderate hemophilia have FVIII or FIX levels between 0.01-0.05 IU/mL, and patients with severe hemophilia have FVIII or FIX levels < 0.01 IU/mL. Patients with moderate or severe hemophilia present with spontaneous bleeding and bleeding after minor trauma, typically in muscles and joints. In this thesis, we will focus on hemophilia A. Hemophilia A has a prevalence of 1:5000 male births, leading to approximately 1600 hemophilia A patients in the Netherlands.[1]

Diagnosis

A patient is suspected of a bleeding disorder based on atypical bleeding with regard to frequency, severity, and/or location. Hemophilia A is diagnosed when FVIII activity measurements are abnormal during hemostatic laboratory workup.[2] It is essential to safeguard accuracy and reproducibility of FVIII activity level measurements as variations in FVIII activity measurements may lead to misclassification of hemophilia severity. Subsequently, this will lead to either under or overtreatment of patients and clinical complications such as joint damage. In addition, in order to monitor factor replacement therapy, reproducible FVIII activity levels are essential to maintain specified FVIII activity levels during bleeding episodes and surgical procedures, according to national guidelines.[3]

FVIII activity measurements are generally performed using one-stage assays (OSA) or chromogenic substrate assays (CSA).[4] The OSA is based on the activated partial throm-

boplastin time (APTT), using the time until clot formation as its endpoint.[5] In the CSA, the coagulation system is triggered resulting in factor Xa (FXa) generation. During the second step of this test, FXa hydrolyses a chromogenic substrate causing a color change, which reflects the amount of FVIII activity in the patient sample.[6,7] As a result of different test methods and endpoints, these assays may lead to different FVIII results in hemophilia patients with varying *F8* mutations.[8]

Treatment

Mainstay of hemophilia A treatment is replacement of the deficient coagulation factor with either intravenously administered factor concentrate also called factor replacement therapy or by administration of desmopressin. Desmopressin increases FVIII by inducing the release of von Willebrand factor from Weibel Palade bodies in the endothelium, and can only be used in non-severe hemophilia patients.[9] Treatment of hemophilia can be divided into prophylaxis to prevent bleeding, or on demand treatment in case of bleeding ór to prevent bleeding during hemostatic challenges such as dental- or surgical procedures.[10] Prophylactic treatment was introduced in 1965 by Ahlberg.[11] It is based on the observation that moderate hemophilia patients with FVIII levels ≥ 0.01 IU/mL have far fewer joint bleeds and develop arthropathy less frequently.[11] Therefore, it was hypothesized that joint bleeding can be prevented in severe hemophilia patients by maintaining FVIII levels above 0.01 IU/mL by regular prophylactic doses of coagulation factor.[12] To achieve this, FVIII concentrate is infused, generally two to four times a week using standard half-life FVIII concentrates. When on demand dosing of FVIII concentrate or desmopressin is administered, specific FVIII levels and ranges are targeted. Which FVIII levels are targeted depends on bleeding severity and location of bleed, type and location of surgery and postsurgical day among others.[2,3]

Interindividual differences

Dosing of FVIII concentrate is challenging. Standard practice is to dose based on bodyweight and crude estimations of *in vivo* recovery and FVIII clearance. The half-life of FVIII is roughly calculated by the formula: half-life = 0.693 * Volume of distribution divided by clearance. Half-life of standard half-life products is estimated at approximately 10.4 hours using the estimated standard half-life clearance of FVIII products of 2.4–3.4 mL/h/kg and a volume of distribution equal to the plasma volume.[13,14] Furthermore, dosing is based on crude estimations of *in vivo* recovery assuming that each unit infused per kg of bodyweight increases FVIII levels by 0.02 IU/mL.[15] However, Bjorkman et al. demonstrated in 152 hemophilia A patients of all ages that a large variation in achieved FVIII levels exists after administration of 50 IU/kg FVIII concentrate.[16] This is caused by large interpatient variability in pharmacokinetic parameters such as clearance and volume of distribution. These differences were associated with patient characteristics such as:

bodyweight, age and height.[16-18] Collins et al. subsequently showed that FVIII concentrate half-life, ranges between 6 and 25 hours in the hemophilia A population, underlining the major challenges when FVIII concentrate dosing is based on bodyweight.[19] Our research group recently reported a cohort of 119 hemophilia A patients undergoing 198 surgeries and showed that 45% of FVIII levels measured were under FVIII target levels during the first 24 hours after surgery resulting in a higher risk of postoperative bleeding.[20] In contrast, 75% of all measured FVIII levels five days after surgery were above FVIII target level with concomitant unnecessary high costs.

Novel treatment strategy: pharmacokinetic (PK)-guided dosing of factor concentrates

Optimization of FVIII concentrate treatment in hemophilia A patients can be achieved by PK-guided dosing. PK is defined as what happens to the drug in a patient's body by processes of absorption, distribution, metabolism and excretion. PK-guided dosing is described as dosing based on the PK parameters of the factor concentrate as derived from an individual patient. Individual PK parameters can be assessed by serial sampling of (ten or more) blood samples and calculating of PK parameters from the measured factor levels. Individual PK parameter estimates can also be obtained by Bayesian forecasting, which can be performed with only a limited number of blood samples (two to three) per patient. Bayesian forecasting however requires the availability of a population PK model. Such a model not only provides typical PK parameter estimates but also their corresponding interindividual variability.

To apply population PK models correctly, they should be constructed from heterogeneous, well-defined populations and constructed with patient data obtained from different settings and under variable circumstances. Not surprisingly, FVIII population PK models were first constructed for prophylactic dosing.[16,21] Our research group was the first to present a perioperative FVIII population PK model for severe and moderate hemophilia A patients.[22] This perioperative model showed large differences in comparison to the Bjorkman et al. prophylactic model as larger volume of distribution (1180 mL/68kg) was observed perioperatively than in the prophylactic setting (240 mL/68 kg).[16,22] Analysis and testing of covariates, which describe the relationship with a specific PK parameter in a population PK model, subsequently leads to explanation of inter-and intraindividual variability. Therefore, leading to more accurate estimations of individual PK parameters and more adequate dosing advices.

The Bayesian forecasting procedure to obtain a dosing regimen works as follows (Figure 1). Firstly, an individual PK profile is constructed. A patient is administered a FVIII concentrate bolus (t=0). At three time points, for example t=4, t=24 and t=48 hours, blood is

drawn and FVIII levels are determined. The available population model contains information from all possible PK profiles (black lines). By combining the individual's measured levels (points) and the population PK model the most probable individual PK profile (red line) is obtained with concomitant individual PK parameters. The availability of these parameters makes it possible to calculate a precise dosing advice for each individual patient, taking specific covariates into account. Despite the fact that PK-guided dosing of factor concentrates using rich sampling was described as efficacious as early as 1993,[23] it has not been applied broadly until more recently in hemophilia.[24] This is due to the prior necessity of at least ten blood samples e.g. time points to calculate patient's PK, in combination with an obligatory factor concentrate washout period, leaving the patient without prophylaxis and unprotected against bleeding. Currently, Bayesian forecasting is increasingly applied since population PK models are increasingly available. In combination with limited blood sampling without application of a factor concentrate wash out period, this technique has emerged as a feasible innovation in hemophilia care. [19,25]

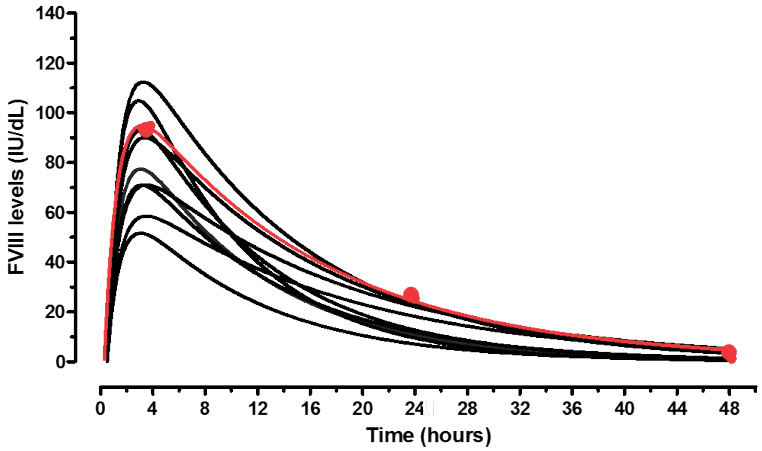


Figure 1. Estimating individual PK parameters using Bayesian analysis. The black lines represent all the information present in the population PK model. The red dots are the measured FVIII levels in an individual patient. Using all information provided, individual PK parameters (red line) can be estimated. Based on the individual PK parameters, a personalized dosing scheme according to targeted FVIII ranges is created.

OUTLINE OF THE THESIS

In this thesis, we will focus on the conditions, strengths, limitations and potential applications of PK-guided dosing of FVIII concentrate in hemophilia A patients.
The thesis is divided into two parts:

1. Evaluation of current diagnostics and treatment monitoring;
2. Implementation of pharmacokinetic-guided dosing of factor VIII concentrate in hemophilia A.

In order to investigate these themes, firstly we will address the pitfalls in the diagnosis of hemophilia A (Chapter 2), and how hemophilia teams and laboratories can avoid these when diagnosing hemophilia A by residual FVIII activity level measurements. In collaboration with the External Quality Assessment Program for Thrombosis and Hemostasis (ECAT) Foundation, we investigate the quality of FVIII measurements in more than 200 laboratories worldwide (Chapter 3). As FVIII measurements are essential to optimize both the diagnosis and quality of treatment monitoring, accuracy of these measurements is of great importance. Implementation of PK-guided dosing is only feasible if data on factor levels observed in the individual patient are precise and reliable. The same applies to the data used to construct population PK models. Therefore, knowledge and expertise on coagulation factor laboratory assays are indispensable when providing PK-guidance of factor concentrate dosing.

Implementation of individualized dosing strategies with FVIII concentrate is the main topic of the second part of this thesis. Firstly, a detailed review discusses the background of PK-guided dosing covering its advantages and limitations (Chapter 4). Subsequently, we describe the design of a randomized controlled trial which compares perioperative PK-guided dosing of FVIII concentrate with standard dosing based on bodyweight in severe and moderate hemophilia A patients in Chapter 5. In Chapter 6, the preliminary results of this unique randomized controlled trial are presented and discussed. As von Willebrand factor (VWF) has a potential effect on FVIII clearance due to its chaperone function, protecting FVIII from proteolytic cleavage in the circulation, VWF will be determined in patients undergoing surgery. Results illustrating VWF kinetics and its role in such a perioperative setting will be evaluated in Chapter 7.

As the prevalence of overweight and obese individuals in the general population is rising, hemophilia A patients are also increasingly overweight and obese. In Chapter 8, we investigate the use of various morphometric variables as substitutions for bodyweight to dose overweight and obese hemophilia A patients. An extremely obese, severe hemophilia A patient who undergoes a laparoscopic sleeve gastrectomy in order to lose weight is followed over time and investigated at subsequent time points to gain insight into the potential effects of significant weight loss on individual FVIII PK parameters (Chapter 9). In the last chapter of this thesis, currently available PK-guided dosing tools will be compared and impact of modeling differences on dosing advices will be analyzed (Chapter 10). Finally, the results of this thesis will be discussed in Chapter 11.

REFERENCES

1. Rosendaal FR, Briet E. The increasing prevalence of haemophilia. Thromb Haemost 1990;63:145.
2. Srivastava A, Brewer AK, Mauser-Bunschoten EP, et al. Guidelines for the management of hemophilia. Haemophilia 2013;19:e1-47.
3. Leebeek FWG, Mauser-Bunschoten EP. Richtlijn diagnostiek en behandeling van hemofilie en aanverwante hemostase stoornissen. Utrecht: Van Zuiden Communications BV; 2009:1-197.
4. Peyvandi F, Oldenburg J, Friedman KD. A critical appraisal of one-stage and chromogenic assays of factor VIII activity. J Thromb Haemost 2016;14:248-61.
5. Over J. Methodology of the one-stage assay of Factor VIII (VIII:C). Scand J of Haematol Suppl 1984:13-24.
6. Barrowcliffe TW. Methodology of the two-stage assay of Factor VIII (VIII:C). Scand J Haematol Suppl 1984;41:25-38.
7. Barrowcliffe TW, Raut S, Sands D, Hubbard AR. Coagulation and chromogenic assays of factor VIII activity: general aspects, standardization, and recommendations. Semin Thromb Hemost 2002;28:247-56.
8. Pavlova A, Delev D, Pezeshkpoor B, Muller J, Oldenburg J. Haemophilia A mutations in patients with non-severe phenotype associated with a discrepancy between one-stage and chromogenic factor VIII activity assays. Thromb Haemost 2014;111:851-61.
9. Svensson PJ, Bergqvist PB, Juul KV, Berntorp E. Desmopressin in treatment of haematological disorders and in prevention of surgical bleeding. Blood Rev 2014;28:95-102.
10. Fijnvandraat K, Cnossen MH, Leebeek FW, Peters M. Diagnosis and management of haemophilia. Bmj 2012;344:e2707.
11. Ahlberg A. Haemophilia in Sweden. VII. Incidence, treatment and prophylaxis of arthropathy and other musculo-skeletal manifestations of haemophilia A and B. Acta Orthop Scand Suppl 1965:Suppl 77:3-132.
12. Manco-Johnson MJ, Abshire TC, Shapiro AD, et al. Prophylaxis versus episodic treatment to prevent joint disease in boys with severe hemophilia. N Engl J Med 2007;357:535-44.
13. Berntorp E, Bjorkman S. The pharmacokinetics of clotting factor therapy. Haemophilia 2003;9:353-9.
14. Collins PW, Bjorkman S, Fischer K, et al. Factor VIII requirement to maintain a target plasma level in the prophylactic treatment of severe hemophilia A: influences of variance in pharmacokinetics and treatment regimens. J Thromb Haemost 2010;8:269-75.
15. Henrard S, Speybroeck N, Hermans C. Body weight and fat mass index as strong predictors of factor VIII in vivo recovery in adults with hemophilia A. J Thromb Haemost 2011;9:1784-90.
16. Bjorkman S, Oh M, Spotts G, et al. Population pharmacokinetics of recombinant factor VIII: the relationships of pharmacokinetics to age and body weight. Blood 2012;119:612-8.
17. Hazendonk H, van Moort I, Mathot RAA, et al. Setting the stage for individualized therapy in hemophilia: What role can pharmacokinetics play? Blood Rev 2018;32:265-71.
18. Kepa S, Horvath B, Reitter-Pfoertner S, et al. Parameters influencing FVIII pharmacokinetics in patients with severe and moderate haemophilia A. Haemophilia 2015.

19. Collins PW, Fischer K, Morfini M, Blanchette VS, Bjorkman S, Group IPSGPEW. Implications of coagulation factor VIII and IX pharmacokinetics in the prophylactic treatment of haemophilia. Haemophilia 2011;17:2-10.

20. Hazendonk HC, Lock J, Mathot RA, et al. Perioperative treatment of hemophilia A patients: blood group O patients are at risk of bleeding complications. J Thromb Haemost 2016;14:468-78.

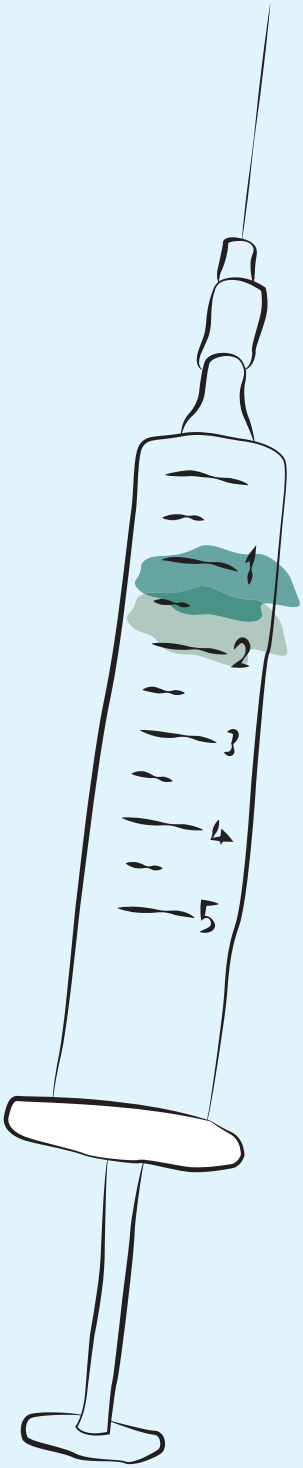
21. Bjorkman S, Folkesson A, Jonsson S. Pharmacokinetics and dose requirements of factor VIII over the age range 3-74 years: a population analysis based on 50 patients with long-term prophylactic treatment for haemophilia A. Eur J Clin Pharmacol 2009;65:989-98.

22. Hazendonk H, Fijnvandraat K, Lock J, et al. A population pharmacokinetic model for perioperative dosing of factor VIII in hemophilia A patients. Haematologica 2016.

23. Carlsson M, Berntorp E, Bjorkman S, Lindvall K. Pharmacokinetic dosing in prophylactic treatment of hemophilia A. Eur J Haematol 1993;51:247-52.

24. Carlsson MB, E; Björkman, S; Lethagen, S; Ljung,R. Improved cost-effectiveness by pharmacokinetic dosing of factor VIII in prophylactic treatment of haemophilia A. Haemophilia 1997;3:96-101.

25. Bjorkman S. Limited blood sampling for pharmacokinetic dose tailoring of FVIII in the prophylactic treatment of haemophilia A. Haemophilia 2010;16:597-605.



2

Pitfalls in the diagnosis of hemophilia: What to do?

van Moort I, Joosten M, de Maat MPM, Leebeek FWG, Cnossen MH.

Pediatr Blood Cancer. 2017 Apr;64(4).

ABSTRACT

Measurements of factor VIII coagulation activity (FVIII:C) may vary and result in misclassification of hemophilia A with delay in initiation of prophylactic treatment. We describe two young brothers who were diagnosed as moderate hemophilia patients and therefore not prophylactically treated with factor VIII concentrate despite frequent bleeding events. These findings emphasize the importance of 1) multiple measurements of FVIII:C by certified laboratories; 2) adjustment of treatment when test results do not correspond to clinical symptoms; 3) relevance of additional DNA mutation analysis in patients with hemophilia A and; 4) treatment in centers with expertise.

INTRODUCTION

Hemophilia A is an X-linked recessive bleeding disorder caused by a deficiency in coagulation factor VIII (FVIII). Measurement of factor VIII coagulant activity (FVIII:C) is fundamental in the diagnosis, classification and treatment of hemophilia A, as prognosis and treatment intensity differs between patient groups. Most severe (FVIII:C < 0.01 IU mL^{-1}) and some moderate (FVIII:C 0.01-0.05 IU mL^{-1}) hemophilia A patients receive intravenously administered prophylaxis with FVIII concentrates to prevent spontaneous bleeding, whereas mild hemophilia patients (FVIII:C 0.05-0.40 IU mL^{-1}) receive desmopressin or FVIII concentrates only in cases of acute bleeding or to prevent bleeding in case of trauma or surgery.[1,2] Furthermore, genetic counseling with factor VIII gene (*F8*) mutation analysis is performed in most patients and/or families to verify diagnosis and to establish carriership.

According to Dutch and World Federation of Hemophilia (WFH) guidelines, prophylaxis is initiated in children with severe hemophilia after their first or second joint bleed.[1,2] Unfortunately, variation in FVIII:C measurements may lead to misclassification of severity type with concomitant delay in initiation of prophylaxis, as more bleeding events will be tolerated in a non-severe hemophilia patient. This brief report aims to emphasize the importance of repeated and reliable FVIII:C testing, the importance of clinical symptoms and the relevance of DNA mutation analysis in hemophilia.

RESULTS

Case presentation

We present two brothers with a delay in diagnosis of severe hemophilia A, from a family with no family history with regard to bleeding disorders.

At the age of ten months, patient A was referred to a hospital after persistent bleeding of his finger after a bite by a house pet. No earlier bleeding was reported and intramuscular vaccinations were performed without problems. After physical examination and laboratory assessments, patient A was diagnosed with moderate hemophilia A, as a FVIII:C of 0.05 IU mL^{-1} was established by one-stage assay. In the five following years, diagnosis was confirmed by four subsequent measurements of FVIII:C, ranging from 0.019-0.05 IU mL^{-1}. To exclude concomitant von Willebrand disease (VWD), von Willebrand factor (VWF) antigen and ristocetin cofactor activity was measured once and revealed plasma concentrations of 1.37 and 1.61 IU mL^{-1}, respectively. In due course, patient A had recurrent joint bleeds in multiple, usually smaller, joints as documented in patient log. The

majority were caused by (minimal) trauma and most were treated with FVIII replacement therapy resulting in more than 50 FVIII infusions at presentation in our clinic. No *F8* mutation analysis was performed.

After diagnosis in patient A, his brother (patient B) was also tested for hemophilia A. A FVIII:C baseline level of 0.03 IU mL^{-1} was found after a single measurement, VWF antigen and ristocetin cofactor activity were not measured. Patient B had few bleeding events and was only sporadically treated with FVIII concentrate until the age of five years. All bleeding events were after trauma. Also in patient B, no *F8* mutation analysis was performed.

At ages of six and five years, both brothers were seen at our hemophilia treatment center after referral due to a further centralization of care of hemophilia patients in the Netherlands.[3] Laboratory analysis also by one-stage assay revealed FVIII:C of <0.01 IU mL^{-1}, indicative of severe hemophilia. Due to clinical phenotype with frequent bleeding and two FVIII:C plasma concentrations <0.01 IU mL^{-1} in both brothers, prophylaxis was initiated immediately at 20 IU kg^{-1} once a week and rapidly extended. Fortunately, no deleterious effects on joint function have yet become visible. Subsequently, *F8* mutation analysis revealed an inversion of intron 22, which is the most common mutation found in severe hemophilia A patients.[4] Targeted mutation analysis in the mother confirmed hemophilia A carriership.

DISCUSSION

This brief report demonstrates a number of important points which can optimize hemophilia care. Firstly, lack of awareness that a significant bleeding phenotype in moderate hemophilia patients indicates necessity of prophylaxis. Secondly, the pitfalls of only sporadic FVIII:C testing and thirdly, omission of DNA analysis to support diagnoses and to safeguard genetic counseling in affected families. Moreover, it underlines the importance of centralization of hemophilia care in order to safeguard expertise.

If hemophilia is suspected due to excessive bleeding or due to a family history of the bleeding disorder, repetitive FVIII:C testing is indicated. Especially if clinical symptoms do not correlate with test results. FVIII:C test results can be influenced by different pre-analytical variables: difficult venipuncture, filling of sodium citrate tube, temperature, storage as well as type of assay.[5-7] These factors may result in unprecise coagulation factor activity measurements. In addition, it is well-known that discrepancies exist between FVIII:C results established by one-stage or chromogenic assay.[8,9] In this brief report,

only one-stage assay was performed to test FVIII:C, venipunctures were performed by experienced medical professionals and samples were most probably processed within two hours. However, inter-and intravariation of test results is unavoidable. Therefore, external quality control assessment programs are essential to improve laboratory performance and reproducibility of test results. However, both laboratories described, participate in such an international program (ECAT external quality assessment program) with excellent performance and Z scores between -2 and 2.

Initiating prophylaxis in patients with moderate hemophilia is not clearly described in international guidelines. The Nordic guideline prescribes primary prophylaxis in moderate hemophilia when FVIII:C/FIX:C is 0.01-0.02 IU mL^{-1}. However, our experience is that most patients with these low plasma concentrations do not experience spontaneous bleeding. WFH guidelines prescribe short-term prophylaxis to decrease bleeding often in combination with intensive physiotherapy.[2] In the Netherlands, patients with moderate hemophilia and multiple spontaneous bleeding episodes are prescribed intermittent 'periodic' prophylaxis. Consequently, amount and dosing interval of treatment is adjusted according to bleeding phenotype.

When diagnosing and classifying hemophilia A, it is also important to consider VWD type 2 Normandy (type 2N), which is always characterized by low FVIII levels. This type of VWD is caused by mutations in the *VWF* gene at the FVIII-VWF binding site, and is able to mimic both moderate and mild hemophilia A.[10,11] As VWF protects FVIII from proteolytic degradation, mutations in the binding site result in excessive FVIII clearance and therefore low FVIII:C plasma concentrations.[12] VWD type 2N can be excluded or diagnosed by FVIII-VWF binding assay.[10]

DNA mutation analysis may also be able to facilitate diagnosis of hemophilia severity. Most frequent mutations in patients with severe hemophilia A are an intron 22 or intron 1 inversion of *F8.*[4,13] When these inversions are not present, complete *F8* gene mutation screening is performed of all exons, exon-intron boundaries and *F8* promotor region by direct Sanger sequencing. The Worldwide Factor VIII Variant Database currently contains more than 2000 *F8* mutations in hemophilia A patients (www.factorviii-db.org). Nevertheless, in 2-18% of patients, no genetic abnormality is observed dependent on type of mutational screening.[14-20] Identical mutations may result in different FVIII:C baseline values, therefore DNA mutation analysis is not always conclusive for hemophilia severity. However, type of mutation is still a strong predictor of the clinical phenotype.[20]

In conclusion, repeated measurements of FVIII:C and von Willebrand factor in certified laboratories, critical appraisal of clinical phenotype and hemophilia severity in centers

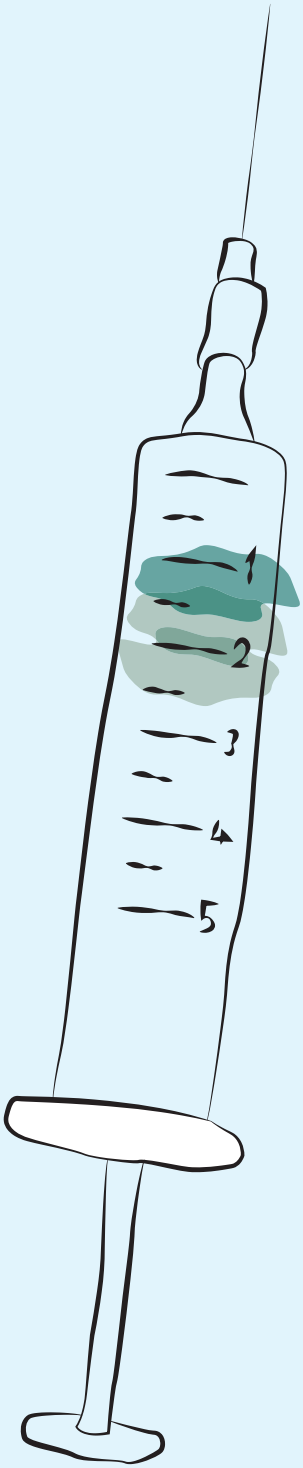
with expertise and DNA mutation analysis are essential in standard care for hemophilia A patients.

REFERENCES

1. Leebeek FWG, Mauser-Bunschoten EP. Richtlijn diagnostiek en behandeling van hemofilie en aanverwante hemostase stoornissen. Utrecht: Van Zuiden Communications BV; 2009:1-197.
2. Srivastava A BA, Mauser Bunschoten EP, Key NS, Kitchen S, Llinas A, Ludlam CA, Mahlangu JN, Mulder K, Poon MC, Street A; Treatment Guidelines Working Group on Behalf of The World Federation Of Hemophilia. Guidelines for the Management of Hemophilia Haemophlia. Montréal, QC: Blackwell Publishing; 2012.
3. Leebeek FW, Fischer K. Quality of haemophilia care in The Netherlands: new standards for optimal care. Blood Transfus 2014;12 Suppl 3:s501-4.
4. Lakich D, Kazazian HH, Jr., Antonarakis SE, Gitschier J. Inversions disrupting the factor VIII gene are a common cause of severe haemophilia A. Nat Genet 1993;5:236-41.
5. Lippi G, Guidi GC, Mattiuzzi C, Plebani M. Preanalytical variability: the dark side of the moon in laboratory testing. Clin Chem Lab Med 2006;44:358-65.
6. Feng L, Zhao Y, Zhao H, Shao Z. Effects of storage time and temperature on coagulation tests and factors in fresh plasma. Sci Rep 2014;4:3868.
7. Favaloro EJ, Meijer P, Jennings I, et al. Problems and solutions in laboratory testing for hemophilia. Semin Thromb Hemost 2013;39:816-33.
8. Pavlova A, Delev D, Pezeshkpoor B, Muller J, Oldenburg J. Haemophilia A mutations in patients with non-severe phenotype associated with a discrepancy between one-stage and chromogenic factor VIII activity assays. Thromb Haemost 2014;111:851-61.
9. Peyvandi F, Oldenburg J, Friedman KD. A critical appraisal of one-stage and chromogenic assays of factor VIII activity. J Thromb Haemost 2016;14:248-61.
10. Nishino M, Girma JP, Rothschild C, Fressinaud E, Meyer D. New variant of von Willebrand disease with defective binding to factor VIII. Blood 1989;74:1591-9.
11. Mazurier C, Dieval J, Jorieux S, Delobel J, Goudemand M. A new von Willebrand factor (vWF) defect in a patient with factor VIII (FVIII) deficiency but with normal levels and multimeric patterns of both plasma and platelet vWF. Characterization of abnormal vWF/FVIII interaction. Blood 1990;75:20-6.
12. Mazurier C, Goudemand J, Hilbert L, Caron C, Fressinaud E, Meyer D. Type 2N von Willebrand disease: clinical manifestations, pathophysiology, laboratory diagnosis and molecular biology. Best Pract Res Clin Haematol 2001;14:337-47.
13. Bagnall RD, Waseem N, Green PM, Giannelli F. Recurrent inversion breaking intron 1 of the factor VIII gene is a frequent cause of severe hemophilia A. Blood 2002;99:168-74.
14. Oldenburg J, Ivaskevicius V, Rost S, et al. Evaluation of DHPLC in the analysis of hemophilia A. J Biochem Biophys Methods 2001;47:39-51.
15. Klopp N, Oldenburg J, Uen C, Schneppenheim R, Graw J. 11 hemophilia A patients without mutations in the factor VIII encoding gene. Thromb Haemost 2002;88:357-60.
16. El-Maarri O, Herbiniaux U, Graw J, et al. Analysis of mRNA in hemophilia A patients with undetectable mutations reveals normal splicing in the factor VIII gene. J Thromb Haemost 2005;3:332-9.
17. Jayandharan G, Shaji RV, Baidya S, Nair SC, Chandy M, Srivastava A. Identification of factor VIII gene mutations in 101 patients with haemophilia A: mutation analysis by inversion screening and

multiplex PCR and CSGE and molecular modelling of 10 novel missense substitutions. Haemophilia 2005;11:481-91.

18. Vinciguerra C, Zawadzki C, Dargaud Y, et al. Characterisation of 96 mutations in 128 unrelated severe haemophilia A patients from France. Description of 62 novel mutations. Thromb Haemost 2006;95:593-9.
19. Bogdanova N, Markoff A, Eisert R, et al. Spectrum of molecular defects and mutation detection rate in patients with mild and moderate hemophilia A. Hum Mutat 2007;28:54-60.
20. Santacroce R, Acquila M, Belvini D, et al. Identification of 217 unreported mutations in the F8 gene in a group of 1,410 unselected Italian patients with hemophilia A. J Hum Genet 2008;53:275-84.



3

Analytical variation in factor VIII one-stage and chromogenic assays: Experiences from the ECAT external quality assessment program

van Moort I, Meijer P, Priem-Visser D, van Gammeren AJ, Péquériaux NCV, Leebeek FWG, Cnossen MH, de Maat MPM.

Haemophilia. 2019 Jan;25(1):162-169.

ABSTRACT

Background Both one-stage (OSA) and chromogenic substrate assays (CSA) are used to measure factor VIII (FVIII) activity. Factors explaining analytical variation in FVIII activity levels are still to be completely elucidated.

Aim The aim of this study was to investigate and quantify the analytical variation in OSA and CSA.

Methods Factors determining analytical variation were studied in sixteen lyophilized plasma samples (FVIII activity <0.01–1.94 IU/ml) and distributed by the ECAT surveys. To elucidate the causes of OSA variation, we exchanged deficient plasma between three company set-ups.

Results On average, 206 (range 164–230) laboratories used the OSA to measure FVIII activity and 30 (range 12–51) used CSA. The CV of OSA and CSA increased with lower FVIII levels (FVIII<0.05IU/ml). This resulted in misclassification of a severe haemophilia A sample into a moderate or mild haemophilia A sample in 4/30 (13.3%) of CSA measurements, while this was 37/139 (26.6%) for OSA. OSA measurements performed with reagents and equipment from Werfen showed slightly lower FVIII activity (0.93, IQR 0.88–0.98 IU/ml) compared to measurements with Stago (1.07, IQR 1.02–1.14 IU/ml) and Siemens (1.03, IQR 0.97–1.07 IU/ml). Part of this difference is explained by the value of the calibrator. For CSA, the measured FVIII levels were similar using the different kits.

Conclusions In the lower range (<0.05 IU/mL), analytical variation of FVIII measurements is high in both OSA and CSA measurements. The variation in FVIII activity levels was partly explained by specific manufacturers. Further standardization of FVIII measurements and understanding of analytical variation is required.

INTRODUCTION

Correct classification of haemophilia A severity is important as treatment intensity is based on categorisation [1]. Severe (factor VIII (FVIII) activity levels <0.01 IU/ml) and some moderate (FVIII activity levels 0.01-0.05 IU/ml) haemophilia patients receive prophylactic replacement therapy to prevent spontaneous bleeding in joints and muscles while mild haemophilia A patients (FVIII activity levels 0.05-0.40 IU/ml) receive desmopressin or replacement therapy only in cases of trauma and/or surgery [1-3]. Measuring FVIII activity levels accurately and reproducibly in different laboratories is therefore essential. We recently showed that despite excellent performance in the ECAT external quality assessment programme, between-laboratory variation may result in different FVIII levels, and consequently, in misclassification of haemophilia severity [4]. Limited between-laboratory variation in FVIII activity levels is also of importance for the monitoring of treatment in patients with haemophilia A, as specific target FVIII activity levels should be maintained around surgery and bleeding episodes [1,2,5].

Two assays are widely used to measure FVIII activity: the one-stage assay (OSA) and the two-stage chromogenic substrate assay (CSA). Most laboratories use the OSA, which is based on the activated partial thromboplastin time (APTT), using the time until clot formation as its endpoint [6]. In the CSA, the coagulation system is triggered resulting in the generation of factor Xa (FXa) [7]. In the second step of this test, FXa hydrolyses a chromogenic substrate causing a colour change, which reflects the amount of FVIII activity left in the patient sample. The endpoint in the CSA differs from that in OSA, as the CSA measures extinction at a plateau phase. Discrepancies in FVIII activity levels have been extensively reported between these two assays, depending on the mutation in *F8* gene [8-10].

Nowadays, reagents and equipment to perform FVIII activity measurements are widely available. The use of varying products may partially explain the between-laboratory variation in FVIII results. However, it is still unclear what the precise impact is of varying in reagents and equipment on the variability of FVIII activity measurements [11-14]. A possible explanation may be that particular companies provide the majority of products applied for the haemostatic testing which is standard in haemophilia. Most reports focus on the specific reagents of one company,[12,15-17] rather than analysing a test system from one company which consists of calibrator, activator, deficient plasma, and equipment. As this is often the case in real life situations, causal factors leading to the variation in FVIII activity levels should be investigated more extensively.

To improve quality of measurements in haemostasis laboratories, laboratories follow international guidelines and participate in external quality control surveys. The data from the ECAT external quality assessments indeed shows that laboratories use all components for the FVIII assays from one company in a majority of cases. Therefore, ECAT data is highly suitable to investigate the influence of company set-ups on FVIII activity level variation. The aim of this study is to investigate and quantify variation in FVIII activity when testing by OSA and CSA in surveys conducted by the ECAT foundation. In addition, we studied effects of replacement of selected reagents in the OSA with those from another company on FVIII results.

MATERIAL AND METHODS

Quantifying variation in FVIII activity measurements

More than 200 laboratories working in the field of haemostasis and thrombosis participate in the ECAT external quality assessment programme for FVIII. Four times per year, two lyophilized plasma samples are distributed. To quantify the variation in FVIII activity measurements, we selected sixteen samples 1) with FVIII activity levels between <0.01 IU/ml and 1.94 IU/ml (consensus values), 2) measured by more than 10 laboratories by OSA or CSA, and 3) measured between 2010 and 2016. As expected, we found that most laboratories use the calibrator, activator, deficient plasma and equipment from one company in the OSA. Therefore, three groups were created from the three largest companies to compare the CVs in the OSA: 1) Siemens, 2) Stago and 3) Werfen.

To investigate the impact of variation on hypothetical haemophilia severity diagnoses which are solely based on laboratory results, FVIII activity levels were subsequently classified according to severity type as stated by the World Federation of Haemophilia [1].

Impact of test system on FVIII activity levels in the OSA

From the ECAT external quality assessment programme, four plasma samples were chosen with different FVIII activity levels to investigate the influence of the test system on the FVIII activity levels. To cover the range of FVIII activity measurements, the following samples from the ECAT surveys were chosen: 1) a severe haemophilia A patient sample (consensus value FVIII<0.01 IU/ml), a mild haemophilia A patient sample (consensus value FVIII 0.16 IU/ml), a borderline haemophilia A/low FVIII activity sample (consensus value FVIII 0.42 IU/ml) and a sample with normal FVIII activity levels (consensus value FVIII 1.00 IU/ml). The FVIII activity levels were measured by laboratories participating in the ECAT surveys. Next, groups were created of laboratories using calibrator, activator, deficient plasma and equipment from one company to investigate the impact of the test

system on FVIII activity levels. When the reported FVIII activity levels were below 0.01 IU/ml, they were considered in the analysis as 0.005 IU/ml. To compare the FVIII activity levels between the three companies we used the Kruskal Wallis test as the data were not normally distributed. All statistics were performed using SPSS statistics for Windows, version 24.0 (IBM Corp, Armonk, NY, USA). A p-value of <0.05 was considered statistically significant.

Impact of test system on FVIII activity levels in the CSA

The impact of different test systems in the CSA was also investigated. FVIII activity levels were compared between Chromogenix Coamatic, Hyphen Biomed and a test system from Siemens in the four plasma samples as described under the subheading of 'Impact of test system on FVIII activity levels in the OSA'. The Kruskal Wallis test was performed to analyse the data.

Contribution of deficient plasma and calibrator

As not all laboratories use complete packages from one manufacturer, deficient plasma or a calibrator from another company may explain the variation in FVIII results. Unfortunately, this could not be investigated in the ECAT surveys, as most laboratories use all the components in the test system from one company. For this reason, we varied in deficient plasma on three different machines and its reagents as shown below in table 1. Calibration curves were created in these set-ups. Using these calibration curves, FVIII activity levels were measured in duplicate in three samples; one sample with normal FVIII activity levels (consensus value FVIII 1.00 IU/ml), mild haemophilia A (consensus value FVIII 0.34 IU/ml) and moderate haemophilia A (consensus value FVIII 0.04 IU/ml).

Table 1. Set-up of the different packages when varying in deficient plasma.

	Company		
	Siemens	*Stago*	*Werfen*
Calibrator	Standard Human Plasma	STA-Unicalibrator	HemosIL Cal Plasma
Activator	FVIII Actin FS	STA-C.K. Prest	APTT-SynthASil
Deficient plasma	FVIII deficient	STA Immunodef VIII	FVIII Def. Plasma
Equipment	CS 5100 Sysmex	STA-R Max	ACL TOP500

The influence of the calibrator was investigated by measuring the FVIII activity levels in duplicates from the calibrator of Werfen (HemosIL Cal Plasma) and Stago (STA-C.K. Prest) in the Siemens set-up as described in table 1. As these calibrators have assigned values, we compared the measured FVIII activity levels of the calibrators with their assigned values.

RESULTS

Quantifying variation in FVIII activity measurements

In the different surveys, on average, 206 (range 164 – 230) laboratories reported results from analyses that used the OSA to measure FVIII activity and 30 (range 12 – 51) laboratories used the CSA. In surveys with lower FVIII activity levels, the CV was higher (figure 1A). When comparing FVIII levels measured by OSA with the CSA, the CV was comparable between the OSA and the CSA. However, the median absolute FVIII activity levels in a sample from a severe haemophilia A patient were similar in the OSA and CSA, with FVIII activity levels of 0.005 IU/ml (IQR 0.005 – 0.03 IU/ml) for the CSA and 0.005 IU/ml (IQR 0.005 – 0.01 IU/ml) for the OSA. When comparing the CV between the laboratories using reagents from three companies for the OSA, similar patterns were observed. However, separation of products from different companies resulted in higher CVs than the overall CV with a CV up to 158% maximally for the Werfen package (figure 1B).

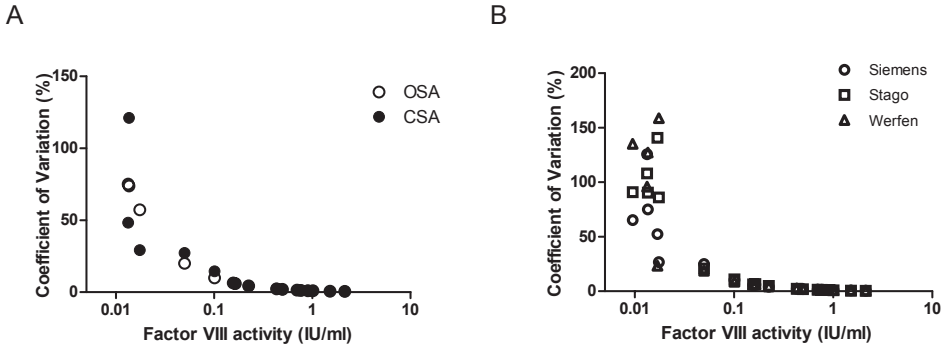


Figure 1. The coefficient of variation (CV) is higher when FVIII activity levels are lower. A)The CVs were calculated for both one-stage assay (OSA) and chromogenic stage assay (CSA). The circles indicate the CVs calculated from measurements with the one-stage assay (OSA). The squares reflect the CVs calculated from measurements with the chromogenic substrate assay (CSA). B) The CV of the OSA was also calculated when FVIII activity levels were measured with products from Siemens (circles), Stago (squares), and Werfen (triangles).

Impact of test system on haemophilia severity classification

The impact of this FVIII variability on haemophilia classification which is solely based on FVIII activity levels is significant. This is illustrated by the fact that the severe haemophilia A sample was classified as moderate in 37/139 (26.6%) of all OSA measurements (figure 2D). When classification is differentiated according to company in samples tested with OSA, 9/45 (20.0%) of the laboratories working with Siemens classified this sample as moderate or mild haemophilia while these percentages were 18/38 (47.4%) for Stago and 10/56 (17.9%) for Werfen. Only a small number of laboratories measured FVIII activity levels with CSA. Overall with CSA, 4/30 (13.3%) classified the severe haemophilia A sample as moderate or mild. When results are differentiated according to company, mis-

classification was observed in 1/8 (12.5%) for Chromogenix, in 2/14 (14.3%) for Hyphen and in 1/8 (12.5%) for CSA testing with Siemens products. In conclusion, laboratories using CSA misclassified severe haemophilia A patients less often. However, the number of CSA measurements is small.

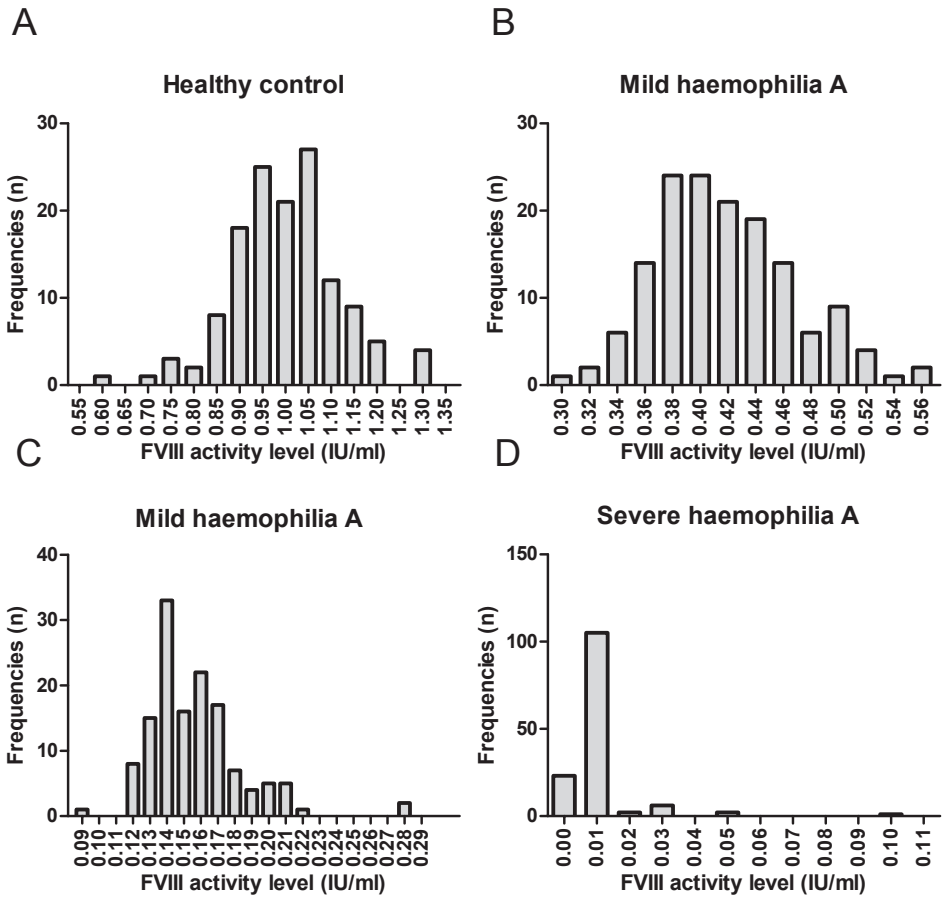


Figure 2. The distribution of the FVIII activity levels measured by OSA. FVIII levels are shown when measured with company set-ups from Siemens, Stago or Werfen.

Impact of test system on FVIII activity levels in the OSA

FVIII activity levels were analysed for the three major companies and shown in figure 3. In a sample from a healthy person (figure 3A), FVIII activity levels measured with products from Werfen (median 0.93, IQR 0.88 – 0.98 IU/ml) were lower than FVIII activity levels measured by products from Stago (median 1.07, IQR 1.02 – 1.14 IU/ml) or Siemens (median 1.03, IQR 0.97 – 1.07 IU/ml). We also observed this trend in a sample with 0.42 IU/ml FVIII (figure 3b). The differences between the three manufacturers in the samples

with lower FVIII activity levels were minimal, however, small differences may have a large clinical impact.

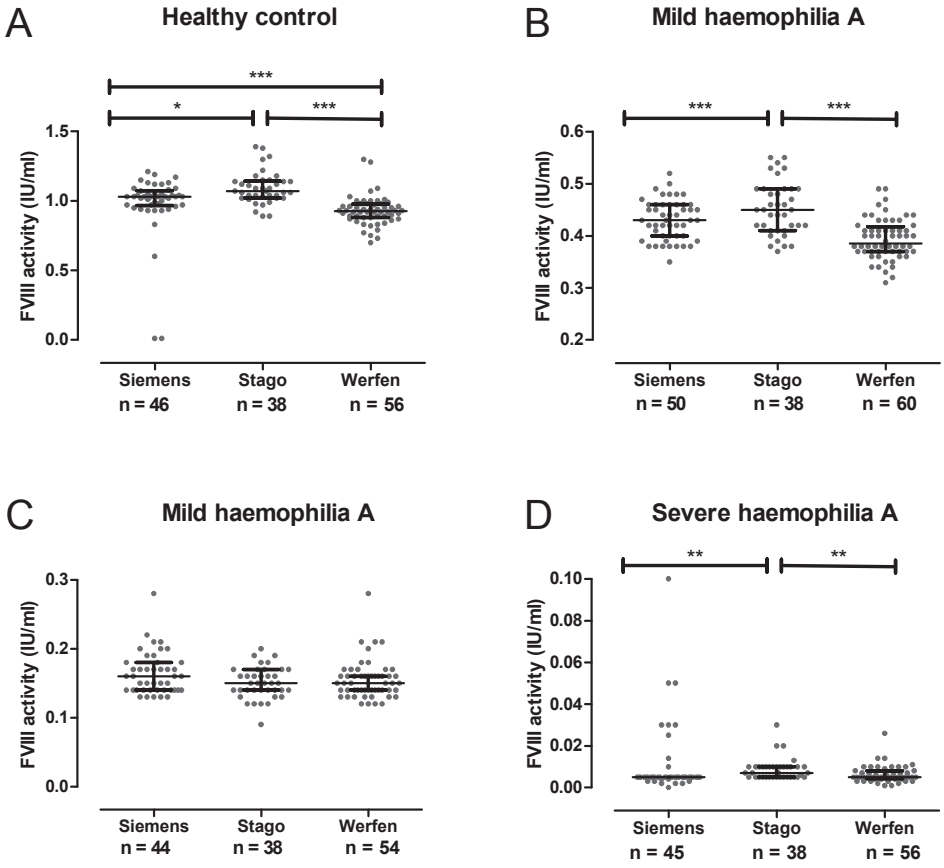


Figure 3. Combination of deficient plasma, equipment, calibrator and activator from Werfen causes lower FVIII activity levels when FVIII >0.40 IU/ml compared to Stago and Siemens. The red dots are the results from each laboratory. The black line represents the median. The error bars represent the interquartile range. Statistical significance is indicated as *p <0.05, **p < 0.01, *** p<0.001.

We also investigated the influence of different activators in the set-up of all products from Siemens. This company had an activator based on ellagic acid and one based on silica. In addition, phospholipid concentrations differ between these activators. We were able to compare these activators since enough participants in the ECAT survey used these activators. We observed equal FVIII activity values between the activators in all four plasma samples (supplementary figure 1).

Impact of test system on FVIII activity levels in the CSA

For the CSA, three kits were most oftenly used: 1) Chromogenix Coamatic (n = 8 – 13), 2) Hyphen Biomed (n = 14 – 23) and 3) FVIII Chromogenic assay from Siemens (n = 7 – 10). We compared the FVIII activity levels obtained by the three most commonly used kits and observed no consistent differences in FVIII activity levels between the kits (figure 4). Some small differences were found as the kit from Siemens had higher FVIII activity levels in the normal sample (median 1.02, IQR 0.98 – 1.09 IU/ml) compared to the kit from Hyphen Biomed (median 0.94, IQR 0.88 -0.98 IU/ml).

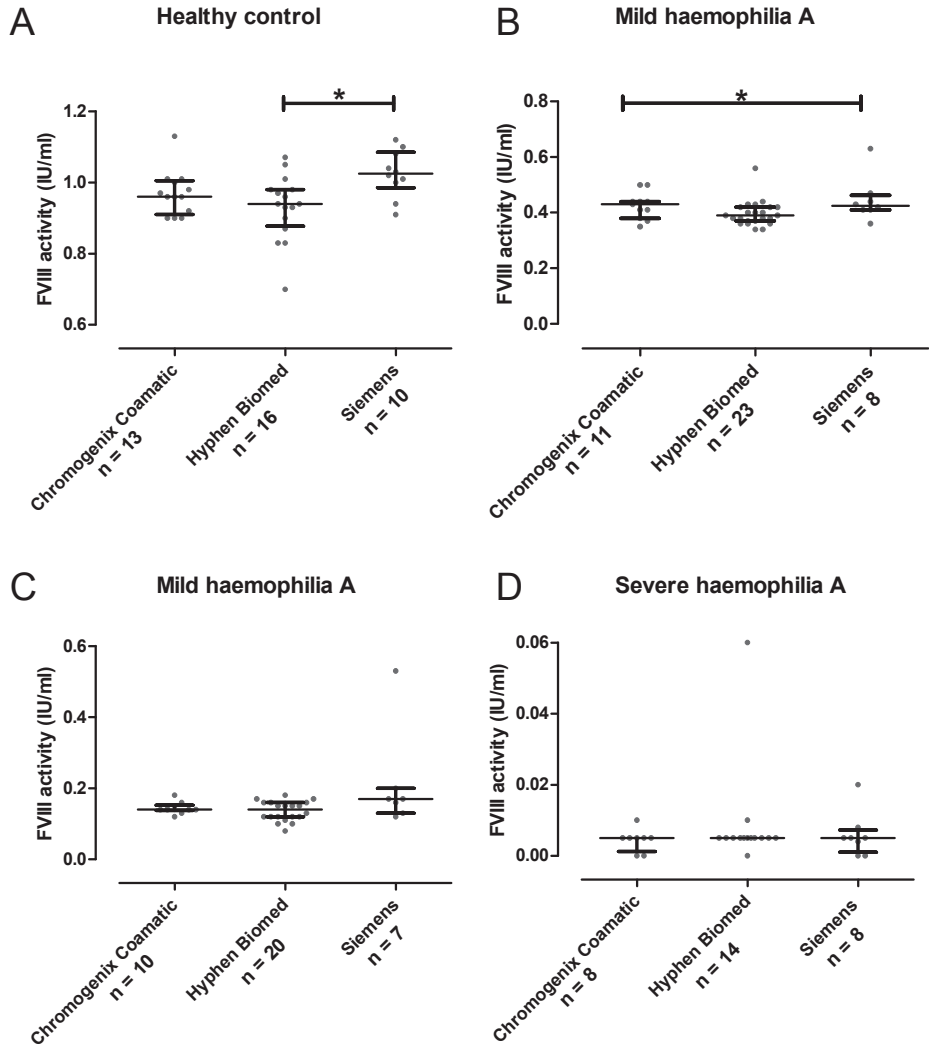


Figure 4. No consistent differences in FVIII activity levels between mostly wide used chromogenic assays. The red dots are the results from each laboratory. The black line represents the median. The error bars represent the interquartile range. Statistical significance is indicated as *p <0.05, **p < 0.01, *** p<0.001.

Effect of deficient plasma on FVIII activity

A possible explanation for the variation in the OSA may be variation in the behaviour of the deficient plasma. Deficient plasma was therefore also exchanged between company set-ups. We observed that using deficient plasma from another company did not influence FVIII activity levels in samples of a moderate haemophilia A patient or in samples containing FVIII activity levels around 0.40 IU/ml FVIII (figure 5). However, in a sample from a healthy person, Stago deficient plasma causes slightly lower FVIII results. For example the FVIII activity level in a Siemens set-up using Stago deficient plasma results

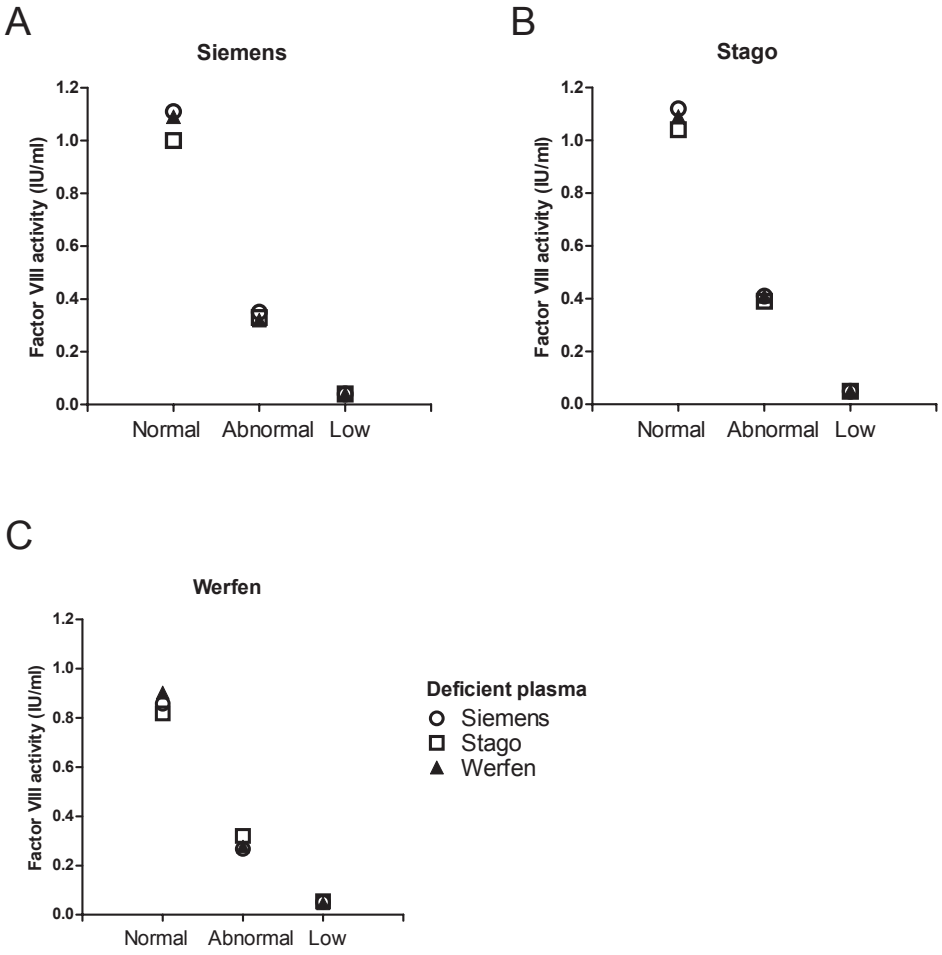


Figure 5. Exchange of deficient plasma into a system set-up with equipment of another company does not change the FVIII activity levels. Deficient plasma was exchanged and used in the OSA set-up of another company. Samples measured with Werfen equipment had lower FVIII activity levels compared to samples measured with Siemens or Stago. Triangles represent FVIII activity levels measured with a deficient plasma from Werfen. Squares represent FVIII activity levels measured with a deficient plasma from Stago. Circles represent FVIII activity levels measured with a deficient plasma from Siemens.

in a FVIII level of 1.00 IU/ml, while Siemens deficient plasma resulted in 1.11 IU/ml and Werfen in 1.09 IU/ml FVIII. More importantly, results obtained with Werfen equipment, were in general lower compared to FVIII results acquired from Stago and Siemens equipment. The average FVIII activity of the normal sample measured with Werfen equipment was 0.86 IU/ml while this was 1.08 IU/ml for Stago and 1.07 IU/ml for Siemens. This experiment shows that not only FVIII deficient plasma but other causes may have an effect on the variation in FVIII measurement.

Differences in calibrator

The influence of the calibrator was determined by measuring the FVIII activity in each calibrator and comparing the measured FVIII activity value to the assigned value from the manufacturer, based on the WHO international standard. The FVIII levels in both the STA-Unicalibrator and the HemosIL calibrator plasmas were measured in duplicates on the Siemens set-up as described in table 1. The assigned calibration value was 1.10 IU/ml and 0.98 IU/ml for the STA-Unicalibrator and the HemosIL, respectively, while the measured FVIII activity levels of these calibrators were 1.21 IU/ml and 1.12 IU/ml. As these values differed from the assigned value, it may be that the calibrator is one of the causes that results in the variation in FVIII activity measurements.

DISCUSSION

The aim of this study was to quantify and understand in more detail the variation in FVIII activity measurements when testing by OSA and CSA in surveys conducted by the ECAT external quality control. We showed that the CV in FVIII measurements has an inverse relationship with FVIII activity levels. In addition, measurements performed with OSA from the Werfen package showed lower FVIII activity levels compared to measurements with the Stago and Siemens package. The explanation may be due to differences in assigned values to the calibrator.

The results of this study showed that the variation between laboratories is higher when FVIII activity levels are lower, both in the OSA and CSA. These results are consistent with the results by Verbruggen et al. in 2008, who also showed a J-shaped relationship between FVIII activity levels and CV, for FVIII results predominantly from the OSA [12]. In their study, the CV increased strongly below 0.20 IU/ml with a maximal CV between 30% and 40%. Our study demonstrated much higher CVs with a maximum of 121%. This may be due to the fact that Verbruggen et al. showed the CVs for samples with FVIII activity levels between 0.10-0.20 IU/ml and not lower. Furthermore, it may be that that haemophilia treatment centres may be more accurate in general and may more often perform both

OSA and CSA. A subanalysis was performed comparing the variability of the two assays with the data from centres carrying out both assays and no difference in CV was observed (supplemental figure 2). The CV increases substantially in samples with low FVIII activity levels (figure 1), although absolute differences in FVIII activity levels remain small. Therefore, it is important to realise, that although these differences are small, they have significant clinical consequences as early initiation of prophylactic treatment is largely dependent on test results and subsequent classification of haemophilia severity.

FVIII activity measurements were slightly lower when measured with products from Werfen, but statistically significant. It was impossible in the ECAT surveys to evaluate the cause of this lower FVIII activity by evaluating each component of the OSA separately, as laboratories often utilise calibrator, activator, deficient plasma and equipment from one manufacturer. We attempted to specify the cause of this variation in FVIII measurements by evaluating deficient plasmas from different companies (figure 5) in separate experiments. No consistent differences were observed when exchanging deficient plasma, e.g. deficient plasma from Stago in a Siemens set-up. Despite the fact that small differences were found, results should be interpreted with caution. In general, a small amount of factor concentrate may still be present in plasma samples derived from severe haemophilia A patients due to prior treatment and an insufficient wash out period, thus influencing FVIII activity levels. In addition, the metrological traceability is only based on a consensus model and no golden standard is available for FVIII measurements. This again raises the question how to perform haemophilia classification based on the measured FVIII levels as it is still unclear which FVIII activity assay is most optimal.

Another cause for the variation in OSA FVIII measurements may be the calibrator. As we found a higher FVIII activity value of the Werfen calibrator in the Siemens set-up, 1.21 IU/ml instead of the assigned 0.98 IU/ml, this may lead to an underestimation of FVIII levels in the Werfen package, explaining the lower FVIII activity results that we have observed. However, as previously mentioned, we do not know the true values. It is important to realise that despite the fact that companies calibrate their reference material against plasma FVIII international standards, differences may still be present in FVIII values between the various test systems.

Several other hypothetical explanations exist which may explain variation in both assays. Firstly, of course, preanalytical variables may influence the measurements [18,19]. However in the ECAT surveys, these preanalytical variables are not applicable as all laboratories receive the same lyophilized plasma sample. Nevertheless, differences in dissolving lyophilized plasma may also be considered a preanalytical variable. Secondly, variation in characteristics of different batches of reagents, deficient plasmas and calibrators

may also cause differences in FVIII activity levels. In the ECAT surveys, many different lot numbers were used by the different laboratories, and therefore we do not expect that typical properties of a single lot will be able to influence the results from the ECAT surveys. Finally, previous studies have shown that some activators (STA Cephascreen (Stago) and Actin FS (Siemens)) are not optimal in diagnosing severe haemophilia A patients which may also have influenced the FVIII activity levels found in this study [12].

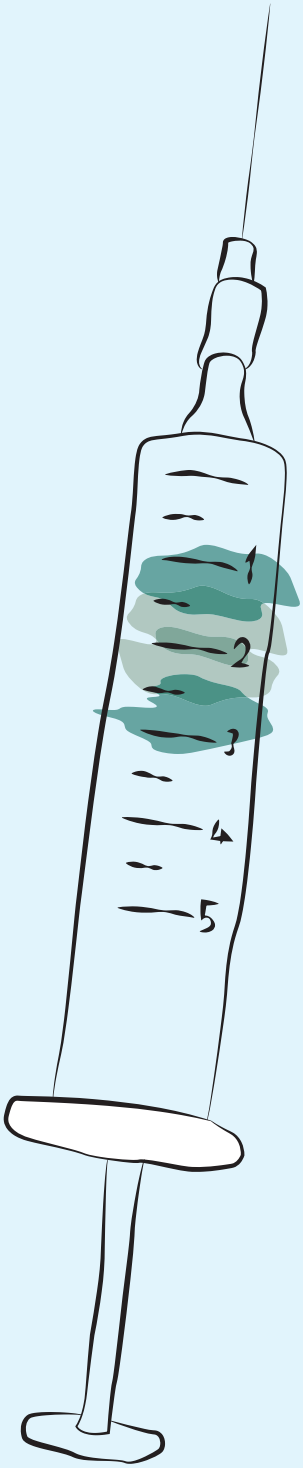
High between-laboratory CVs may influence diagnoses of haemophilia A patients between hospitals as reported previously [4]. Already, small absolute differences in FVIII activity may result in misclassification and suboptimal treatment. This emphasizes the importance of the following three aspects in haemophilia management 1) performance of other relevant tests such as DNA mutation analysis aid in classification as well as repeated testing, taking lowest levels as basis for treatment; 2) adjustment of treatment is obligatory when test results do not correspond with clinical symptoms; and 3) treatment of haemophilia patients in certified and specialized centres in which (paediatric) haematologists specialized in rare bleeding disorders and the diagnostic criteria and clinical presentation of these disorders is of utmost importance. Laboratories should also be aware that incorrect patient diagnosis is still possible despite excellent analytical performance in quality control surveys. In addition, to reduce the large between laboratory CV both in the OSA and CSA, standardization is required for example by an external quality control as the ECAT foundation. Current developments in method harmonization may also reduce the large between-laboratory variability.

In conclusion, FVIII activity levels are negatively associated with CV for both the OSA and CSA. The variation in the OSA may be attributed to the different components used in current FVIII assays. As no golden standard is available for FVIII measurements, it is not possible to judge which result is superior. Future studies focusing on standardization of FVIII measurements and in depth education on available tests are required to further improve haemophilia diagnosis and patient management.

REFERENCES

1. Srivastava A, Brewer AK, Mauser-Bunschoten EP*, et al.* Guidelines for the management of hemophilia. *Haemophilia* 2013; 19**:** e1-47.
2. Leebeek FWG, Mauser-Bunschoten EP. *Richtlijn diagnostiek en behandeling van hemofilie en aanverwante hemostase stoornissen.* . Utrecht: Van Zuiden Communications BV, 2009.
3. Fijnvandraat K, Cnossen MH, Leebeek FW, Peters M. Diagnosis and management of haemophilia. *Bmj* 2012; 344**:** e2707.
4. van Moort I, Joosten M, de Maat MP, Leebeek FW, Cnossen MH. Pitfalls in the diagnosis of hemophilia severity: What to do? *Pediatr Blood Cancer* 2017; 64.
5. Armstrong E, Astermark J, Baghaei F*, et al.* Nordic Hemophilia Guidelines. Nordic Hemophilia Council guideline working group, 2015: 1-93.
6. Over J. Methodology of the one-stage assay of Factor VIII (VIII:C). *Scand J of Haematol Suppl* 1984**:** 13-24.
7. Rosén S FP, Andersson M, Vinazzer H. A new chromogenic assay for determination of human factor VIII:C activity. *Triplett DA, ed Advances in Coagulation Testing Skokie, IL: College of American Pathologists*, 1986: 255-260.
8. Peyvandi F, Oldenburg J, Friedman KD. A critical appraisal of one-stage and chromogenic assays of factor VIII activity. *J Thromb Haemost* 2016; 14**:** 248-261.
9. Pavlova A, Delev D, Pezeshkpoor B, Muller J, Oldenburg J. Haemophilia A mutations in patients with non-severe phenotype associated with a discrepancy between one-stage and chromogenic factor VIII activity assays. *Thromb Haemost* 2014; 111**:** 851-861.
10. Van Moort I, Cnossen MH, De Maat MPM. Measurement of factor VIII for the diagnosis of hemophilia A. *Special Issue ECAT foundation* 2015; 5**:** 19-21.
11. Kitchen S, Signer-Romero K, Key NS. Current laboratory practices in the diagnosis and management of haemophilia: a global assessment. *Haemophilia* 2015; 21**:** 550-557.
12. Verbruggen B, Meijer P, Novakova I, Van Heerde W. Diagnosis of factor VIII deficiency. *Haemophilia* 2008; 14 Suppl 3**:** 76-82.
13. Mackie I, Cooper P, Lawrie A*, et al.* Guidelines on the laboratory aspects of assays used in haemostasis and thrombosis. *Int J Lab Hematol* 2013; 35**:** 1-13.
14. Kitchen SM, A; Echenagucia, M; . Diagnosis of Hemophilia and other Bleeding Disorders. A Laboratory Manual, 2nd edition. Montreal: World Federation of Hemophilia, 2010.
15. Kluft C, van Leuven CJ. Consequences for the APTT due to direct action of factor XIa on factor X, resulting in bypassing factors VIII-IX. *Thromb Res* 2015; 135**:** 198-204.
16. Lawrie AS, Kitchen S, Efthymiou M, Mackie IJ, Machin SJ. Determination of APTT factor sensitivity--the misguiding guideline. *Int J Lab Hematol* 2013; 35**:** 652-657.
17. Toulon P, Eloit Y, Smahi M*, et al.* In vitro sensitivity of different activated partial thromboplastin time reagents to mild clotting factor deficiencies. *Int J Lab Hematol* 2016; 38**:** 389-396.
18. Zhao Y, Lv G. Influence of temperature and storage duration on measurement of activated partial thromboplastin time, D-dimers, fibrinogen, prothrombin time and thrombin time, in citrate-anticoagulated whole blood specimens. *Int J Lab Hematol* 2013; 35**:** 566-570.

19. Favaloro EJ, Meijer P, Jennings I, *et al.* Problems and solutions in laboratory testing for hemophilia. *Semin Thromb Hemost* 2013; 39**:** 816-833.



4

Setting the stage for individualized therapy in hemophilia: What role can pharmacokinetics play?

Hazendonk HCAM, **van Moort I**, Mathôt RAA, Fijnvandraat K, Leebeek FWG, Collins PW, Cnossen MH for the OPTI-CLOT study group.

Blood Rev. 2018 Jul;32(4):265-271.

ABSTRACT

Replacement therapy with clotting factor concentrates (CFC) is the mainstay of treatment in hemophilia. Its widespread application has led to a dramatic decrease in morbidity and mortality in patients, with concomitant improvement of quality of life. However, dosing is challenging and costs are high. This review discusses benefits and limitations of pharmacokinetic (PK)-guided dosing of replacement therapy as an alternative for current dosing regimens. Dosing of CFC is now primarily based on body weight and based on its in vivo recovery (IVR). Benefits of PK-guided dosing include individualization of treatment with better targeting, more flexible blood sampling, increased insight into association of coagulation factor levels and bleeding, and potential overall lowering of overall costs. Limitations include a slight burden for the patient, and availability of closely collaborating, experienced clinical pharmacologists.

HEMOPHILIA AND CURRENT TREATMENT

Background of the disease

Hemophilia A and B are X-linked inherited bleeding disorders characterized by deficiencies of factor VIII (FVIII) and factor IX (FIX), respectively. Prevalence is estimated at 1 in 5,000 male births for hemophilia A and 1 in 30,000 male births for hemophilia B.[1,2] FVIII and FIX enhance formation of thrombin and consequently stabilize the hemostatic clot by increased fibrin formation. Disease severity is classified according to residual FVIII or FIX coagulation activity in plasma.[3] Mild hemophilia patients have FVIII or FIX levels of 0.05-0.40 IU mL^{-1}, moderate patients FVIII or FIX levels of 0.01-0.05 IU mL^{-1} and severe patients FVIII or FIX levels of less than 0.01 IU mL^{-1}. Mild hemophilia is characterized by an increased risk of bleeding after trauma or surgery. Moreover, severe as well as moderate hemophilia patients suffer from spontaneous bleeding or bleeding after minimal trauma in muscles and/or joints, potentially resulting in disabling arthropathy.[4] Strikingly, bleeding phenotype differs between hemophilia patients with identical baseline FVIII or FIX levels and is probably influenced by inter-individual variation in patient characteristics such as age, body weight, modifying factors within the hemostatic system, behavioral factors and daily (sporting) activities and other yet unidentified factors.[5-10] In addition, it may be influenced by inter-individual variation of half-life of clotting factor concentrates (CFC) administered either prophylactically or on demand (Table 1).

Table 1. Factors influencing bleeding phenotype in hemophilia patients

Patient characteristics	Hemostatic factors	Pharmacokinetics of treatment
Age*	FVIII and FIX plasma levels	Clearance (Cl)
Body weight	FVIII and FIX gene mutation	Volume of distribution (Vd)
Other morphometric variables	Blood group*	Half-life (T1/2)
Joint status	von Willebrand factor*	In vivo recovery (IVR)
General (muscle) condition	Thrombin generation and fibrinolysis	
Daily (sporting) activities	Unidentified hemostatic factors	
Behavioral factors		
Adherence to treatment		
Miscellaneous		

*influencing factor for hemophilia A patients only.

Current treatment with replacement therapy

Replacement therapy with CFC can be given to prevent spontaneous or repetitive bleeding (prophylaxis), or "on demand" to treat acute bleeding and prevent bleeding at the time of dental or surgical procedures. Current CFCs are either of recombinant or plasma-derived origin. Prophylaxis is the mainstay of treatment in hemophilia. Its introduction has dramatically changed the lives of many hemophilia patients. Consequently, hemophilia has evolved from a crippling disease with a shortened life expectancy into a disease with a normal life expectancy, significantly less joint arthropathy and acceptable quality of life.[11,12]

Prophylaxis

Prophylaxis was introduced in 1965 by Ahlberg and is based on the observation that moderate hemophilia patients with FVIII or FIX levels above 0.01 IU mL^{-1} have far fewer joint bleeds and less subsequent arthropathy.[13] Therefore, it was reasoned that joint bleedings could be prevented in severe hemophilia by keeping FVIII and FIX levels above 0.01 IU mL^{-1}. To achieve this, CFCs must be regularly infused generally two to four times a week in hemophilia A and one to three times a week in hemophilia B.[14-17] Prophylactic treatment profoundly reduces frequency of bleeding and improves joint status as demonstrated by Manco Johnson et al. in a randomized controlled trial.[11] Various guidelines for prophylaxis are available of which Table 2 shows a selection of those most often applied. The efficacy of prophylaxis in preventing joint bleedings is largely dependent on maintaining minimal FVIII and FIX trough levels of 0.01 IU mL^{-1} in the patient. Moreover, time spent below trough levels is associated with number of bleeding events.[18] However, in standard clinical practice, trough levels are rarely measured and dose and

Table 2. Prophylactic dosing regimens for hemophilia A and B.

Prophylaxis	Hemophilia A		Hemophilia B	
	Dose (IU kg^{-1})	**Frequency dosing** (n/ week)	**Dose** (IU kg^{-1})	**Frequency dosing** (n/ week)
***Utrecht protocol**-Dutch* (Low dose prophylactic regimen) [14]	15-30	three	15-30	two
***Malmö protocol** – Nordic* (High dose prophylactic regimen) [17]	25-40	three	25-40	two
UKHCDO [16]	25-50	four	Not provided*	
WFH [15]	According to Utrecht or Malmö protocol		According to Utrecht or Malmö protocol	

*Recommendations for patients with hemophilia B are not provided given the paucity of published evidence.

frequency of prophylactic infusions are only adjusted when spontaneous or frequent bleeding occurs.

On demand treatment

When patients are treated "on demand" either for acute bleeding or in a dental and/or surgical setting, dosing of CFC is aimed to achieve FVIII and FIX levels above a certain threshold/ trough and below a certain maximum to avoid waste of CFC and high costs without clinical effect according to various guidelines (Table 3).

More specifically, when acute bleeding occurs FVIII and FIX peak levels are generally considered particularly important, although they are rarely monitored. Targeted peak levels are dependent on both severity and location of bleeding. In Dutch guidelines[14], FVIII or FIX peak levels of 0.30 IU mL^{-1} for minor bleeds, 0.50 IU mL^{-1} for severe bleeds and 1.00 IU mL^{-1} for life threatening bleeds are targeted. In severe or life threatening bleeds, it is more important to take trough levels into account. These FVIII and FIX levels are sometimes monitored but often merely estimated, and maintained based on the opinions of the treating physician. In the perioperative setting, mainly trough levels are considered important. Although, at initiation of surgery a specific peak FVIII and FIX range is targeted according to all guidelines. Overall, targeted perioperative FVIII and FIX trough levels depend on the invasiveness of the dental and/or surgical procedure and postoperative day, with e.g. Dutch guidelines prescribing FVIII or FIX trough levels of 0.80-1.00 IU mL^{-1} during the first 24 hours after surgery; 0.50-0.80 IU mL^{-1} 1 to 5 days (24-120 hours) after surgery; and 0.30-0.50 IU mL^{-1} >5 days after surgery (Table 3).

Peak FVIII and FIX levels are estimated based on average in vivo recovery (IVR) of FVIII or FIX concentrates and amounts of CFC (IU) infused per kilogram body weight. This IVR-based dosing originates from studies that show that each infused unit of CFC per kilogram results in a mean increase of 0.02 IU mL^{-1} for FVIII and 0.01 IU mL^{-1} for FIX.[9,19] Application of this formula only provides a rough estimate of the maximum plasma concentration of FVIII and FIX after infusion. More explicitly, it does not take the pharmacokinetics (PK) of administered CFC of the individual patient into account, e.g., clearance, volume of distribution, and half-life (Figure 1). Application of these PK parameters results in a more precise estimate of peak FVIII and FIX but also enables calculation of FVIII or FIX levels and the formulation of recommendations on frequency and timing of dosing of FVIII and FIX concentrates.

When describing PK of the various CFC in hemophilia, differences are apparent between products. In both recombinant and plasma-derived FVIII concentrates, average half-life is estimated at 10.4 hours [95% CI 7.5-16.5] in adults and 9.4 hours [95%CI 7.4-13.1] in

Table 3. Target ranges with peak and trough FVIII and FIX levels and duration of administration according to a selection of available guidelines.

		Hemophilia A		Hemophilia B	
		Predefined target ranges (IU mL^{-1})	**Duration** (days)	**Predefined target ranges** (IU mL^{-1})	**Duration** (days)
Dutch [14]					
	Major surgery				
	Preoperative	*0.80-1.00*		0.80-1.00	
	Postoperative	0.80-1.00	1	0.80-1.00	1
		0.50-0.80	2-5	0.50-0.80	2-5
		0.30-0.50	>6	0.30-0.50	>6
	Minor surgery				
	Preoperative	*0.80-1.00*		0.80-1.00	
	Postoperative	>0.50	Depending on procedure	>0.50	Depending on procedure
Nordic [17]					
	Major surgery				
	Preoperative	*0.70-1.00*		*0.70-1.00*	
	Postoperative	0.60-0.80	1-3	0.60-0.80	1-3
		0.40-0.60	4-6	0.40-0.60	4-6
		0.30-0.40	7-9	0.30-0.40	7-9
	Minor surgery				
	Preoperative	*>0.50*		*>0.50*	
	Postoperative		1-5 depending on procedure		1-5 depending on procedure
WFH [15]		*No significant resource constraints*			
	Major surgery				
	Preoperative	*0.80-1.00*		*0.60-0.80*	
		0.60-0.80	1 - 3	0.40-0.60	1 - 3
	Postoperative				
		0.40-0.60	4 - 6	0.30-0.50	4 - 6
		0.30-0.50	7 - 14	0.20-0.40	7 -14
	Minor surgery				
	Preoperative	*0.50-0.80*		*0.50-0.80*	
	Postoperative	0.30-0.80	1 - 5	0.30-0.80	1 - 5
		Significant resource constraints			
	Major surgery				
	Preoperative	*0.60-0.80*		*0.50-0.70*	
	Postoperative	0.30-0.40	1 - 3	0.30-0.40	1 - 3
		0.20-0.30	4 - 6	0.20-0.30	4 - 6
		0.10-0.20	7 - 14	0.10-0.20	7 -14
	Minor surgery				
	Preoperative	*0.40-0.80*		*0.40-0.80*	
	Postoperative	0.20-0.50	1 - 5	0.20-0.50	1 - 5

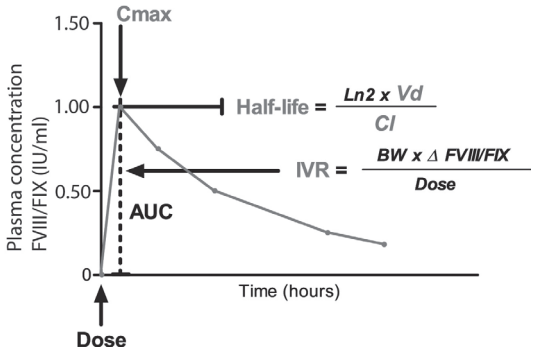


Figure 1. Description of pharmacokinetic (PK) parameters using non-compartimental principles. After bolus infusion of clotting factor concentrate, FVIII or FIX plasma levels increase until the maximum concentration is reached (**Cmax**). The in vivo recovery (**IVR**) is calculated by body weight (**BW**) (kilograms) x observed increase in FVIII/FIX plasma levels divided by the dose. **Half-life** is derived from the clearance (**Cl**) and volume of distribution (**Vd**) and is defined as the time required for the concentration to halve. Finally, area under de curve (**AUC**) is the integral of the concentration-time curve.

children.[20] This lower half-life in children can be explained by a higher clearance of FVIII in childhood probably due to the fact that VWF levels increase with age.[21] Contrastingly, no relationship between age and terminal half-life is observed for FIX concentrates.[22] Differences in PK of current FVIII and FIX concentrates are also significant. FVIII clearance is lower than FIX clearance (2.4-3.4 mL h^{-1} kg^{-1} versus 3.8-8.4 mL h^{-1} kg^{-1}) [23], due to the binding of FVIII to its carrier protein VWF which protects FVIII from proteolytic degradation.[24,25]

Although, FVIII has a lower clearance in comparison to FIX, FIX has a much larger volume of distribution (Vd). This larger volume of distribution of FIX is due to FIX binding to the vascular endothelium and diffusion into interstitial fluid on account of its lower molecular weight when compared to FVIII (FIX: 57 kDa ; FVIII: 280 kDa).[26,27] This results in a longer half-life for FIX compared to FVIII (18-34 hours and 11-16 hours, respectively) as half-life is calculated roughly by t1/2 = 0.693*Volume of distribution (Vd)/ clearance (CL).[23]

Limitations of current treatment guidelines

Underlying the presently used dosing calculations is the assumption that all patients demonstrate similar PK of administered CFCs. However unfortunately, this is not the case. Bjorkman et al. were the first to report the significant inter-individual variations in PK after the administration of a standard bolus of FVIII or FIX concentrate in a large population. Significant differences were observed with regard to in vivo recovery (IVR), clearance and half-life with FVIII half-life varying from 6-25 hours and FIX half-life from 25-56 hours between individuals.[8,20,28,29] Collins et al. showed that the efficacy of prophylactic treatment is based on time spent above certain FVIII trough levels [30] and that

therefore half-life and frequency of CFC dosing are more important than IVR of CFCs. Despite these findings, current treatment guidelines for replacement therapy are still based on IVR-based dosing regimens, which do not take the inter-individual variation of pharmacokinetics of CFCs into account.

Furthermore, as is observed in the general population, obesity also increasingly occurs in hemophilia patients.[31] This will result in in higher FVIII and FIX consumption if prophylactic and on demand treatment is persistently based on body weight and IVR-based dosing regimens. Importantly, increasing body weight is not linearly associated with increasing volume of distribution as assumed by IVR-based dosing regimens.[32] Therefore, these higher costs of treatment may not be necessary to safeguard hemostasis. Obviously, current global constraints of health care budgets, obligates hemophilia communities worldwide to generate dosing algorithms in hemophilia with optimal results for patients and minimal costs for society.

Moreover in the perioperative setting, we recently demonstrated that current dosing leads to significantly lower and higher FVIII and FIX levels than targeted in hemophilia A and B [33], (Hazendonk et al. in preparation). In moderate and severe hemophilia A patients, a large proportion of trough and steady state FVIII levels were found to be below or above predefined target ranges. Specifically, 45% of FVIII measurements were below the FVIII target range within first 24 hours after surgery and 75% above the target range during hospitalization more than six days after surgery.[33] Potentially, more optimal maintenance of perioperative target ranges could result in a reduction of 44% of CFC consumption, when ignoring logistical aspects of care.[33] In a recent retrospective study on perioperative management in moderate and severe hemophilia B patients, 60% of FIX measurements were below target and 59% FIX levels above target during hospitalization more than six days after surgery (Hazendonk et al. in preparation). Although the terminology of under- and overdosing suggests putting the patient at risk which is not the case as perioperative complications were minimal, these data do underline the limitations of current dosing algorithms primarily based on body weight using IVR-based dosing, as well as potential cost-effectiveness of alternative algorithms.

PHARMACOKINETIC (PK)-GUIDED DOSING IN HEMOPHILIA

Principles of PK-guided dosing

To address inter-individual differences in PK of CFC and to employ more effective dosing, PK-guided dosing is a potential strategy. PK-guided dose-calculations are based on individual PK parameters in relationship to the population PK model and obtained

by Bayesian analysis using statistical software (NONMEM®). In a population PK model for CFC, relationships between dose and achieved FVIII or FIX levels are described by PK parameters of all individuals in the population under review. This makes it possible to describe both inter-individual and intra-individual variability within this population dataset. In general, an important condition for implementing PK-guided dosing, is that intra-individual variability is smaller than inter-individual variability. Identified covariates explaining variability can be used to further improve constructed models, while unknown factors are labeled as residual errors. The principal strength of PK-guided dosing is that a population PK model not only represents identified covariates influencing PK parameters, but also takes the unknown modifiers of PK into account as they are described by the population data included in the model.

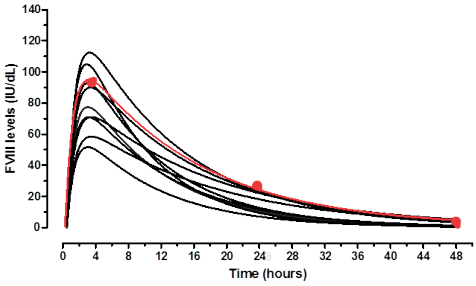
Importantly, Bayesian adaptive dosing is only possible when population PK models are representative of the individual patient and her or his specific clinical setting. Constructed models should therefore comprise a wide variation in patient-related (age, body weight, endogenous baseline FVIII/FIX, blood group) and circumstance-related factors (prophylaxis, on demand dosing during hemostatic challenges such as acute bleeding and surgery). For example, the recently published perioperative FVIII population PK model showed a significantly larger peripheral volume of distribution in comparison to the prophylactic PK model by Bjorkman et al. (1180 ml/68 kg versus 240 ml/68 kg).[8,34] Further, to optimize current population models it is important to include often underrepresented patient populations, such as children and overweight/obese patients since PK parameters in these populations may differ significantly.

Construction of individual PK profiles and population PK models

Extensive work performed by Bjorkman et al. has made PK-guided prophylactic dosing with limited blood sampling in hemophilia possible.[35] Prior to the construction of these population PK models, individual PK curves were constructed through extensive blood sampling (>10 samples), with an obligatory wash-out period, leaving the patient at potential risk of bleeding. Currently, individual PK profiles for FVIII and FIX can fortunately be constructed with limited blood sampling and without a wash out period (Table 4).[35-37] Different PK sampling models exist for rFIX and pdFIX, as it was already previously shown, that the PK of these two products differ.[9] Using Bayesian analysis and a representative population PK model, individual PK estimates can be iteratively updated, providing prophylactic dosing advice and prediction of achieved FVIII and FIX levels.[38]

Perioperatively, several research groups have estimated preoperative loading doses of FVIII and FIX after constructing individual PK profiles.[39-43] However, until recently it was not possible to iteratively dose patients in the perioperative setting owing to the lack of

Table 4. Limited blood sampling strategies to construct individual PK curves.
The upper panel shows a graphic example of a Factor VIII (FVIII) concentrate PK profile. A FVIII concentrate bolus is administrated followed by FVIII measurements (red points). Using a population PK model, FVIII plasma levels (red line) are calculated using individual PK parameter estimates derived from Bayesian analysis. To estimate FIX PK, similar principles are applied, although FIX blood sampling occurs at different longer time points as FIX concentrate half-life is longer.



	Bolus infusion ($IUkg^{-1}$)	**FVIII or FIX measurements**
Factor VIII (FVIII) *(Björkman et al.[35])*	50	T=4, T=24, T=48 hours
Plasma derived Factor IX (FIX) *(Brekkan et al.[36])*	50	T=48, T=72 hours ór T=54, T=78 hours
Recombinant Factor IX (FIX) *(Preijers et al.[37])*	100	One sample post infusion, two samples between T=72 and T=80 hours.

population PK models for this specific setting. Construction of perioperative PK population models for both moderate and severe hemophilia A [34] and B, mild hemophilia A [44] and in the near future for von Willebrand disease [45] will eventually make this possible for several bleeding disorders.

The most important covariate in FVIII population PK models for hemophilia A patients, will most likely be von Willebrand factor (VWF) as patients with blood group O have 25% lower VWF levels. This is supported by findings that blood group O versus non-O is a significant covariate of clearance in the perioperative setting, with 26% higher clearance rates for patients with blood group O.[45] Furthermore, it was also shown by Kepa et al. that blood group was associated with FVIII half-life.[46] However, this effect of blood group O was not previously observed in a steady state prophylactic setting [47] and therefore not considered to be a covariate in available prophylactic population PK models. Most likely, this difference can be explained by an increase of VWF due to inflicted endothelial damage and its role in the acute phase reaction after surgery.[48]

Pharmacogenomics may also play an important role in the PK of coagulation factor concentrates. Many genes are known to modify the hemostatic system and the clearance

of coagulation factors. As VWF serves as carrier protein for FVIII, mutations in the binding site of VWF to FVIII can result in lower levels of FVIII, also known as von Willebrand disease type 2N. In addition, the R1205H mutation in the D3 domain of VWF, also known as VWD Vicenza, results in reduced plasma VWF levels with ultra-large VWF multimers and therefore leading to an accelerated clearance of both VWF and FVIII.[49] Although, not only mutations in the *VWF* gene influence FVIII levels as ABO blood group has also a strong relation with FVIII levels. Blood group O is associated with lower FVIII levels compared to other blood types even when adjusted for VWF antigen.[44] In addition, the CHARGE has consortium reported multiple genetic loci in clearance receptors of VWF and/or FVIII which were associated with FVIII levels, for example *STXBP5* and *SCARA5.*[50] Furthermore, polymorphisms in one of the clearance receptors of FVIII/VWF, *LRP1* gene, are also associated with both FVIII and VWF plasma levels.[51]

Benefits of PK-guided treatment

As early as 1997, Carlsson et al. showed benefits of a PK-guided dosing approach for prophylaxis.[7] This small study was designed as a randomized cross-over study comparing PK-guided dosing of prophylaxis with standard prophylactic dosing in 14 individuals during a period of two times six months. Strikingly, a reduction of CFC administration of 30% was achieved. The number of reported bleedings was similar in both treatment arms.[7] Such a reduction can have a significant financial impact, since annual costs for replacement therapy in the Netherlands amount to more than 126 million euros.[52] Before drawing conclusions, however, it is important to prospectively evaluate these outcomes of PK-guided dosing in adequately designed and powered studies.[53] Currently, a randomized controlled trial comparing PK-guided perioperative treatment of CFC in moderate and severe hemophilia A patients is in place to analyze the amount of CFC administered, time spent to achieve targeted FVIII levels, as well as staff investment and costs, all in accordance with the economic health principles established by Hakkaart-van Rooijen.[53,54] This study may result in clear conclusions regarding the cost effectiveness of PK-guided dosing.

Another benefit of PK-guided dosing is that both prophylactic and “on demand” dosing will be based on actual FVIII and FIX trough and peak levels or FVIII and FIX levels predicted by population PK models, instead of current FVIII and FIX estimates based on IVR-based dosing. Furthermore, FVIII and FIX sampling can be made flexible and not necessarily fixed at certain time points before or after infusion, once models are in place. Moreover, PK-guidance will optimize dosing as knowledge will increase with regard to the relationship between FVIII and FIX levels and bleeding in individual patients and patient groups. In addition, an increase in dosing will not only depend on actual bleeding and a reduction of dosing can be considered by the treating professional in

consultation with patients and parents. Importantly, the dose and frequency of CFC of patients on prophylaxis should only be reduced if clinically justified and impact should

Table 5. Benefits and limitations of PK-guided dosing based on population PK models as alternative for body weight and IVR-based dosing regimens

Advantages	Conditions and remarks
1. Better targeting of FVIII and FIX levels with minimal burden to patients.	Although limited blood sampling decreases frequency of blood sampling, two/three samples for individual PK profile are still obligatory. Population PK models should however be representative of population and specific setting.
2. More flexible blood sampling	Once PK-guided dosing is in place.
3. Insight into association of FVIII and FIX trough and peak levels and bleeding Future possibilities to construct both PK and pharmacodynamic (PD) models and to further understand pathophysiology of hemostasis	Further development of global hemostatic assays measuring hemostatic potential also essential.
4. Possibility to potentially lower FVIII and FIX target values, due to more reliable targeting	Intensive collaboration between experienced clinical pharmacologist and (pediatric) hematologist necessary. Realization of risks and intensive monitoring of clinical impact of lowering of FVIII and FIX levels obligatory.
5. Facilitation of actual personalization of treatment according to lifestyle	Continuous adaptation of dosing regimen according to lifestyle also necessary. Individual PK profiles must be potentially repeated every 3-4 years. Adherence must be discussed.
6. Potential reduction of overall treatment costs	Prospective studies to evaluate actual impact on costs are obligatory.
7. Further enrichment of population PK models	Intensive collaboration between experienced clinical pharmacologist and (pediatric) hematologist necessary.

be monitored with regard to bleeding events, bleeding pattern and joint status (Table 5).

Over time, more exact targeting of FVIII and FIX levels may also lead to reliably lowering of target levels of treatment. Especially in hemophilia B, studies and clinical experience suggest that lower target levels may be acceptable.[15,55] In a recent retrospective study on perioperative management in moderate and severe hemophilia B patients, 60% of FIX measurements were below target, without clinical relevant bleeding and independent of the severity of surgical procedures (Hazendonk et al. in preparation). Srivastava et

al. showed that lower trough FVIII (0.20-0.40 IU mL^{-1}) and FIX (0.15-0.30 IU mL^{-1}) levels 0-72 hours after surgery were not accompanied by bleeding complications since only one patient experienced bleeding due to a lack of surgical hemostasis.[55] Furthermore, International WFH guidelines for perioperative treatment in hemophilia A and B patients recommend FIX levels 0.20 IU mL^{-1} lower than FVIII levels (Table 3).[15] In countries with significant financial constraints, even lower FIX target ranges are suggested.[15] Interestingly, various European guidelines do not differ regarding perioperative target ranges for hemophilia A and B [14,17,56] as reported in a survey by the European Therapy Standardization Board in 2009.[57]

PK-guided dosing will also facilitate individualization of dosing according to individual lifestyle and activities, therefore achieving true personalization of treatment. When targeting weekly FVIII and FIX levels, personal activities and preferences should be taken into account, as bleeding risk is closely related to these factors.[58-60] Moreover, nonadherence should be discussed as implementation of minimal dosing schemes may lead to an increased risk of bleeding.[61-63] Patients and families should be aware of time points when factor concentrate levels are low or high and consider additional dosing when bleeding risk is significant.

All benefits of PK-guided dosing are also applicable with regard to upcoming enhanced half-life (EHL) products. Moreover, costs of treatment will directly depend on the dose and frequency of treatment and therefore on individual PK and population PK parameters. Furthermore, the ongoing discussion of the association of trough levels and the role of peak levels with regard to bleeding will be made more transparent. This is especially relevant in EHL products as higher troughs will be possible and treatment peaks will be less frequent.[64]

Limitations of PK-guided treatment

Important limitations with regard to PK-guided dosing include the requirement of close collaboration with a clinical pharmacologist with expertise in PK modeling. Furthermore, time investments by patients, parents and medical professionals may be substantial as individual PK profiles must be performed regularly (every three to four years depending on patient characteristics) and perioperative PK-guided iterative dosing requires daily dosing recommendations. Solutions to overcome these limitations are the availability of web portal-based consultancies for PK-guided dosing advice, as established by Iorio [21], for instance, and as developed by a pharmaceutical company for the prophylactic setting.[65] Both initiatives to implement a closer collaboration and to educate both professionals and patients are valuable for future patient care. Transparency and reliability

of the data used to construct underlying population models are of course of crucial importance in such settings.

FUTURE ROLE FOR PK-GUIDED DOSING OF FACTOR CONCENTRATES IN HEMOPHILIA CARE AND RESEARCH PERSPECTIVE

Replacement therapy has led to the high standard of hemophilia care in high-income countries. However, recent studies show that treatment is suboptimal; although bleeding is rare, both under- and overdosing of CFC occur. We believe that PK-guided dosing as the alternative to body weight and IVR-based dosing, will play an important role in further individualization of therapy. We have summarized the anticipated improvements in Table 5.

Future research should include studies prospectively validating constructed population PK models but also combining PK with pharmacodynamic data (e.g. bleeding events, global hemostatic test results) and simulations to determine minimal FVIII and FIX levels required for adequate hemostasis in the individual patient and in populations. These data may subsequently support studies aiming at lower target levels in specific bleeding disorders.

There are no suggestions that implementation of PK-guided dosing will lead to an increased risk of inhibitor development. It is well-known that risk factors of development are peak treatment moments in younger children, which is often the case in the perioperative period. Use of PK-guided dosing may be able to prevent extreme peaks. However, future studies will be needed to prove such a hypothesis.

CONCLUSION

We believe that PK-guided dosing deserves attention as a means of ensuring the individualization of treatment in hemophilia since benefits are significant and limitations can be overcome. The burden for patients and parents appears to be minimal. Accordingly, we call on patients, medical professionals, clinical pharmacologists, hemostatic laboratories and pharmaceutical companies to join hands in applying this approach for all CFCs, in hemophilia and other bleeding disorders requiring CFC replacement therapy.

REFERENCES

1. Mannucci PM, Tuddenham EG. The hemophilias--from royal genes to gene therapy. N Engl J Med 2001;344:1773-9.
2. Rosendaal FR, Briet E. The increasing prevalence of haemophilia. Thromb Haemost 1990;63:145.
3. White GC, 2nd, Rosendaal F, Aledort LM, et al. Definitions in hemophilia. Recommendation of the scientific subcommittee on factor VIII and factor IX of the scientific and standardization committee of the International Society on Thrombosis and Haemostasis. Thromb Haemost 2001;85:560.
4. Fischer K, Collins P, Bjorkman S, et al. Trends in bleeding patterns during prophylaxis for severe haemophilia: observations from a series of prospective clinical trials. Haemophilia 2011;17:433-8.
5. Aronstam A, McLellan DS, Wassef M, Mbatha PS. Effect of height and weight on the in vivo recovery of transfused factor VIII C. J Clin Pathol 1982;35:289-91.
6. Bjorkman S, Carlsson M, Berntorp E, Stenberg P. Pharmacokinetics of factor VIII in humans. Obtaining clinically relevant data from comparative studies. Clin Pharmacokinet 1992;22:385-95.
7. Carlsson M, Berntorp E, BjÖRkman S, Lethagen S, Ljung R. Improved cost-effectiveness by pharmacokinetic dosing of factor VIII in prophylactic treatment of haemophilia A. Haemophilia 1997;3:96-101.
8. Bjorkman S, Folkesson A, Jonsson S. Pharmacokinetics and dose requirements of factor VIII over the age range 3-74 years: a population analysis based on 50 patients with long-term prophylactic treatment for haemophilia A. Eur J Clin Pharmacol 2009;65:989-98.
9. Bjorkman S. Pharmacokinetics of plasma-derived and recombinant factor IX - implications for prophylaxis and on-demand therapy. Haemophilia 2013;19:808-13.
10. Matucci M, Messori A, Donati-Cori G, et al. Kinetic evaluation of four Factor VIII concentrates by model-independent methods. Scand J Haematol 1985;34:22-8.
11. Manco-Johnson MJ, Abshire TC, Shapiro AD, et al. Prophylaxis versus episodic treatment to prevent joint disease in boys with severe hemophilia. N Engl J Med 2007;357:535-44.
12. Peyvandi F, Garagiola I, Young G. The past and future of haemophilia: diagnosis, treatments, and its complications. Lancet 2016;388:187-97.
13. Ahlberg A. Haemophilia in Sweden. VII. Incidence, treatment and prophylaxis of arthropathy and other musculo-skeletal manifestations of haemophilia A and B. Acta Orthop Scand Suppl 1965:Suppl 77:3-132.
14. Leebeek FWG, Mauser-Bunschoten EP, Editors. Richtlijn Diagnostiek en behandeling van hemofilie en aanverwante hemostasestoornissen. Van Zuiden Communications BV 2009:1-197.
15. Srivastava A, Brewer AK, Mauser Bunschoten EP, et al. Guidelines for the management of hemophilia. Montréal, Canada: Blackwell Publishing; 2012.
16. Richards M, Williams M, Chalmers E, et al. A United Kingdom Haemophilia Centre Doctors' Organization guideline approved by the British Committee for Standards in Haematology: guideline on the use of prophylactic factor VIII concentrate in children and adults with severe haemophilia A. Br J Haematol 2010;149:498-507.

17. Nordic Hemophilia Guidelines. Nordic Hemophilia Council guideline working group, 2015. (Accessed 8 November 2016, 2017, at http://www.nordhemophilia.org/library/Files/PDF-skjol/NordicGuidelinesCongenitalHaemophilia_2017.pdf.)
18. Collins PW, Blanchette VS, Fischer K, et al. Break-through bleeding in relation to predicted factor VIII levels in patients receiving prophylactic treatment for severe hemophilia A. J Thromb Haemost 2009;7:413-20.
19. Ingram GI. Calculating the dose of factor VIII in the management of haemophilia. Br J Haematol 1981;48:351-4.
20. Collins PW, Bjorkman S, Fischer K, et al. Factor VIII requirement to maintain a target plasma level in the prophylactic treatment of severe hemophilia A: influences of variance in pharmacokinetics and treatment regimens. J Thromb Haemost 2010;8:269-75.
21. Sanders YV, Giezenaar MA, Laros-van Gorkom BA, et al. von Willebrand disease and aging: an evolving phenotype. J Thromb Haemost 2014;12:1066-75.
22. Bjorkman S, Shapiro AD, Berntorp E. Pharmacokinetics of recombinant factor IX in relation to age of the patient: implications for dosing in prophylaxis. Haemophilia 2001;7:133-9.
23. Berntorp E, Bjorkman S. The pharmacokinetics of clotting factor therapy. Haemophilia 2003;9:353-9.
24. Lenting PJ, van Mourik JA, Mertens K. The life cycle of coagulation factor VIII in view of its structure and function. Blood 1998;92:3983-96.
25. Lenting PJ, CJ VANS, Denis CV. Clearance mechanisms of von Willebrand factor and factor VIII. J Thromb Haemost 2007;5:1353-60.
26. Bjorkman S, Carlsson M, Berntorp E. Pharmacokinetics of factor IX in patients with haemophilia B. Methodological aspects and physiological interpretation. Eur J Clin Pharmacol 1994;46:325-32.
27. Heimark RL, Schwartz SM. Binding of coagulation factors IX and X to the endothelial cell surface. Biochem Biophys Res Commun 1983;111:723-31.
28. Bjorkman S, Folkesson A, Berntorp E. In vivo recovery of factor VIII and factor IX: intra- and inter-individual variance in a clinical setting. Haemophilia 2007;13:2-8.
29. BjÖRkman S, Carlsson M. The pharmacokinetics of factor VIII and factor IX: methodology, pitfalls and applications. Haemophilia 1997;3:1-8.
30. Collins PW, Fischer K, Morfini M, Blanchette VS, Bjorkman S, International Prophylaxis Study Group Pharmacokinetics Expert Working G. Implications of coagulation factor VIII and IX pharmacokinetics in the prophylactic treatment of haemophilia. Haemophilia 2011;17:2-10.
31. Hofstede FG, Fijnvandraat K, Plug I, Kamphuisen PW, Rosendaal FR, Peters M. Obesity: a new disaster for haemophilic patients? A nationwide survey. Haemophilia 2008;14:1035-8.
32. Feldschuh J, Enson Y. Prediction of the normal blood volume. Relation of blood volume to body habitus. Circulation 1977;56:605-12.
33. Hazendonk HC, Lock J, Mathot RA, et al. Perioperative treatment of hemophilia A patients: blood group O patients are at risk of bleeding complications. J Thromb Haemost 2016;14:468-78.
34. Hazendonk H, Fijnvandraat K, Lock J, et al. A population pharmacokinetic model for perioperative dosing of factor VIII in hemophilia A patients. Haematologica 2016;101:1159-69.

35. Bjorkman S. Limited blood sampling for pharmacokinetic dose tailoring of FVIII in the prophylactic treatment of haemophilia A. Haemophilia 2010;16:597-605.

36. Preijers T, Hazendonk HCAM, Fijnvandraat K, Leebeek FWG, Cnossen MH. In silico evaluation of limited blood sampling strategies for individualized recombinant factor IX prophylaxis in hemophilia B patients. Haemophilia 2016;22:100-1.

37. Brekkan A, Berntorp E, Jensen K, Nielsen EI, Jonsson S. Population pharmacokinetics of plasma-derived factor IX: procedures for dose individualization. J Thromb Haemost 2016;14:724-32.

38. Boeckmann AJ, Sheiner LB, Beal SL. NONMEM Users Guide. NONMEM Project Group 2011;University of California at San Francisco:1-165.

39. Batorova A, Martinowitz U. Intermittent injections vs. continuous infusion of factor VIII in haemophilia patients undergoing major surgery. Br J Haematol 2000;110:715-20.

40. Négrier C, Lienhart A, Meunier S. Surgeries in patients with haemophilia A performed with a continuous infusion of Recombinate. J Thromb Haemost 2001;(Suppl1): P0829.

41. Stieltjes N, Altisent C, Auerswald G, et al. Continuous infusion of B-domain deleted recombinant factor VIII (ReFacto) in patients with haemophilia A undergoing surgery: clinical experience. Haemophilia 2004;10:452-8.

42. Ragni MV. Platelet VIII pack evades immune detection. Blood 2016;127:1222-4.

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

44. Franchini M, Capra F, Targher G, Montagnana M, Lippi G. Relationship between ABO blood group and von Willebrand factor levels: from biology to clinical implications. Thromb J 2007;5:14.

45. Witmer C, Young G. Factor VIII inhibitors in hemophilia A: rationale and latest evidence. Therapeutic advances in hematology 2013;4:59-72.

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

47. Bjorkman S, Oh M, Spotts G, et al. Population pharmacokinetics of recombinant factor VIII: the relationships of pharmacokinetics to age and body weight. Blood 2012;119:612-8.

48. Kahlon A, Grabell J, Tuttle A, et al. Quantification of perioperative changes in von Willebrand factor and factor VIII during elective orthopaedic surgery in normal individuals. Haemophilia 2013;19:758-64.

49. Gezsi A, Budde U, Deak I, et al. Accelerated clearance alone explains ultra-large multimers in von Willebrand disease Vicenza. J Thromb Haemost 2010;8:1273-80.

50. Smith NL, Chen MH, Dehghan A, et al. Novel associations of multiple genetic loci with plasma levels of factor VII, factor VIII, and von Willebrand factor: The CHARGE (Cohorts for Heart and Aging Research in Genome Epidemiology) Consortium. Circulation 2010;121:1382-92.

51. Morange PE, Tregouet DA, Frere C, et al. Biological and genetic factors influencing plasma factor VIII levels in a healthy family population: results from the Stanislas cohort. Br J Haematol 2005;128:91-9.

52. Nederlandse Zorgautoriteit. Onderzoek naar de toegankelijkheid en betaalbaarheid van geneesmiddelen in de medisch specialistische zorg. https://www.nza.nl/publicaties/1048188/Onderzoeksrapport__Toegankelijkheid_en_betaalbaarheid_van_geneesmiddelen_in_de_medisch_specialistis29-06-2015:1-110.

53. Hazendonk HC, van Moort I, Fijnvandraat K, et al. The “OPTI-CLOT” trial. A randomised controlled trial on periOperative PharmacokineTIc-guided dosing of CLOTting factor concentrate in haemophilia A. Thromb Haemost 2015;114:639-44.

54. Hakkaart-van Roijen L, Tan SS, Bouwmans CAM. Handleiding voor kostenonderzoek: Methoden en standaard kostprijzen voor economische evaluaties in de gezondheidszorg. Instituut voor Medical Technology Assessment Erasmus Universiteit Rotterdam 2010:1-127.

55. Srivastava A, Chandy M, Sunderaj GD, et al. Low-dose intermittent factor replacement for postoperative haemostasis in haemophilia. Haemophilia 1998;4:799-801.

56. Australian Haemophilia Centre Directors’ Organisation. Guideline for the management of patients with haemophilia undergoing surgical procedures. 2010:1-13.

57. Hermans C, Altisent C, Batorova A, et al. Replacement therapy for invasive procedures in patients with haemophilia: literature review, European survey and recommendations. Haemophilia 2009;15:639-58.

58. Lindvall K, Astermark J, Bjorkman S, et al. Daily dosing prophylaxis for haemophilia: a randomized crossover pilot study evaluating feasibility and efficacy. Haemophilia 2012;18:855-9.

59. Valentino LA, Pipe SW, Collins PW, et al. Association of peak factor VIII levels and area under the curve with bleeding in patients with haemophilia A on every third day pharmacokinetic-guided prophylaxis. Haemophilia 2016;22:514-20.

60. Broderick CR, Herbert RD, Latimer J, et al. Association between physical activity and risk of bleeding in children with hemophilia. Jama 2012;308:1452-9.

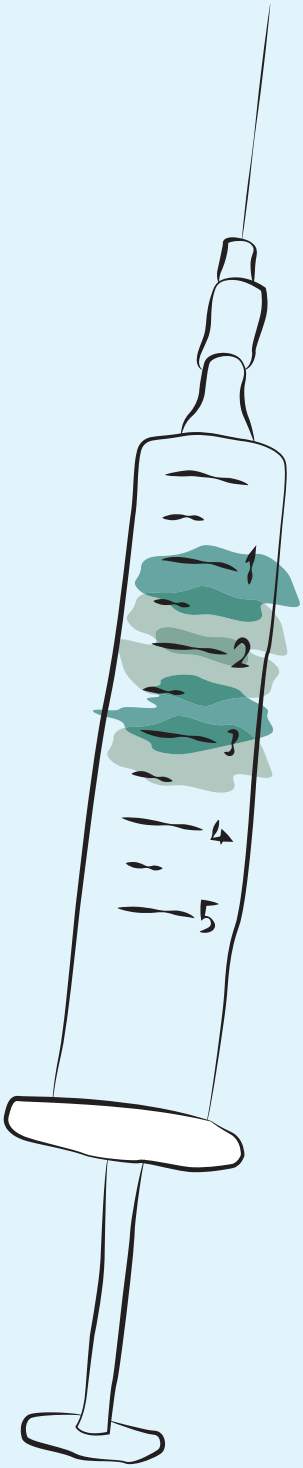
61. Fischer K, Lewandowski D, Janssen MP. Modelling lifelong effects of different prophylactic treatment strategies for severe haemophilia A. Haemophilia 2016;22:e375-82.

62. Lock J, Raat H, Duncan N, et al. Adherence to treatment in a Western European paediatric population with haemophilia: reliability and validity of the VERITAS-Pro scale. Haemophilia 2014;20:616-23.

63. Lock J, de Bekker-Grob EW, Urhan G, et al. Facilitating the implementation of pharmacokinetic-guided dosing of prophylaxis in haemophilia care by discrete choice experiment. Haemophilia 2016;22:e1-e10.

64. Collins P, Chalmers E, Chowdary P, et al. The use of enhanced half-life coagulation factor concentrates in routine clinical practice: guidance from UKHCDO. Haemophilia 2016;22:487-98.

65. myPKFiT user guide. Westlake Village, CA: Baxter Healthcare Corporation; 2014.



5

The OPTI-CLOT trial. Study design of a randomized controlled trial on perioperative pharmacokinetic-guided dosing of factor VIII concentrate in hemophilia A

Hazendonk HCAM, **van Moort I**, Fijnvandraat K, Kruip MJHA, Laros-van Gorkom BAP, van der Meer FJM, Meijer K, Peters M, Schutgens REG, Zwaan CM, Driessens MH, Polinder S, Leebeek FWG, Mathôt RAA, Cnossen MH.

Thromb Haemost. 2015 Aug 31;114(3):639-44.

SUMMARY

Background Haemophilia A is an X-linked inherited, rare bleeding disorder, caused by a deficiency of coagulation factor VIII (FVIII). Previous studies in prophylactic dosing have demonstrated that FVIII consumption can be significantly reduced by individualizing dosing based on combined analysis of individual pharmacokinetic (PK) profiling and population PK data (Bayesian analysis). So far, no studies have been performed that address perioperative concentrate consumption using iterative PK-guided dosing based on a PK population model.

Methods The "OPTI-CLOT" trial is an open-label, prospective, multicentre randomized controlled superiority trial (RCT), aiming to detect a 25% difference in perioperative FVIII concentrate consumption with iterative Bayesian PK-guided dosing in comparison to the standard dosing procedure. Sixty haemophilia A patients ≥ 12 years of age, with FVIII plasma levels ≤ 0.05 $IUml^{-1}$ will be included requiring FVIII replacement therapy administered either by continuous or bolus infusion for an elective, low or medium risk surgical procedure.

Results The proposed study aims to investigate a novel perioperative iterative PK-guided dosing strategy, based on a recently constructed perioperative PK population model. This model will potentially decrease underdosing and overdosing of clotting factor concentrate and is expected to overall reduce FVIII consumption by minimally 25%. Moreover, participating hospitals will gain experience with PK-guided dosing, facilitating future implementation of this intervention which is expected to optimize current care and reduce costs of treatment.

INTRODUCTION

Haemophilia A is an X-linked inherited bleeding disorder, caused by a deficiency of coagulation factor VIII (FVIII). Patients with severe (FVIII <0.01 $IUml^{-1}$) and moderate severe (FVIII 0.01-0.05 $IUml^{-1}$) haemophilia A experience spontaneous bleeding, mainly in muscles and joints, or bleeding after minor trauma. Prophylactic substitution of FVIII concentrate intravenously, several times a week, aiming for a trough level of above 0.01 $IUml^{-1}$, generally prevents severe joint damage and subsequent long term disability. Surgery necessitates an intensive regimen of factor replacement therapy, as factor levels are normalized for 7-14 days. Therefore, overall in the perioperative period, a patient may consume up to 15% of his regular annual use of clotting factor concentrate. Annually, overall costs of haemophilia treatment In the Netherlands are estimated at €130 million, of which more than 90% consists of costs for concentrates [1-3].

In the perioperative period, target trough levels dictated by National and International Guidelines are exceeded in the perioperative setting. This seems due to difficulties in targeting these levels but also due to the fact that doctors are inclined to dose generously in order to avoid low factor activity levels and subsequent bleeding risk [4-6].Treatment is extremely effective as perioperative bleeding is rare in haemophilia patients in countries where concentrates are adequately available. Refining of perioperative dosing strategies seems warranted to optimize replacement therapy in the individual haemophilia patient and to increase quality of care by avoiding both under dosing and overdosing.

Rationale: Pharmacokinetic-guided dosing may be cost-reductive

Large inter-individual differences in the pharmacokinetics (PK) of FVIII concentrates have been demonstrated, making PK-guided dosing a potential strategy to improve clinical outcome and possibly cost-effectiveness of dosing in prophylactic, on demand and perioperative settings [7]. Bayesian PK-guided dosing is the combined analysis of individual PK parameters in regard to the population parameters. Using this approach only three samples are needed to describe a complete PK curve [8].

Using a Bayesian approach in the prophylactic setting, Carlsson et al. [9] reported a dose reduction of 30% without an increase in bleeding in a randomized cross-over study of two six months' periods, comparing PK-guided dosing with standard dosing. However, prophylactic population PK models cannot be extrapolated to the perioperative situation, as surgery forms an uncomparable haemostatic challenge. Moreover, Longo et al. reported excessive FVIII consumption and clearance in 50% of surgical hemophilia patients due to unidentified factors [10].

Current perioperative management in haemophilia

In surgery, earlier studies on perioperative management in haemophilia have compared continuous infusion with intermittent bolus infusions. Batorova et al. [11] reviewed eleven case series and concluded that continuous infusion of FVIII and factor IX is safe and effective in situations requiring intensive replacement therapy such as surgery and major bleedings. Strikingly, there was a wide variation in targeted haemostatic levels, dosage regimens, modes and duration of therapy. Eight of these studies concluded that continuous infusion is more cost-effective than intermittent bolus administration by reduction of total clotting factor consumption [12-19]. In two studies, bolus injections were prospectively compared to adjusted continuous infusions, demonstrating 30-36% reduction of FVIII consumption in the latter [12,19]. Preoperative PK profiling was reported in 3 out of 8 haemophilia A case series, but was not specified. [13-15,17,19,20]. A perioperative study by Batorova and Martinowitz [12] showed that if targeted FVIII levels are actually maintained, a reduction of consumption and therefore costs of up to 70% may be attained. Mulcahy et al. [15] retrospectively evaluated adjusted continuous FVIII infusions in a single centre study and concluded that close monitoring of FVIII levels in itself with strict regulation of infusion rate alone, may significantly reduce FVIII consumption.

So far, iterative Bayesian PK-guided perioperative dosing has not been performed as perioperative PK population models for clotting disorders have not been available. Recently, we have however constructed a population PK model from retrospective perioperative FVIII data [21], facilitating Bayesian adaptive dosing of FVIII in the perioperative setting. We hypothesize that application of this approach may improve quality of haemophilia care as both underdosing and overdosing are avoided. Potentially, it will also decrease FVIII consumption and lower costs of treatment.

OBJECTIVE

The objective of this study is to investigate whether perioperative PK-guided dosing of FVIII concentrate in haemophilia A patients receiving FVIII replacement therapy using a Bayesian approach, leads to a significant reduction in perioperative clotting factor consumption.

METHODS

Trial design

The “OPTI-CLOT” trial is a prospective, multicentre randomized controlled superiority trial (RCT), aiming to detect a difference in perioperative FVIII consumption with iterative PK-guided dosing in comparison to the standard dosing procedure, the latter primarily

based on body weight. FVIII levels will be targeted in the middle of each target range defined in the perioperative guidelines of the National Haemophilia Consensus, unless stated otherwise by treating physician.

In all patients six weeks before surgery, a preoperative PK profile will be constructed using only three blood samples without a wash out period but with exact notation of prior doses in the week before PK profiling; individual PK parameters will be assessed by Bayesian analysis (Figure 1). Subsequently, patients will be randomized after stratification according to mode of FVIII administration (continuous or bolus), type of surgical procedure (low or medium risk) and treatment centre as is usual in a multicentre trial and subsequently allocated to one of two perioperative treatment arms: (A) the intervention arm in which dosing is adjusted on basis of the individual preoperative PK parameter estimates and iterative perioperative Bayesian analysis; or (B) the standard treatment arm in which dosing regimen is established by the physician according to

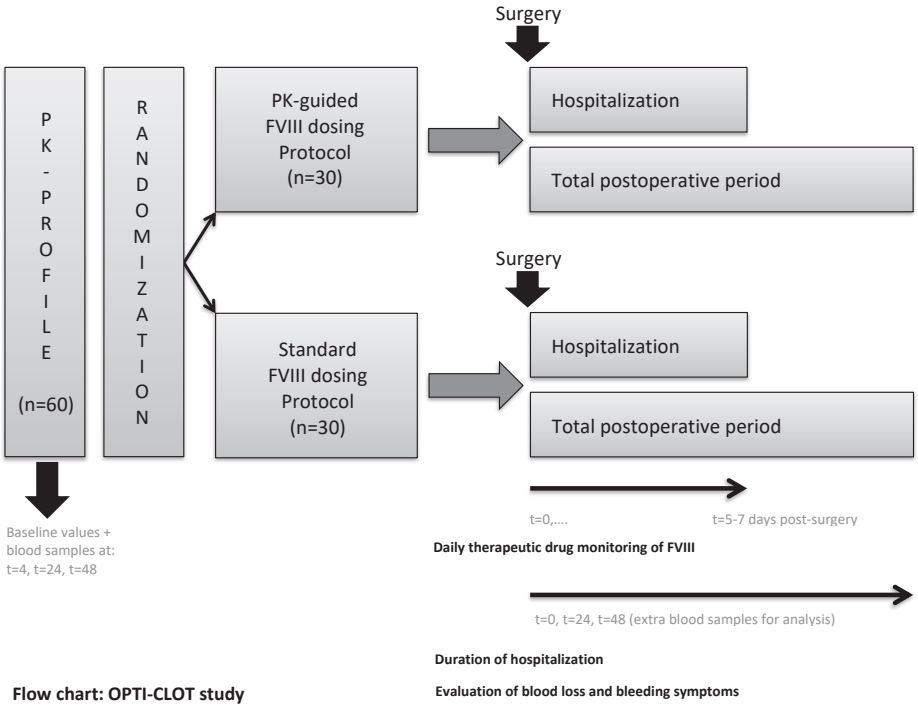


Figure 1. Flowchart. After inclusion a PK profile will be constructed preoperatively prior to stratified randomization; individual PK parameters will be assessed using Bayesian analysis. In the intervention arm dosing will be administered based on the individual PK parameter estimates. With daily FVIII levels available the bayesian approach will be used to iteratively adjust the daily dose. In the standard treatment arm dosing will be set by the treating physician according to the standard dosing regimen described in the National Haemophilia Consensus in the Netherlands based primarily on bodyweight with target plasma FVIII values as set in the Consensus, unless otherwise specified. Adjustments will be performed by the treating physician without knowledge of the preoperative profile of the patient.

the standard dosing regimen. At least 60 patients will be needed to detect a difference of minimally 25% in consumption of FVIII concentrate during the perioperative period between treatment arms. Safety is guarded by generally daily monitoring of FVIII levels, as is now standard procedure. Perioperative haemostasis will be assessed during the entire perioperative period.

Study population

Patients will be included from six large Academic Haemophilia Treatment Centres in the Netherlands: Erasmus University Medical Centre Rotterdam, Academic Medical Centre Amsterdam, Radboud university medical centre, University Medical Centre Utrecht, University Medical Centre Groningen and Leiden University Medical Centre.

Inclusion criteria:

- Severe and moderate haemophilia A patients with FVIII levels ≤0.05 $IUml^{-1}$;
- ≥ 12 years of age at inclusion date;
- Undergoing elective, low or medium risk surgery as defined by surgical risk score (Koshy et al.[22]);
- Under replacement therapy with FVIII concentrate, (plasma derived or recombinant) by continuous or bolus infusion;
- Written informed consent, according to METC guidelines.

Exclusion criteria:

- Patient with other congenital or acquired haemostatic abnormalities.
- Withdrawal of (parental) informed consent.
- Due to additional effect on FVIII clearance: detectable FVIII inhibiting antibodies (>0.2 Bethesda Units) at study inclusion.
- General medical conditions which may interfere with participation in the study.

Outcome measures

Primary endpoint:

1. Total amount of infused FVIII concentrate (IU per kilogram) during the perioperative period per postoperative day (up to 14 days after surgery);

Secondary endpoints:

1. Achieved FVIII levels ($IUml^{-1}$) after FVIII infusion.
2. Duration of hospitalization (day of release - day of surgery/ start of continuous or bolus FVIII infusion);
3. Perioperative haemostasis as quantified by standardized form;

4. Effects of baseline VWF antigen, propeptide values and blood type on FVIII clearance and identification of other potential modifiers;
5. Economic analysis of costs in both treatment arms.

Interventions

Overall treatment protocol in all patients, independent of treatment arm

In all patients, a PK profile will be constructed six weeks before surgery prior to stratified randomization. After a standard bolus of FVIII of 50 IU per kilogram, FVIII levels will be measured in three blood samples of 1.8 ml citrate blood, withdrawn at t=4, t=24 and t=48 hours after FVIII clotting factor concentrate infusion, according to Björkman et al. [8]. The individual PK parameters are assessed by Bayesian analysis. During PK profiling, exact information on three prior FVIII doses before the standardized FVIII infusion will be gathered to predict residual FVIII level in each individual patient. Treating physicians and nurses will be blinded for PK profile results in both arms.

In both treatment arms, target ranges for FVIII values will be applied as set by the National Haemophilia Consensus aiming for the middle of each range, unless stated otherwise by treating physician (table 1) [4]. In all patients in both treatment arms, FVIII levels will be monitored as is momentarily standard clinical practice, which is generally daily, during the perioperative period.

Table 1. Target trough levels of FVIII in the perioperative period according to the National Haemophilia Consensus

Time postoperative		Target range FVIII levels ($IUml^{-1}$)	OPTI-CLOT Target FVIII level ($IUml^{-1}$)
Day 1	0-24 hours	0.80-1.00	0.90
Day 2-5	24-120 hours	0.50-0.80	0.65
Day >6	> 120 hours	0.30-0.50	0.40

Specification of treatment arms

Trial/ Intervention arm:

The FVIII dose prior to the start of the surgery will be based on the required FVIII target level according to the National Consensus and individual PK parameters as obtained from the preoperative PK profile according to Björkman et al.[8] and exact information of three prior doses of FVIII concentrate preceding the date of surgery. Subsequent doses will be adjusted iteratively using Bayesian analysis on basis of daily therapeutic moni-

toring of FVIII using our own perioperative population PK model based on retrospective data of severe and moderate severe haemophilia patients undergoing surgery[21].

Daily therapeutic monitoring of FVIII is part of current standard clinical care and FVIII values will be analysed locally. Results will be blinded for the treating physician and nurses. The FVIII levels and administered FVIII doses will be communicated by the laboratories of each specific site with the clinical pharmacologist. The clinical pharmacologist is responsible for the calculation of the individual dose using the Bayesian approach. The clinical pharmacologist will send the FVIII dosing recommendations to the treating physician, who will subsequently adjust dosing. Target levels according to Consensus or otherwise set by the treating physician will be safeguarded by an unblinded haematologist with experience in haemophilia treatment.

Trial/ Standard treatment arm:

The standard perioperative dosing regimen, as described by the Consensus, consists of a FVIII bolus dose directly prior to surgery of 50 IU kg^{-1}, followed by either continuous infusion or intermittent daily bolus infusions. The rate of infusion (IU $hour^{-1}$) is obtained by multiplying the patient's bodyweight (kg) with clearance (3-4 ml kg^{-1} $hour^{-1}$) and target FVIII level (IU ml^{-1}). Subsequent FVIII clotting factor concentrate dosing will be based on daily monitoring of FVIII levels and adjusted according to doctor's opinion, based on a standard clearance of 3-4 ml kg^{-1} $hour^{-1}$ and FVIII levels are targeted in the middle of each target range as set by the National Haemophilia Consensus, which decrease per postoperative day (Table 1) [4]. Dosing adjustments will be performed by the treating physician on basis of the daily monitored FVIII levels, without knowledge of PK parameters collected in the individual preoperative PK profile.

Bayesian-analysis

In the treatment arm individual PK parameters will be assessed iteratively by application of Bayesian analysis as implemented in the NONMEM® software (Icon, Dublin, Ireland). For Bayesian analysis a population PK model will be used constructed on the basis of the PK data from an earlier retrospective perioperative study [21]. Based on the derived individual Bayesian PK parameters an optimal dosing scheme will be calculated during perioperative use, taking mode of administration of concentrates (continuous or bolus infusion) and desired target FVIII value into account. As for continuous infusions, individual estimates for clearance are needed. With regard to intermittent bolus infusions, estimates for both clearance and volume of distribution are needed.

Sample size

Based on earlier data and economic relevance, we aim to detect a difference in mean FVIII concentrate use in IU kg^{-1} which equals 150 IU kg^{-1} or a 25% reduction in use of clotting factor. To study this with a power of 80% and a two-sided α of 0.05, a sample size of minimally 60 patients is necessary. To allow for dropouts, 65 patients will be included in the total study. One patient per centre, except Erasmus MC (n=5) will be treated according to protocol to test local logistical processes. These patients will not be evaluated with regard to primary endpoints, only with regard to secondary endpoints.

Economic analysis will be performed from a health care perspective taking all health care costs into account. We will calculate and compare the costs of PK profiling consisting of: bolus of 50 IU kg^{-1} FVIII minus prophylactic doses required, time invested by haemophilia caretakers, two and three FVIII test samples (in standard clinical practice, usually one in vivo recovery FVIII level is performed), generation of PK profile by Bayesian analysis and perioperative iterative Bayesian analysis to the standard dosing procedure consisting of: establishment of treatment protocol by haemophilia expert, and perioperative dosing adjustment calculations by haemophilia expert. Theoretical consequences of PK profiling on prophylactic dosing regimen in the following years, at one year after surgery, both reduction and increase of dosing, will also be taken into account in this economic evaluation. Actual medical costs will be calculated by multiplying volume of health care use with corresponding unit prices. Costs will be valued using the National guidelines for economic evaluation studies [23].

CONCLUSION

The proposed study aims to investigate an innovative individualized perioperative PK-guided dosing strategy, which is expected to reduce FVIII consumption and therefore costs by minimally 25%. Strengths of the study are the number of participating centres and study design. The application of both perioperative Bayesian guided dosing and a specific perioperative population PK model has not been performed before. Furthermore, the inclusion of children is important, as inter-individual clearance of clotting factor is exceptionally variable at younger ages. As a direct consequence of the study, participating hospitals will gain experience with PK-guided dosing facilitating future implementation of this promising intervention.

REFERENCES

1. Johnson KA, Zhou ZY. Costs of care in hemophilia and possible implications of health care reform. Hematology Am Soc Hematol Educ Program 2011;2011:413-8.
2. Schramm W, Berger K. Economics of prophylactic treatment. Haemophilia 2003;9 Suppl 1:111-5; dicussion 6.
3. Feldman BM, Aledort L, Bullinger M, et al. The economics of haemophilia prophylaxis: governmental and insurer perspectives. Proceedings of the Second International Prophylaxis Study Group (IPSG) symposium. Haemophilia 2007;13:745-9.
4. Leebeek FWG, Mauser-Bunschoten EP, Editors. Richtlijn Diagnostiek en behandeling van hemofilie en aanverwante hemostasestoornissen. Van Zuiden Communications BV 2009:1-197.
5. Lock J, Hazendonk HCAM, Bouzariouh H, et al. A retrospective observational multicenter cohort study on peri-operative Factor VIII consumption in Hemophilia A ("OPTI-CLOT" studies). J Thromb Haemost 2013;11 Suppl. 2:Abstract PB 2.36-2.
6. Hazendonk HCAM, Lock J, Fijnvandraat K, et al. A retrospective observational multicenter study on peri-operative Factor IX consumption in Hemophilia B ("OPTI-CLOT" studies). J Thromb Haemost 2013;11 Suppl. 2:Abstract PA 2.07-1.
7. Collins PW, Fischer K, Morfini M, Blanchette VS, Bjorkman S, International Prophylaxis Study Group Pharmacokinetics Expert Working G. Implications of coagulation factor VIII and IX pharmacokinetics in the prophylactic treatment of haemophilia. Haemophilia 2011;17:2-10.
8. Bjorkman S. Limited blood sampling for pharmacokinetic dose tailoring of FVIII in the prophylactic treatment of haemophilia A. Haemophilia 2010;16:597-605.
9. Carlsson M, Berntorp E, BjÖRkman S, Lethagen S, Ljung R. Improved cost-effectiveness by pharmacokinetic dosing of factor VIII in prophylactic treatment of haemophilia A. Haemophilia 1997;3:96-101.
10. Longo G, Messori A, Morfini M, et al. Evaluation of factor VIII pharmacokinetics in hemophilia-A subjects undergoing surgery and description of a nomogram for dosing calculations. Am J Hematol 1989;30:140-9.
11. Batorova A, Martinowitz U. Continuous infusion of coagulation factors: current opinion. Curr Opin Hematol 2006;13:308-15.
12. Batorova A, Martinowitz U. Intermittent injections vs. continuous infusion of factor VIII in haemophilia patients undergoing major surgery. Br J Haematol 2000;110:715-20.
13. Srivastava A. Choice of factor concentrates for haemophilia: a developing world perspective. Haemophilia 2001;7:117-22.
14. Dingli D, Gastineau DA, Gilchrist GS, Nichols WL, Wilke JL. Continuous factor VIII infusion therapy in patients with haemophilia A undergoing surgical procedures with plasma-derived or recombinant factor VIII concentrates. Haemophilia 2002;8:629-34.
15. Mulcahy R, Walsh M, Scully MF. Retrospective audit of a continuous infusion protocol for haemophilia A at a single haemophilia treatment centre. Haemophilia 2005;11:208-15.
16. Negrier C, Lienhart A, Meunier S. Surgeries in patients with haemophilia A performed with a continyous infusion of Recombinate. J Thromb Haemost 2001;Suppl 1:P0829.

17. Stieltjes N, Altisent C, Auerswald G, et al. Continuous infusion of B-domain deleted recombinant factor VIII (ReFacto) in patients with haemophilia A undergoing surgery: clinical experience. Haemophilia 2004;10:452-8.

18. Perez Bianco R, Primiani L, Neme D, Candela M. Continuous infusion of factor VIII for major orthopedic surgery in persons with haemophilia A. Haemophilia : the official journal of the World Federation of Hemophilia 2004;10:PO38.

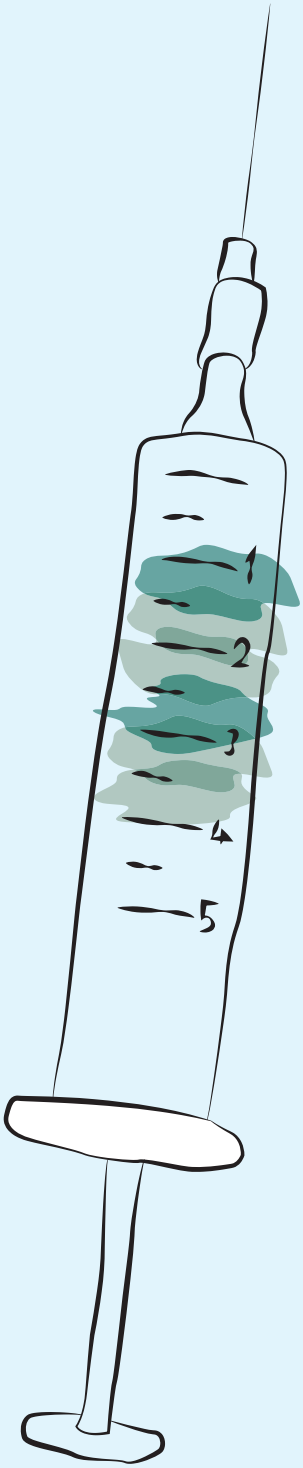
19. Bidlingmaier C, Deml MM, Kurnik K. Continuous infusion of factor concentrates in children with haemophilia A in comparison with bolus injections. Haemophilia 2006;12:212-7.

20. Batorova A, Martinowitz U. Continuous infusion of coagulation factors. Haemophilia 2002;8:170-7.

21. Hazendonk HCAM, Lock J, Fijnvandraat K, et al. Population pharmacokinetics in hemophilia A: Towards individualization of perioperative replacement therapy. . Bari International Conference Abstract book 2014:PO.04.17 - 91.

22. Koshy M, Weiner SJ, Miller ST, et al. Surgery and anesthesia in sickle cell disease. Cooperative Study of Sickle Cell Diseases. Blood 1995;86:3676-84.

23. Hakkaart-van Roijen L, Tan SS, Bouwmans CAM. Handleiding voor kostenonderzoek: Methoden en standaard kostprijzen voor economische evaluaties in de gezondheidszorg. Instituut voor Medical Technology Assessment Erasmus Universiteit Rotterdam 2010:1-127.



6

A randomized controlled trial comparing perioperative dosing of factor VIII concentrate in hemophilia A based on pharmacokinetics with standard treatment (OPTI-CLOT trial)

van Moort I, Preijers T, Bukkems LH, Hazendonk HCAM, van der Bom JG, Laros-van Gorkom BAP, Beckers EAM, Nieuwenhuizen L, van der Meer FJM, Ypma P, Coppens M, Fijnvandraat K, Schutgens REG, Meijer K, Leebeek FWG, Mathôt RAA, Cnossen MH, for the OPTI-CLOT study group.

Submitted

ABSTRACT

Background Dosing of replacement therapy with factor VIII (FVIII) concentrate in hemophilia A patients in the perioperative setting is challenging. Under- and overdosing of FVIII concentrate should be avoided to minimize perioperative bleeding risk and redundant treatment costs. We hypothesized that dosing based on an individual patient's pharmacokinetics (PK) of FVIII concentrate instead of bodyweight which is standard treatment, will reduce FVIII consumption and improve the accuracy of attained FVIII levels.

Aim To compare PK-guided dosing of FVIII concentrate with standard treatment in perioperative hemophilia A patients.

Methods In a multicenter, open-label, randomized controlled trial, patients were assigned to the PK-guided or standard treatment arm in a 1:1 ratio with stratification for mode of FVIII concentrate administration and complexity of surgical procedure. Primary endpoint was FVIII concentrate consumption. Secondary endpoints included achievement of FVIII levels, bleeding, and duration of hospitalization.

Results Sixty-six hemophilia A patients were included. Perioperative FVIII concentrate consumption during the perioperative period was similar in the PK-guided arm (mean: 365±202 IU/kg) and the standard treatment arm (mean: 379±202 IU/kg) (P=0.90). PK-guided treatment resulted in FVIII measurements 12% below, 69% within and 19% above the prespecified FVIII target range, while this was respectively 15%, 37% and 48% for standard treatment (P<0.001). No differences were observed in bleeding events or duration of hospitalization between treatment arms.

Conclusion This first randomized controlled trial demonstrates that perioperative PK-guided dosing leads to similar perioperative FVIII consumption but more optimal achievement of FVIII target ranges (Netherlands Trial Registry: NL3955).

INTRODUCTION

Hemophilia A is an X-linked bleeding disorder, caused by a deficiency of coagulation factor VIII (FVIII). Severity is categorized according to residual FVIII level. The clinical phenotype is characterized by severe bleeding, typically in muscles and joints. Replacement therapy with FVIII concentrate is administered intravenously, both prophylactically to prevent bleeding and on demand when bleeding occurs or when patients undergo medical interventions. In general, FVIII concentrate dosing is based on bodyweight, while aiming for FVIII target ranges defined in clinical guidelines.[1,2]

In the perioperative setting, high FVIIII levels are prescribed for longer time periods and frequently monitored to assure sufficient FVIII is administered. Previous studies have reported that standard perioperative dosing based on bodyweight results in a majority of FVIII levels below or above predefined target ranges.[3-7] Depending on postoperative day, it has been reported that 7-45% of achieved FVIII levels are under and 33-75% are above FVIII target ranges.[6] The large interindividual differences in the pharmacokinetics (PK) of factor concentrates are most probably causative as these are not taken into account in current dosing strategies.[8,9] It is important to decrease this under- and overdosing of FVIIII concentrate to minimize perioperative bleeding risk and redundant treatment which is associated with a higher risk of thrombosis and high medication costs.[1,10-12]

Recent studies show that PK-guided iterative adaptive dosing of factor concentrates is a promising innovative approach.[9,13,14] However, effects on clinical and economic outcomes are yet to be established.[15,16] Therefore, we performed a randomized controlled trial in severe and moderate hemophilia A patients to compare PK-guided perioperative treatment with standard FVIII replacement therapy to evaluate effect on FVIII concentrate consumption and on attainment of FVIII levels.

METHODS

Study design

The OPTI-CLOT trial is a multicenter, open-label, randomized controlled trial comparing PK-guided perioperative dosing of standard half-life FVIII concentrates with routine dosing based on bodyweight in severe and moderate hemophilia A patients.[17] The study was approved by the Medical Ethical Committee of Erasmus University Medical Center Rotterdam, the Netherlands (Netherlands Trial Registry number: NL3955) and by the boards of participating hospitals. The trail was supervised by the leading investigators, i.e. a pediatric hematologist and a clinical pharmacologist. Data analyses were conducted by

the study coordinator, supported by a statistician, and a clinical pharmacologist. One pilot patient per participating hemophilia treatment center with exclusion of primary treatment site, was allocated to the PK-guided treatment arm to test the logistics involved with iterative PK-guidance of dosing.

Patients and randomization

Between May 1st 2014 and March 1st 2020, hemophilia patients with FVIII levels ≤0.05 IU/mL, ≥12 years old, and planned for elective surgery were enrolled from all hemophilia treatment centers in the Netherlands. Detailed information with regard to inclusion and exclusion criteria is found in the appendix and in our previously published trial design paper.[17] After written informed consent, medical site staff registered patients in the Trans European Network for Clinical Trials Services (TENALEA), a web-based registration and randomization system.[18] Patients were randomly assigned to treatment arms in a 1:1 ratio, after stratification for mode of FVIII concentrate administration i.e. intermittent bolus versus continuous infusion, and complexity of surgical procedure i.e. low risk versus medium risk surgery, as these factors are known to influence factor concentrate consumption.[16,19,20] Surgical risk was categorized according to the International Classification of Diseases (9th revision) diagnosis codes for procedures based on the complexity of the surgery.[19]

Study interventions

Both treatment arms

In all patients, a preoperative individual PK profile was obtained in steady non-bleeding state after bolus infusion of approximately 50 IU/kg of various standard half-life FVIII concentrates. FVIII activity one-stage assay measurements were performed at approximately t=4, 24 and 48 hours after bolus infusion.[14] FVIII levels were measured locally and results were sent to the clinical pharmacologist for analyses. Treating (pediatric) hematologists and hemophilia teams were blinded for all preoperative individual PK profile results to ensure there was no indication of patient's FVIII PK. Blood sampling was performed before surgery to determine the FVIII peak level, directly after surgery and daily thereafter if possible, up to a maximum of 14 days after surgery. In most patients, treatment with FVIII concentrate was continued after hospital discharge at home with bolus infusions. FVIII trough levels were determined if possible and when indicated by the hematologist.

Standard treatment arm

In the standard treatment arm, FVIII dosing regimens were based on bodyweight and established by the hematologist according to clinical guidelines as shown in supplementary table 1.[1]

Pharmacokinetic (PK)-guided treatment arm

In the PK-guided treatment arm, the FVIII concentrate loading dose was calculated using the patient's preoperative individual PK profile.[8,9] Consecutive FVIII doses were then also iteratively adjusted after application of *maximum a posteriori* (MAP) Bayesian forecasting that estimates individual PK parameters in order to meticulously calculate the required dosing regimen to achieve FVIII target levels.[16] All FVIII levels measured in the intervention arm were blinded for the treatment team.

Outcomes

The primary study endpoint was FVIII concentrate consumption during the total perioperative period, defined as all FVIII concentrate doses until 14 days after initiation of surgery. Secondary endpoints were accuracy of achieved FVIII levels in relationship to target ranges, safety, e.g. bleeding and thrombosis, and the duration of hospitalization. FVIII target ranges were prespecified by hematologists and defined in clinical guidelines (supplementary table 1). However, when indicated the hematologist was able to deviate from these guidelines. The middle of the prespecified target range was used to evaluate how accurate FVIII levels were targeted. Perioperative bleeding was based on the definition of clinically relevant bleeding as defined by the International Society of Thrombosis and Haemostasis (ISTH).[21] For our analyses, this specifically included bleeding complications involving a hemoglobin decrease of ≥ 1.24 mmol/L, and/or necessitating additional FVIII concentrate treatment, and/or red blood cell transfusion, and/or a second surgical intervention, and/or prolongation of hospitalization. Bleeding events were recorded up to 10 weeks after surgery.

Statistical analysis

Statistical analysis demonstrated that a sample size of 60 patients, 30 patients in each treatment arm, stratified for mode of factor concentrate administration and complexity of surgical procedure, was required to detect a 25% reduction in FVIIII concentrate consumption with a power of 80% and a two-sided α of 0.05. The pilot patients were not randomized and therefore not included in primary endpoint analyses, but were used to evaluate secondary endpoints.

The primary study endpoint, i.e. the difference in FVIII concentrate consumption up to 14 days after surgery between the two treatment arms, was analyzed with a multivari-

ate linear regression model. Analyses were adjusted for the possible confounders: age, bodyweight, blood group, mode of FVIII concentrate administration, and complexity of surgery.

The accuracy of achieved FVIII trough levels was analyzed with a chi-square test. In addition, the total number of FVIII levels according to day after surgery below, within or above FVIII target range were analyzed by multivariate linear regression and adjusted for possible confounders. Bleeding and thrombotic events in the perioperative period were analyzed using Fisher exact's testing. The duration of hospitalization was compared in both treatment arms by multivariate linear regression. Number of days of hospital admission was log-transformed as data were not normally distributed. Hospital admission was also adjusted for confounders. All statistical analyses were two-sided and performed using R software v3.6.1 (R Core Team (2019)) and SPSS Statistics v25.0 (IBM Corp. (2017)).

RESULTS

Participants

In total, 66 patients were included of which 34 patients were assigned to the intervention arm and 32 to the standard treatment arm (Figure 1). The Medical Ethical Committee of Erasmus University Medical Center permitted dispensation for the 67th patient and study finalization during the COVID-19 pandemic. The two groups were well-balanced at baseline (Table 1) and all 66 patients completed the entire study protocol.

FVIII concentrate consumption

In the total perioperative period, FVIII concentrate consumption was similar in the PK-guided treatment arm (365 IU/kg ±202, mean±SD) and the standard treatment arm (379 IU/kg ±202) (adjusted difference = -6 IU/kg, 95% confidence interval -88 to 100 IU/kg) as depicted in Figure 2. Additional posthoc analyses were performed as previous studies showed under dosing mostly in the first 24 hours and overdosing in the period 24-120 hours, and 120 hours after surgery. No association between PK-guided dosing and FVIII consumption was demonstrated during the first 24 hours after surgery (adjusted difference = -4 IU/kg, 95% CI -31 – 24 IU/kg), from 24 to 120 hours after surgery (adjusted difference = - 5 IU/kg, 95% CI -45 – 35 IU/kg) and after 120 hours following surgery (adjusted difference = 14 IU/kg, 95% CI -29 – 58 IU/kg).

As depicted in supplementary Table 2, age was associated with FVIII concentrate consumption, as an increase of one year in age was associated with a decrease of 4 IU/kg (95% CI -7 – -1 IU/kg) FVIII concentrate in the perioperative period. Low risk surgeries

had a lower, but not statistically significant, mean total FVIII concentrate consumption of 310 ± 231 IU/kg when compared to medium risk surgeries of 443 ± 132 IU/kg consumption (adjusted difference = 112, 95% CI -3 – 228 IU/kg, supplementary Table 2). The mean FVIII consumption in surgeries with intermittent bolus infusions was 302±210 IU/kg and therefore lower, although not statistically significant, than the FVIII consumption in surgeries with continuous infusion, 452±159 IU/kg (adjusted difference = 119, 95% CI -1 – 239 IU/kg). Most surgeries with continuous infusion of FVIII concentrate were categorized as medium surgical risk (25/31), whereas most surgeries with bolus infusions were low surgical risk (28/35).

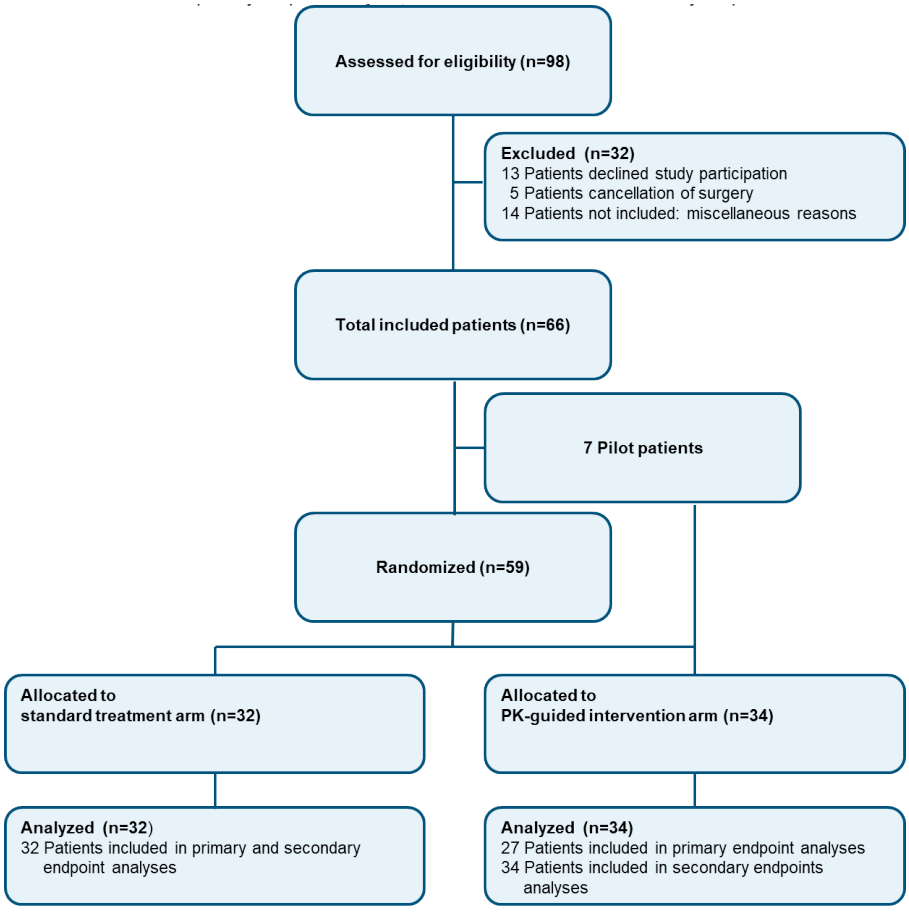


Figure 1. Enrollment in the randomized controlled OPTI-CLOT trial.
Medical ethical approval required addition of one pharmacokinetic (PK)-guided pilot patient per participating center to test logistics of PK-guided dosing. These pilot patients were not randomized and therefore not included in primary endpoint analyses, but were used to evaluate secondary endpoints.

Table 1. Patient characteristics in the perioperative OPTI-CLOT randomized controlled trial: Pharmacokinetic (PK)-guided dosing versus standard dosing of FVIII concentrate.

	PK-guided treatment	Standard treatment	Total
Patients (n)	34	32	66
Age (years)	49.8 (36.3 – 63.7)	47.6 (34.8 – 59.1)	49.1 (35.0 – 62.1)
Bodyweight (kg)	83.0 (74.1 – 95.0)	88.2 (73.3 – 96.6)	86.7 (73.9 – 95.4)
Blood group			
O	21 (61.8)	19 (59.4)	40 (60.6)
non O	13 (38.2)	13 (40.6)	26 (39.4)
Hemophilia severity			
Severe	22 (64.7)	22 (64.7)	44 (66.7)
Moderate	12 (35.3)	10 (31.3)	22 (33.3)
Factor concentrate			
Octocog alfa [#]	8 (23.5)	10 (31.3)	18 (27.3)
Octocog alfa*	11 (32.4)	9 (28.1)	20 (30.3)
Moroctocog alfa^	3 (8.8)	1 (3.1)	4 (6.1)
Plasma derived FVIIII concentrate[&]	2 (5.9)	1 (3.1)	3 (4.5)
Turoctocog alfa[##]	10 (29.4)	11 (34.4)	21 (31.8)
Mode of administration			
Bolus	19 (55.9)	16 (50.0)	35 (53.0)
Continuous	15 (44.1)	16 (50.0)	31 (47.0)
Surgical risk			
Low	17 (50.0)	17 (53.1)	34 (51.5)
Medium	17 (50.0)	15 (46.9)	32 (48.5)
Type of surgical procedure			
General	1 (2.9)	1 (3.1)	2 (3.0)
Colorectal	2 (5.9)	1 (3.1)	3 (4.5)
Neurological	3 (8.8)	0 (0.0)	3 (4.5)
Orthopedic	12 (35.3)	19 (59.4)	31 (47.0)
Urology	3 (8.8)	1 (3.1)	4 (6.0)
Ear-nose-throat	1 (2.9)	1 (3.1)	2 (3.0)
Eye	2 (5.9)	0 (0.0)	2 (3.0)
Miscellaneous	10 (29.5)	9 (28.2)	19 (28.8)

Table 1. Patient characteristics in the perioperative OPTI-CLOT randomized controlled trial: Pharmacokinetic (PK)-guided dosing versus standard dosing of FVIII concentrate. (*continued*)

	PK-guided treatment	Standard treatment	Total
Days of hospitalization	3.0 (0.0 – 8.0)	4.5 (0.3 – 10.5)	3.5 (0.0 – 9.0)
Days of treatment required	10.5 (5.8 – 11.0)	10.0 (7.3 – 13.0)	10.0 (6.8 – 11.3)
No. of patients with complication			
Bleeding	6 (17.6)	3 (9.4)	9 (13.6)
Thrombosis	0 (0.0)	0 (0.0)	0 (0.0)
Number of FVIII measurements	6 (3.3 – 10.0)	7.5 (3.8 - 10.0)	6.5 (3.3 – 10.0)

[#]Kogenate®; *Advate®; ^Refacto AF®; [&]Aafact®; [##]NovoEight®
Values given in No. (%) or median (interquartile range).

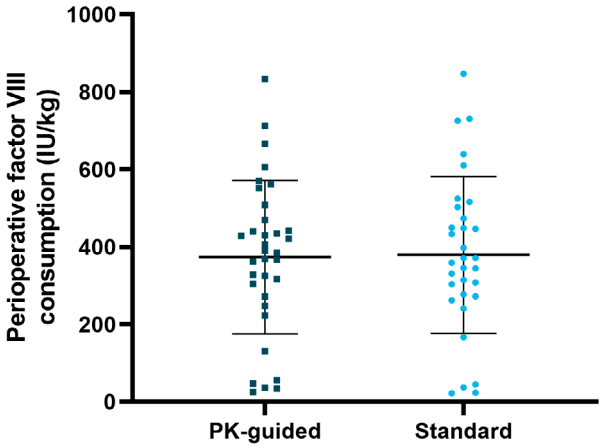


Figure 2. Pharmacokinetic (PK)-guided FVIII concentrate dosing leads to similar and not lower FVIII concentrate consumption in the perioperative period. For each plot, whiskers depict the standard deviation of the data, whereas the black horizontal line in the middle depicts the mean. Each dot or square represents the perioperative FVIII consumption in IU/kg of one individual patient. The perioperative period was defined as time from day of surgery until 14 days after surgery.

Analyses of FVIII achievement within the desired range

PK-guided treatment was associated with a higher number of FVIII levels within the prespecified FVIII range during follow up as visualized in Figure 3 and described in supplementary Table 3. In total, the number of FVIII measurements (Table 1) within targeted range for PK-guided treatment as compared to standard treatment was observed to be 113/164 (69%) versus 58/155 (37%), respectively (p<0.001). In addition, PK-guided treatment resulted in 3.3±2.7 days within targeted range compared to standard treatment

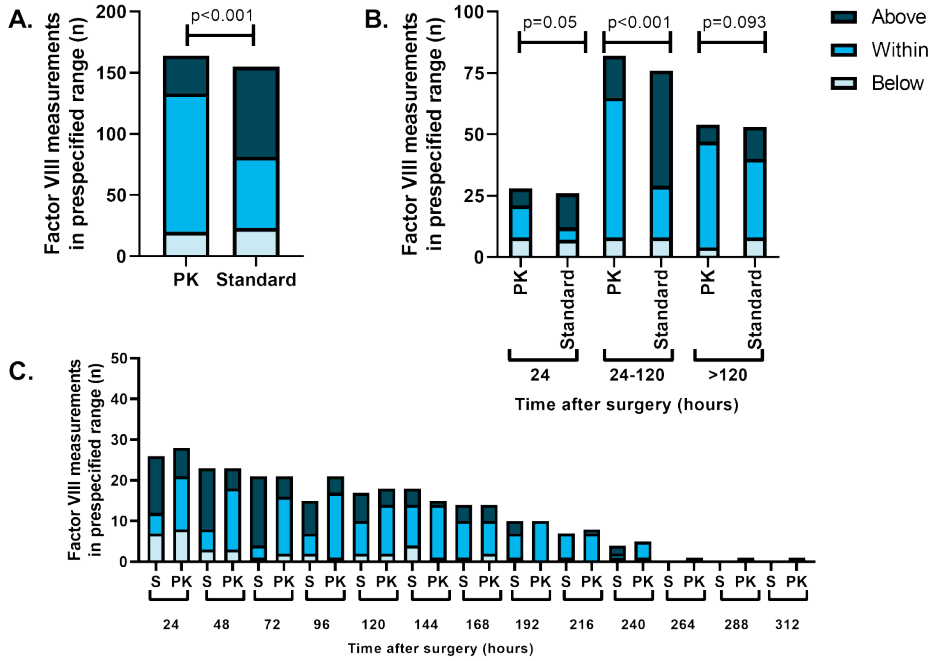


Figure 3. Pharmacokinetic (PK)-guided treatment is associated with a higher number of measured FVIII levels in the predefined FVIII range in comparison to standard treatment based on bodyweight. A) Results of all FVIII trough levels measured in the perioperative period randomized to PK-guided or standard treatment arm B) Results of all FVIII trough measurements in randomized treatment arms and time periods in hours after surgery. C) Results of all FVIII trough measurements in randomized treatment arms and time points in hours after surgery. Statistical analysis was performed using Chi-squared tests. S or Standard corresponds with standard treatment. PK stands for pharmacokinetic-guided treatment. For each FVIII level measurement, it was calculated whether the FVIII level was above (dark blue), within (turquoise) or below (light blue) the prespecified target range based on perioperative guidelines or expert opinion.

with 1.8±1.7 days (adjusted difference 1.5, 95% CI 0.6 – 2.4). Overdosing was less with PK-guided treatment (0.91±1.1 days) versus standard treatment (2.3±2.0 days) (adjusted difference -1.3, 95% CI -1.9 – -0.8), while PK-guided treatment did not reduce under dosing (0.6±0.6 days) compared to standard treatment (0.8±1.1 days) (adjusted difference -0.1, 95% CI -0.6 – 0.3). Figure 4 further shows the degree of attainment of FVIII levels, in which the ratio of measured divided by prespecified FVIII level ideally is one. The ratio of measured FVIII levels divided by prespecified target levels (mean ±SD) was calculated as 1.10±0.33 and 1.31±0.50 for respectively the PK-guided dosing arm and standard treatment arm (supplementary statistical section).

Safety

Bleedings were documented until ten weeks after surgery. In total, nine out of 66 patients experienced a clinically relevant bleed (13.6%) at a median of 7.0 days, as described in supplementary Table 4. The number of bleeds were not associated with either PK-guided treatment (6/34) or standard treatment (3/32, p = 0.269). No cases of thrombosis or

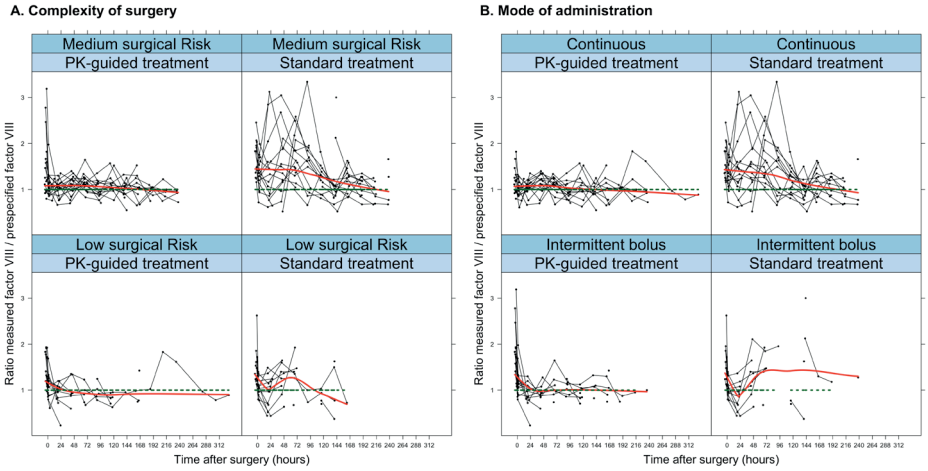


Figure 4. Pharmacokinetic (PK)-guided treatment is associated with improved achievement of prespecified FVIII levels in comparison with standard treatment based on bodyweight. A) Stratification for complexity of surgery i.e. low versus medium surgical risk surgery; B) Stratification for mode of administration of FVIII concentrate i.e. continuous versus bolus infusion. Spaghetti plots showing ratio between measured FVIII and prespecified target FVIII values in perioperative period. Each patient is represented by a black line. The red line indicates the local regression or LOESS line, which follows densest part of the data. The green dotted line is situated at y=1, and represents the most ideal situation in which measured FVIII levels are equal to targeted FVIII levels.

death were reported. The median duration of hospitalization was 3.0 days (IQR: 0.0 – 8.0) in the PK-guided treatment arm and 4.5 days (IQR: 0.3 – 10.5) in the standard treatment arm. The duration of hospitalization was not associated with mode of administration (adjusted difference = 0.82, 95% CI 0.57 - 1.19) when adjusted for confounders.

DISCUSSION

In this first unique randomized controlled trial on the efficacy and impact of PK-guided treatment in severe and moderate hemophilia A patients undergoing surgery, we demonstrate that PK-guided treatment results in similar consumption of FVIII replacement therapy while significantly improving the achievement of FVIII levels within the desired ranges. Therefore, PK-guided treatment optimizes and personalizes hemophilia treatment in patients undergoing surgery.

A recent retrospective study by Hazendonk et al., that evaluated perioperative data of 119 severe and moderate hemophilia A patients undergoing 198 surgeries, showed that 45% of measured FVIII levels were below desired range during the first 24 hours after surgery and that 75% were above the desired range 120 hours after surgery.[6] Based on these data, we assumed that patient safety and quality of care will improve by more op-

timal achievement of FVIII target ranges through implementation of PK-guided dosing. Moreover, we calculated that the actual achievement of desired FVIII levels, would lead to a cost reduction of up to 44% on expensive FVIII concentrate medication. However, in our current randomized controlled trial, we show that consumption is similar and not reduced, while FVIII levels are significantly more often within the prespecified target range.

Possible explanations why no difference in FVIII concentrate consumption is demonstrated are diverse. Firstly, the use of higher FVIII doses to achieve more precise target FVIII levels in the first 24 hours after surgery by PK-guided dosing, using higher FVIII concentrate doses may theoretically have counterbalanced a reduction in consumption >120 hours after surgery. We were not able to prove this hypothesis due to limited FVIII measurements at the end of the perioperative period as depicted in Figure 3. More specifically, the median number of 6.5 FVIII measurements per patient (PK guided arm: 6.0; standard treatment arm: 7.5) in the total study corresponds with a median of only 96-120 hours after surgery. Secondly, the difference in the median hospitalization period of 3.5 days (IQR: 0.0 – 9.0) in the OPTI-CLOT trial and 9 days (IQR 5.0 – 12.0) in the retrospective study may partially explain the lack of reduction of FVIII concentrate consumption. This decrease in duration of hospital admission seems related to a general trend of shorter admissions as no difference in surgical risk was observed between treatment arms. No association was found between earlier discharge with a concomitant switch to intermittent bolus infusion and a higher consumption of FVIII concentrate at the end of the 14 day study period.

Importantly, our study is the first to demonstrate the impact of PK-guided treatment in a perioperative setting within a methodologically correct design. Prior, this was done in two prophylaxis studies with small patient numbers.[22,23] But we also realize that our study has some limitations, mainly due to the real world setting of the study. Firstly, standard dosing was performed by (pediatric) hematologists who were not blinded for FVIII doses and FVIII measurements. Therefore, a Hawthorne effect may have resulted in stricter and more precise dosing in the standard treatment arm, as well as the desire to do better than the results of the retrospective multicenter study, in which the same hemophilia treatment centers participated.[24] In the retrospective study by Hazendonk et al, which was conducted without such a performance bias, only 22% of all measured FVIII levels were within prespecified FVIII target range during the perioperative period.[6] Therefore, the fact that within the OPTI-CLOT trial this increased to 69% in the PK-guided arm, versus 37% in the standard treatment arm illustrates that PK-guided dosing secures more accurate achievement of desired FVIII levels. Secondly, the lack of consecutive FVIII measurements at the end of a patient's hospitalization is a caveat.

We assume that due to real-life patient and doctor-related factors less pro-active FVIII monitoring was performed to unburden both the patient and organization of the intensive intravenous blood sampling required at the beginning of each hospitalization period. Thirdly, although PK-guided treatment resulted in more optimal achievement of FVIII target ranges, the shorter although not statistically significant hospitalization period in the PK-guided treatment patient may have caused bias. A shorter stay with more FVIII measurements may indicate less time between the measurements and more opportunity to adapt dosing schedules. However, FVIII measurements were scheduled daily in this trial and the PK-guided treatment arm only includes two additional patients (n=34 versus n=32). Taking these factors into account, time periods are similar between FVIII measurements and FVIII measurement per patient and therefore comparable (Table 1). Finally, the frequency of bolus administrations per day and subsequent FVIII doses are considerably influenced by practical issues and cannot be endlessly adapted. Less frequent dosing leads to higher doses when aiming for prespecified FVIII target ranges, dosing in high frequency with lower doses is often logistically difficult.

In conclusion, PK-guided treatment decreases under-and overdosing of hemophilia patients while using similar amounts of FVIII replacement therapy compared with standard treatment. Importantly, we show that PK–guided treatment results in more optimal achievement of prespecified FVIII ranges by more accurate perioperative dosing and hence optimization of treatment for patients.

ACKNOWLEDGMENTS

This study is part of the research program of the international multicenter ‘OPTI-CLOT’ consortium (Patient tailOred PharmacokineTIc-guided dosing of CLOTting factor concentrates and desmopressin in bleeding disorders), which aims to implement PK-guided dosing of factor replacement therapy and desmopressin by initiating studies that describe the impact of PK-guided dosing, by constructing prophylactic and on-demand dosing population PK models, and by evaluating the cost-effectiveness of a PK-guided approach. A complete list of the members of the ‘OPTI-CLOT’ research program is available in the appendix. Most importantly, we are grateful to all patients and family members who participated so enthusiastically in this trial. We also intensely thank all hemophilia teams, nurses and research coordinators for their indispensable assistance . Furthermore, we thank N. van Leeuwen and D. Nieboer for their statistical advice, I. van Vliet A. Koolma and C.M. Zwaan for trial support, and J.M. Heijdra, L.M. Schütte for their help and assistance during the trial period.

REFERENCES

1. Leebeek FWG, Mauser-Bunschoten EP. Richtlijn diagnostiek en behandeling van hemofilie en aanverwante hemostase stoornissen. Utrecht: Van Zuiden Communications BV; 2009:1-197.
2. Srivastava A BA, Mauser Bunschoten EP, Key NS, Kitchen S, Llinas A, Ludlam CA, Mahlangu JN, Mulder K, Poon MC, Street A; Treatment Guidelines Working Group on Behalf of The World Federation Of Hemophilia. Guidelines for the Management of Hemophilia Haemophlia. Montréal, QC: Blackwell Publishing; 2012.
3. Batorova A, Martinowitz U. Intermittent injections vs. continuous infusion of factor VIII in haemophilia patients undergoing major surgery. Br J Haematol 2000;110:715-20.
4. Dingli D, Gastineau DA, Gilchrist GS, Nichols WL, Wilke JL. Continuous factor VIII infusion therapy in patients with haemophilia A undergoing surgical procedures with plasma-derived or recombinant factor VIII concentrates. Haemophilia 2002;8:629-34.
5. Bidlingmaier C, Deml MM, Kurnik K. Continuous infusion of factor concentrates in children with haemophilia A in comparison with bolus injections. Haemophilia 2006;12:212-7.
6. Hazendonk HC, Lock J, Mathot RA, et al. Perioperative treatment of hemophilia A patients: blood group O patients are at risk of bleeding complications. J Thromb Haemost 2016;14:468-78.
7. Schutte LM, de Rooij N, Hazendonk H, et al. Current dosing practices for perioperative factor VIII concentrate treatment in mild haemophilia A patients result in FVIII levels above target. Haemophilia 2019;25:960-8.
8. Bjorkman S, Folkesson A, Jonsson S. Pharmacokinetics and dose requirements of factor VIII over the age range 3-74 years: a population analysis based on 50 patients with long-term prophylactic treatment for haemophilia A. Eur J Clin Pharmacol 2009;65:989-98.
9. Bjorkman S, Oh M, Spotts G, et al. Population pharmacokinetics of recombinant factor VIII: the relationships of pharmacokinetics to age and body weight. Blood 2012;119:612-8.
10. Johnson KA, Zhou ZY. Costs of care in hemophilia and possible implications of health care reform. Hematology Am Soc Hematol Educ Program 2011;2011:413-8.
11. Shrestha A, Eldar-Lissai A, Hou N, Lakdawalla DN, Batt K. Real-world resource use and costs of haemophilia A-related bleeding. Haemophilia 2017.
12. Zhou ZY, Koerper MA, Johnson KA, et al. Burden of illness: direct and indirect costs among persons with hemophilia A in the United States. J Med Econ 2015;18:457-65.
13. Hazendonk H, van Moort I, Mathot RAA, et al. Setting the stage for individualized therapy in hemophilia: What role can pharmacokinetics play? Blood Rev 2018;32:265-71.
14. Bjorkman S. Limited blood sampling for pharmacokinetic dose tailoring of FVIII in the prophylactic treatment of haemophilia A. Haemophilia 2010;16:597-605.
15. Sheiner LB. The population approach to pharmacokinetic data analysis: rationale and standard data analysis methods. Drug Metab Rev 1984;15:153-71.
16. Hazendonk H, Fijnvandraat K, Lock J, et al. A population pharmacokinetic model for perioperative dosing of factor VIII in hemophilia A patients. Haematologica 2016;101:1159-69.
17. Hazendonk HC, van Moort I, Fijnvandraat K, et al. The “OPTI-CLOT” trial. A randomised controlled trial on periOperative PharmacokineTIc-guided dosing of CLOTting factor concentrate in haemophilia A. Thromb Haemost 2015;114:639-44.

18. ALEA Tools for Clinical Trials. 2018. (Accessed 02-07-2020, 2020, at https://www.aleaclinical.eu/.)

19. Koshy M, Weiner SJ, Miller ST, et al. Surgery and anesthesia in sickle cell disease. Cooperative Study of Sickle Cell Diseases. Blood 1995;86:3676-84.

20. Mulcahy R, Walsh M, Scully MF. Retrospective audit of a continuous infusion protocol for haemophilia A at a single haemophilia treatment centre. Haemophilia 2005;11:208-15.

21. Kaatz S, Ahmad D, Spyropoulos AC, Schulman S, Subcommittee on Control of A. Definition of clinically relevant non-major bleeding in studies of anticoagulants in atrial fibrillation and venous thromboembolic disease in non-surgical patients: communication from the SSC of the ISTH. J Thromb Haemost 2015;13:2119-26.

22. Carlsson MB, E; Björkman, S; Lethagen, S; Ljung,R. Improved cost-effectiveness by pharmacokinetic dosing of factor VIII in prophylactic treatment of haemophilia A. Haemophilia 1997;3:96-101.

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

24. Sedgwick P, Greenwood N. Understanding the Hawthorne effect. Bmj 2015;351:h4672.

Table S1. Predefined factor VIII ranges in the perioperative period as described in the National Hemophilia Consensus[2] and subsequent OPTI-CLOT trial factor VIII target values.

Time point after surgery			
Day	***Hours***	***Pre-specified factor VIII range (IU/mL)***	***OPTI-CLOT trial Target value factor VIII (IU/mL)***
1	*0-24*	0.80-1.00	0.90
2-5	*24-120*	0.50-0.80	0.65
≥6	*>120*	0.30-0.50	0.40

Table S2. Pharmacokinetic (PK)-guided dosing is not associated with perioperative factor VIII concentrate consumption. Multivariate linear regression modeling was used to determine the associations with primary endpoint perioperative factor VIII concentrate consumption (IU/kg) and dosing strategy adjusted for multiple variables.

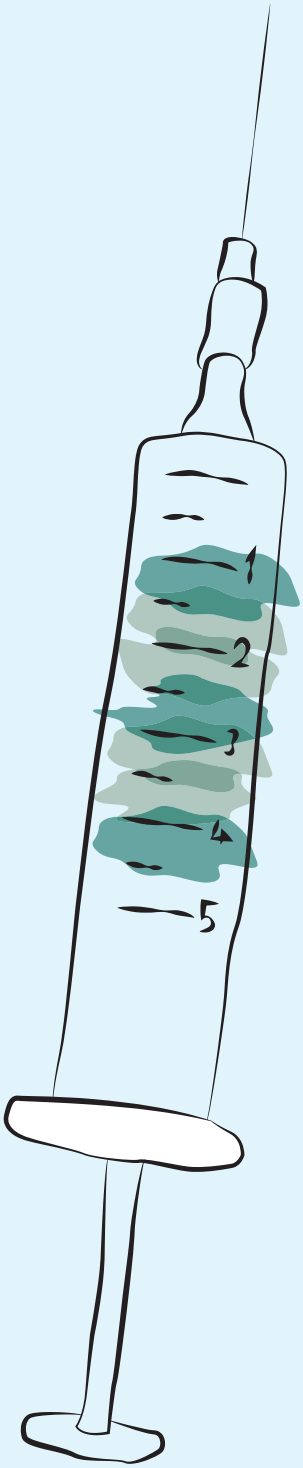
	Coefficient	95% Confidence interval	P-value
Constant	299	17 - 582	
Dosing strategy, PK-guided dosing	6	-88 - 100	0.897
Age (years)	-4	-7 - 1	0.018
Bodyweight (kg)	-1	-4 - 1	0.298
Blood group, group O	66	-28 - 161	0.163
Factor VIII administration, continuous infusion	112	-3 - 228	0.057
Surgical risk, medium risk	119	-1 - 238	0.052

Table S3. Number of factor VIII trough measurements above, within or below pre-specified factor VIII ranges visualized for pharmacokinetic (PK)-guided and standard dosing treatment arms. Values given in No. (%). From postoperative day 11, factor VIII measurements from only one patient were available, statistical analyses were discontinued after this time point. Statistical differences were calculated using Chi-squared tests.

		PK-guided treatment	Standard treatment	Total	P-value
Total					
	Above	31 (18.9)	74 (47.8)	105 (32.9)	<0.001
	Within	113 (68.9)	58 (37.4)	171 (53.6)	
	Below	20 (12.2)	23 (14.8)	43 (13.5)	
0 – 24 hours					
	Above	7 (25.0)	14 (53.9)	21 (38.9)	0.053
	Within	13 (46.4)	5 (19.2)	18 (33.3)	
	Below	8 (28.6)	7 (26.9)	15 (27.8)	
24 – 120 hours					
	Above	17 (20.7)	47 (61.8)	64 (40.5)	<0.001
	Within	57 (69.5)	21 (27.6)	78 (49.4)	
	Below	8 (9.8)	8 (10.5)	16 (10.1)	
>120 hours					
	Above	7 (13.0)	13 (24.5)	20 (18.7)	0.093
	Within	43 (79.6)	32 (60.4)	75 (70.1)	
	Below	4 (7.4)	8 (15.1)	12 (11.2)	

Table S4. Description of postoperative bleeding events in the randomized controlled OPTI-CLOT trial. All bleedings, both related and not related to the surgical procedure were recorded until 10 weeks after surgery. Grading was performed according to the common terminology criteria for adverse events version 4.0 (CTCAE v4.0). Abbreviations: PK = pharmacokinetic-guided treatment arm' Standard = standard dosing treatment arm based on bodyweight.

Patient	Treatment arm	Day of onset of bleeding	CTCAE v4.0	Blood group	Description
		Hours after surgery; days (n)	*Grade*	*(O vs non-O)*	
1	PK	72 (3)	2	O	Postoperative hemorrhage in knee three days after total knee replacement, consequently re-operation was necessary. Bleeding stopped after re-operation.
2	PK	384 (16)	2	O	Postoperative hemorrhage in knee 16 days after total knee replacement.
3	Standard	24 (1)	4	Non-O (B)	Postoperative hemorrhage around site of incision one day after tonsillectomy, leading to intensive care admission.
4	Standard	168 (7)	2	O	Central line insertion. Seven days after placement mild blood leakage from insertion site.
5	PK	384 (16)	2	Non-O (A)	Postoperative hemorrhage e.g. hematuria 16 days after transurethral prostate resection.
6	PK	216 (9)	1	O	Postoperative hemorrhage in lower arm nine days after ulnaris nerve release surgery under factor VIII prophylaxis.
7	PK	336 (14)	2	Non-O (AB)	Hematuria, 14 days after trigger finger surgery, bleed not related to surgery.
8	Standard	Directly after surgery	1	O	Leakage of the surgical wound.
9	PK	7	1	O	Postoperative hemorrhage, subcutaneous bleed in lower leg seven days after total knee replacement.



7

Von Willebrand factor and factor VIII clearance in perioperative hemophilia A patients (OPTI-CLOT trial)

van Moort I, Bukkems LH, Heijdra JM, Schutgens REG, Laros-van Gorkom BAP, Nieuwenhuizen L, van der Meer FJM, Fijnvandraat K, Ypma P, de Maat MPM, Leebeek FWG, Meijer K, Eikenboom J, Mathôt RAA, Cnossen MH, for the OPTI-CLOT study group.

Thromb Haemost. 2020 Jul;120(7):1056-1065

ABSTRACT

Background Von Willebrand factor (VWF) is crucial for optimal dosing of factor VIII (FVIII) concentrate in hemophilia A patients as it protects FVIII from premature clearance. To date, it is unknown how VWF behaves and what its impact is on FVIII clearance in the perioperative setting.

Aim Investigate VWF kinetics (VWF antigen (VWF:Ag), VWF glycoprotein Ib binding (VWF:GP1bM) and VWF propeptide (VWFpp) in severe and moderate perioperative hemophilia A patients included in the randomized controlled perioperative OPTI-CLOT trial.

Methods Linear mixed effects modeling was applied to analyze VWF kinetics. One-way and two-way ANOVA were used to investigate perioperative VWF propeptide/VWF antigen ratios and associations with surgical bleeding.

Results Fifty-nine patients with median age of 48.8 years (IQR: 34.8–60.0) were included. VWF:Ag and VWF:GPIbM increased significantly postoperatively. Both blood type non-O or medium risk surgery were associated with higher VWF:Ag and VWF:GPIbM levels compared to blood type O and low risk surgery. VWFpp/VWF:Ag was significantly higher immediately after surgery than 32-57 hours after surgery (p<0.001). Lowest VWF:Ag quartile (0.43–0.92 IU/mL) was associated with an increase of FVIII concentrate clearance of 26 mL/h (95% CI 2–50 mL/h) compared to highest VWF antigen quartile (1.70–3.84 IU/mL). VWF levels were not associated with perioperative bleeding $F(4,227)=0.54$, $p=0.710$.

Conclusion VWF:Ag and VWF:GPIbM levels increase postoperatively, most significantly in patients with blood type non-O or medium risk surgery. Lower VWF antigen levels did not lead to clinically relevant higher FVIII clearance. VWF antigen or VWF:GPIbM levels were not associated with perioperative hemorrhage.

INTRODUCTION

A deficiency of coagulation factor VIII (FVIII) leads to diagnosis of hemophilia A, an X-linked bleeding disorder characterized by bleeding typically in joints and muscles, or bleeding after minor trauma and/or surgery. Mainstay of treatment is replacement therapy with FVIII concentrates which is administered both prophylactically in more severely affected patients, and on demand to treat bleeding events or to prevent bleeding during dental or surgical procedures in all patient categories [1]. Previously, we reported a study in 119 hemophilia A patients undergoing 198 surgeries and showed that perioperative FVIII concentrate dosing is challenging using current guidelines based on bodyweight [2]. In this retrospective study, 45% of all FVIII levels measured in the first 24 hours after surgery were below target levels as prescribed in Dutch guidelines [3], with a hypothetical higher risk of bleeding. In addition, 75% of FVIII levels measured 120 hours after surgery were above targeted FVIII levels with concomitantly unnecessary higher treatment costs. As von Willebrand factor (VWF) protects FVIII from proteolytic cleavage, premature activation and clearance from the circulation, VWF is crucial to achieve adequate FVIII levels during FVIII concentrate dosing. Importantly, ratio between VWF propeptide (VWFpp) and VWF antigen (VWF:Ag) can be used as a marker for both VWF synthesis, secretion and clearance. More specifically, VWF:Ag and VWFpp are secreted equimolarly but are independently cleared with different half-lives of 8-12 hours and 2 hours, respectively [4,5]. We hypothesized that specific knowledge on how VWF behaves and influences FVIII clearance in perioperative hemophilia A patients is relevant to optimize FVIII dosing.

The role of VWF in the perioperative period has previously been investigated in 30 healthy individuals, mainly women, undergoing orthopedic surgery by Kahlon et al. [6]. This report showed that both VWF:Ag and VWF ristocetin cofactor activity (VWF:RCo) decrease during a surgical procedure and increase directly afterwards with concomitant decrease and increase of FVIII levels. These results however cannot be translated to our population due to gender differences, as primarily men are diagnosed with hemophilia. In addition, levels of VWFpp were not measured in these patients. Moreover, FVIII in our population is derived from replacement therapy and therefore not released due to endogenous mechanisms.

Therefore, we aimed to investigate VWF kinetics in perioperative hemophilia A patients and the influence of VWF on FVIII clearance. This will provide novel insights into: 1) factors that modify VWF levels; 2) influence of VWF on FVIII concentrate pharmacokinetic parameters, especially FVIII clearance; and 3) association of VWF levels with perioperative bleeding.

PATIENTS AND METHODS

Patients

Patients were diagnosed with severe or moderate hemophilia A and included in the perioperative OPTI-CLOT trial [7]. The OPTI-CLOT trial is a randomized controlled trial, which aims to compare pharmacokinetic (PK)-guided FVIII concentrate dosing with dosing based on bodyweight (standard treatment). Patients are stratified according to surgical risk (medium versus low risk) and mode of FVIII concentrate administration (bolus administration versus continuous infusion). All patients had baseline (lowest) FVIII activity levels ≤ 0.05 IU/mL, were ≥12 years of age, did not have FVIII inhibitory antibodies (Bethesda Units; BU < 0.2 IU) and underwent elective surgery. Patients were enrolled from six Academic Hemophilia Treatment Centers in the Netherlands (Erasmus University Medical Center Rotterdam, University Medical Center Groningen, University Medical Center Utrecht, Radboud university medical center Nijmegen / Maxima Medical Center, Veldhoven, Leiden University Medical Center/ Haga Hospital, The Hague, Amsterdam University Medical Centers). This study was approved by the Institutional Review Board of the Erasmus University Medical Center and all patients gave written informed consent before enrollment according to the declaration of Helsinki.

Patient and surgical characteristics were collected and included blood type, age, bodyweight, body mass index (BMI), ideal bodyweight, FVIII concentrate consumption, surgical risk score and perioperative hemorrhage [8,9]. Perioperative hemorrhage was based on the definition for a clinically relevant bleed as stated by the International Society of Thrombosis and Hemostasis (ISTH). More specifically for our analyses, this included bleeding complications either leading to hemoglobin decrease of ≥ 1.24 mmol/L, necessitating additional FVIII concentrate treatment and/or red blood cell transfusion, and/or a second surgical intervention and/or prolongation of hospitalization.

Blood sampling and laboratory measurements

Blood samples were drawn at baseline (≤ 3 days before surgery), immediately after first dose of FVIII concentrate (t=15-30 minutes), postoperatively in recovery room, beginning of first day after surgery (t=16-33 hours) and at the beginning of second postoperative day (t=33-57 hours). FVIII levels were measured locally at each treatment center, using a one-stage clotting assay. VWF:Ag, VWF:GPIbM and VWFpp were measured at two central laboratories (VWF:Ag and VWF:GPIbM in Erasmus University Medical Center Rotterdam; VWFpp in Leiden University Medical Center). VWF:Ag was measured using polyclonal rabbit anti-human VWF antibody and horseradish peroxidase conjugated anti-human VWF antibody (DakoCytomation, Glostrup, Denmark) in an enzyme-linked immunoassay. VWF activity was measured as VWF:GPIbM. VWF:GPIbM was measured with the In-

novance VWF Ac reagent (Siemens Healthcare Diagnostics, The Hague, The Netherlands) on a Sysmex CS 5100 (Sysmex, EttenLeur, The Netherlands) using the manufacturer's protocol. In this test, polystyrene particles coated with anti-GPIb monoclonal antibodies were added and particle agglutination was measured as a change in turbidity. VWFpp was determined by enzyme-linked immunoassay using Sanquin antibodies (Amsterdam, The Netherlands) [10].

Population pharmacokinetic modeling

Individual FVIII PK parameters e.g. clearance and volume of distribution were estimated using nonlinear mixed-effects modeling software NONMEM v7.4 (ICON Development Solutions, Ellicott City, MD, USA). To determine perioperative FVIII concentrate PK parameters, our published perioperative population PK model for FVIII concentrate dosing in severe and moderate hemophilia A patients was utilized [11]. The following PK parameters were estimated: clearance (CL), inter-compartmental clearance (Q), volume of distribution of central (V1), peripheral (V2) compartment, and elimination half-life (T1/2). R software v3.6.1 (R Core Team (2019)) and Xpose v4.5.3 were used for data exploration and model diagnostics [12].

Statistical analyses

Descriptive statistics were expressed as medians and interquartile range (IQR), or as numerical counts with percentages. To identify effect of different variables in the perioperative period on VWF:Ag or VWF:GPIbM, a linear mixed-effects model was applied on log transformed VWF:Ag or VWF:GPIbM using the lme4 package in R. In this model with log(VWF:Ag) or log(VWF:GPIbM) as outcome, relationships with blood type, surgical risk, BMI and age were investigated. This method was also used to identify VWF effect on FVIII PK parameters in the perioperative period. One-way ANOVA was used to identify statistical differences in ratios VWFpp/VWF:Ag or VWF:Ag/VWF:GPIbM in the perioperative period, both log transformed as a result of non-normality. A two-way ANOVA was applied on log transformed VWF:Ag and/or VWF:GPIbM and their association with postoperative hemorrhage. Posthoc tests were performed with a Bonferroni correction. A p-value of 0.05 was considered statistically significant. All statistical analyses were performed using R software v3.6.1 (R Core Team (2019)).

RESULTS

Patients characteristics

Table 1 presents general characteristics of the study population. In this analysis, a total of 59 patients were included from the perioperative OPTI-CLOT trial of which 38 patients

(64.4%) had severe hemophilia A. Median age was 48.8 years old (IQR: 34.8 – 60.0 years) with a median bodyweight of 87.0 kg (IQR 50.4 – 133.5 kg). Nine of the 59 patients experienced a postoperative bleeding event.

Table 1. General characteristics of the study population

	No. (%) or median [IQR]
Patient characteristics	
Total no. of patients	59
Age (years)	48.8 [34.8 – 60.0]
Severe hemophilia (FVIII<0.01 IU/mL)	38 (64.4%)
Blood group O	34 (57.6%)
Height (cm)	178 [172 – 185]
Bodyweight (kg)	87.0 [74.2 – 95.3]
Body Mass Index (kg/m2)	26.4 [23.3 – 29.7]
Ideal bodyweight (kg)	71.0 [66.8 – 76.3]
History of inhibiting FVIII antibodies	11 (18.6%)
Baseline VWF:Ag (IU/mL)	1.09 [0.88 – 1.42]
Baseline VWF:GPIbM (IU/mL)	0.89 [0.65 – 1.25]
Clotting factor VIII concentrates	
Octocog alfa [#]	17
Octocog alfa*	20
Moroctocog alfa^	4
Plasma derived FVIII concentrate[&]	3
Turoctocog alfa[##]	15
Surgical characteristics	
Surgical risk**	
Low	30
Medium	29
Mode of FVIII concentrate administration	
Bolus	30
Continuous	29
Postoperative hemorrhage	
No	50
Yes	9

[#]Kogenate®; *Advate®; ^Refacto AF®; [&]Aafact®; [##]NovoEight®
**Surgical risk was defined according to Koshy et al [8]. Low surgical risk includes e.g. porth-a-cat removal/insertion and dental surgery. Medium risk includes e.g. total hip or knee replacement and tonsillectomy.

Perioperative VWF and FVIII levels

In the perioperative period, patients were treated with FVIII concentrate, aiming for target FVIII levels as stated in Dutch guidelines. Figure 1 shows that FVIII increases, as well

as VWF:Ag and VWF:GPIbM levels. As is depicted in Figure 1B, VWF:Ag increased postoperatively from preoperative median of 1.09 IU/mL (IQR: 0.88 – 1.42) to a postoperative median of 1.53 IU/mL (IQR: 1.14 – 1.82) 48 hours after surgery with significant interpatient variability. Interpatient variability and increase of VWF:GPIbM was even greater, as at average two-fold differences were observed for each postoperative patient (Figure 1C) with preoperative median values of 0.89 IU/mL (IQR: 0.65 – 1.25) to a postoperative median of 1.74 IU/mL (IQR: 1.04 – 2.57) 48 hours after surgery. In contrast, VWFpp only increased immediately postoperatively. In the majority of patients, rapidly decreasing VWFpp levels were observed during the first day following surgery (t=16.0 - 32.7 hours).

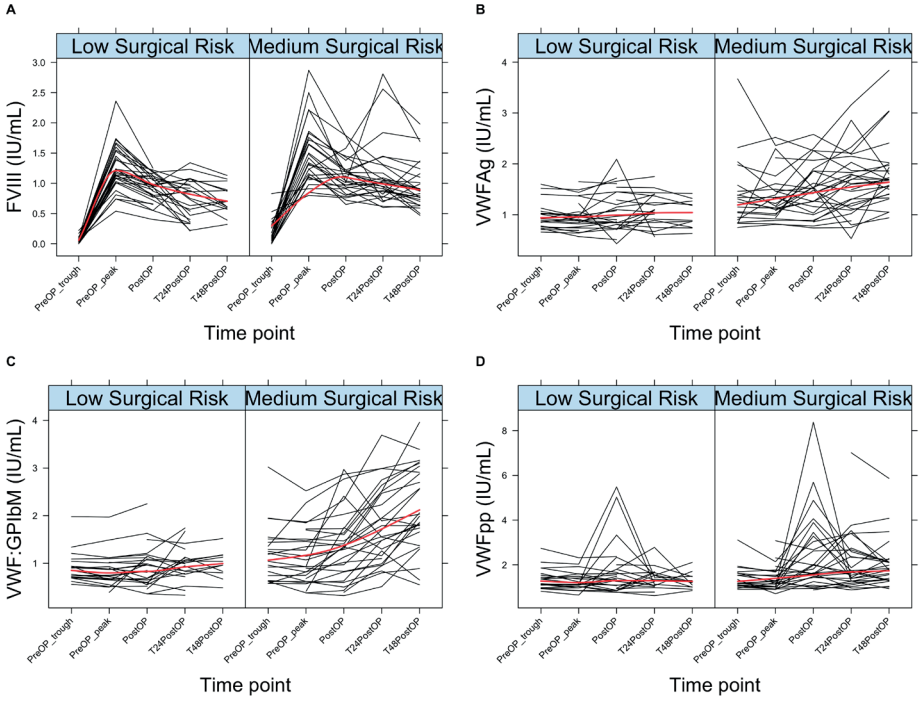


Figure 1. Factor VIII (FVIII) and von Willebrand factor (VWF) in the perioperative period stratified by surgical risk score. Spaghetti plots of A) FVIII; B) VWF:Ag; C) VWF:GPIbM; D) VWFpp. Each patient is represented by a black line. The red line indicates the local regression or LOESS line, which follows densest part of the data.

Figure 2A and 2C show fluctuations per individual of VWFpp/VWF:Ag ratio and local regression or locally estimated scatterplot smoothing (LOESS) line over time. A LOESS line is a non-parametric approach which aims to create a smooth line through all the data points available by fitting multiple regressions in local neighborhood. The VWFpp/VWF:Ag ratio differed between subsequent time points as determined by one-way ANOVA ($F(4,290) = 4.21$, $p = 0.003$). VWFpp/VWF:Ag was higher immediately after surgery when compared to 48 hours after surgery ($p < 0.001$), supporting an increased acute

production and/or release of large amounts of VWFpp due to surgical intervention. Figure 2B and 2D show that VWF:Ag/VWF:GPIbM ratios decrease slightly over time with statistically significant differences when calculated over the total perioperative period as determined by one-way ANOVA ($F(4,232) = 4.25$, $p = 0.002$). Bonferroni posthoc testing showed that VWF:Ag/VWF:GPIbM is also statistically significant when a) preoperative VWF:Ag/ VWF:GPIbM is compared to VWF:Ag/VWF:GPIbM 48 hours after surgery ($p = 0.004$); b) and VWF:Ag/VWF:GPIbM immediately after surgery is compared to VWF:Ag/ VWF:GPIbM 48 hours postoperative ($p = 0.024$).

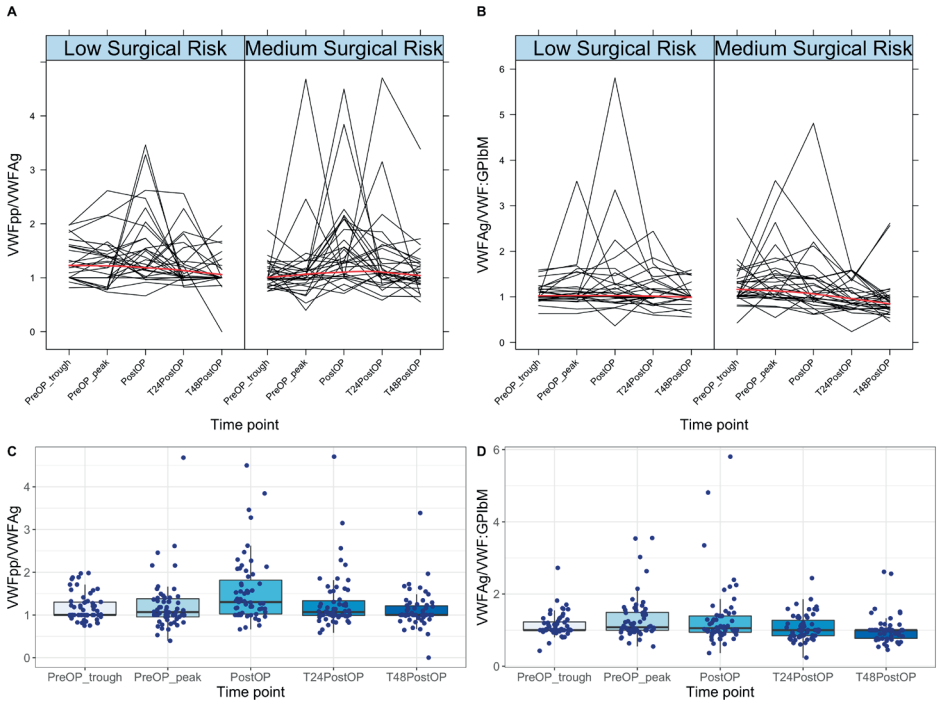


Figure 2. Ratios of VWFpp/VWF:Ag and VWF:Ag/VWF:GPIbM differ in the perioperative period.
Figure A and B are spaghetti plots of VWFpp/VWF:Ag and VW:Ag/ VWF:GPIbM in the perioperative period. Each patient is represented by a black line. The red line indicates local regression or LOESS line, which follows the densest part of the data. A) VWFpp/VWF:Ag ratio, which can be used as a measure for VWF secretion in the acute phase. VWFpp/VWF:Ag is higher immediately after surgery compared to ratios before the surgery. B) VWF:Ag/VWF:GPIbM ratio represents also the acute phase response of VWF. This ratio decreases over time. C and D) boxplots of VWFpp/VWF:Ag and VW:Ag/ VWF:GPIbM ratios for each perioperative time point. For each boxplot, whiskers depict 2.5th and 97.5th percentile of the data, whereas the box depicts the interquartile range. Median of data is depicted by black horizontal line inside the boxplot.

VWF dynamics in perioperative setting

To analyze how VWF levels e.g. VWF:Ag and VWF:GPIbM evolve over time due to alterations in synthesis, secretion and clearance, a linear mixed effect model was created. As VWF:Ag was not distributed normally, a log transformation was performed. In this model

with log(VWF:Ag) as outcome, relationships with blood type, surgical risk, BMI and age were analysed Time was set at t=0 at moment of first incision by the operating surgeon and considered a nonlinear function in the model. Firstly, the most extensive model with interaction terms between time and blood type, time and age, and age and BMI was investigated. A model with both random intercepts and random slopes was proven not superior to only random intercepts when testing with a restricted maximum likelihood test (p = 0.42). Therefore, analyses were continued with the extensive model with only random intercepts. Subsequently, all interaction terms were removed from the random intercept model to investigate if interaction terms improved the model. Models were fitted under maximum likelihood as the F-test could not be computed and denominator degrees of freedom could not be (reliably) defined. The likelihood ratio test (LRT) also showed that interaction terms were not able to improve the model (p = 0.14). Finally, the nonlinear characteristic of the time variable was investigated by creating a model with a linear function of time. Comparing these models with LRT resulted in a statistically significant difference (p = 0.037), meaning that nonlinear terms of time were important contributors to the model. Model assumptions were evaluated with residual plots, and did not show violation of model assumptions, as is documented in supplementary Figure 2.

The final model describing log(VWF:Ag) in the perioperative period is demonstrated in table 2. The expected difference in log(VWF:Ag) between patients with blood type O and non O is -0.16 (95% CI -0.30 - -0.01) if patients are comparable with regard to age, bodyweight, BMI, surgical risk and when sampled at identical time points during perioperative follow-up. Transformation of data results in exp(-0.16) = 0.86. Clinically this means that perioperative hemophilia patients with blood type O may have 14% less VWF:Ag when compared to patients with blood type non O, if all the other variables are kept constant. The expected difference in log(VWF:Ag) between medium and low risk surgical procedures was 0.29 (95% CI 0.13 – 0.43) if patients were comparable with regard to age, bodyweight, BMI, surgical risk and when sampled at identical time points during perioperative follow-up. Transformation of data resulted in exp(0.29)=1.33. When translated into clinical terms, this means that patients with a medium surgical risk may have 33% higher VWF:Ag levels compared to patients undergoing low surgical risk surgery if all other variables are kept constant. The influence of perioperative timing is reflected in the effect plot, which is included in supplementary Figure 1. Similar results were obtained when creating a linear mixed effect model of log(VWF:GPIbM) of which results are included in supplementary table 1 and supplementary Figure 3. Figure 3 also depicts impact of blood type (Figure 3A) and surgical risk (Figure 3B) and on FVIII, VWF:Ag and VWF:GPIbM. In this figure, it is clearly demonstrated that blood type non O

and medium surgical risk result in higher VWF:Ag and VWF:GPIbM levels when compared to blood type O and/ or low surgical risk .

Table 2. Associations between the determinants blood type, surgical risk, age, BMI and the outcome log(VWF:Ag). Linear mixed-effects modeling was used to determine the associations with the outcome log(VWF:Ag). Time was set at t=0 at moment of first incision by the operating surgeon and was defined as a non-linear function. Especially blood type non-O and medium surgical risk were associated with higher VWF:Ag levels perioperatively.

Fixed effects	Coefficient	95% Confidence interval	P-value
Intercept	-0.132	-0.543 – 0.28	0.535
Time since start surgery (hours)	0.002	0.001 – 0.004	0.000
Time since start surgery^2 (hours)	0.000	0.000 – 0.000	0.040
Blood type, type O	-0.157	-0.305 – -0.008	0.042
Surgical Risk, medium risk	0.286	0.134 – 0.438	0.001
Age (years)	0.003	-0.002 – 0.008	0.188
BMI (kg/m^2)	0.003	-0.010 – 0.015	0.682

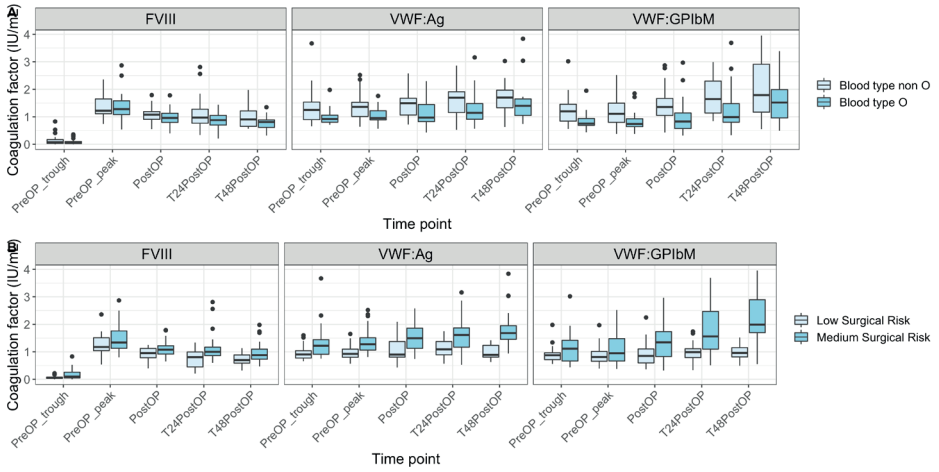


Figure 3. Both blood type and surgical risk affect perioperative VWF:Ag and VWF:GPIbM levels. For each boxplot, whiskers depict 2.5th and 97.5th percentile of the data, whereas the box depicts interquartile range. Median of data is depicted by the black horizontal line inside the boxplot. A) shows boxplots over time separated by blood type. Hemophilia A patients with blood type non O have higher VWF:Ag and VWF:GPIbM levels compared to patients with blood type O. B) shows lower FVIII levels for surgeries with a lower surgical risk, as the Dutch guidelines advise lower FVIII target levels. Both VWF:Ag and VWF:GPIbM are lower postoperatively in low risk surgeries.

Individual VWF and FVIII PK parameters

The PK parameters clearance, volume of distribution (central and peripheral) and elimination half-life were calculated using a perioperative FVIII population PK model [13]. As this model is only valid starting from initiation of surgery, PK parameters were

calculated from first postoperative time point onwards until 48 hours after surgery. Figure 4 shows no differences between time points for clearance (one-way ANOVA (F(2,152) = 0.02, p = 0.98), volume of distribution (one-way ANOVA (F(2,152) = 0.12, p = 0.89) and elimination half-life (one-way ANOVA (F(2,152) = 0.43, p = 0.65). To investigate how FVIII clearance evolves over time and to evaluate VWF:Ag influence on clearance, another linear mixed effect model was created. This final model with FVIII clearance as outcome, time as a linear function (in hours) and VWF:Ag (in IU/mL) was divided into four categories (quartiles), both were added as fixed effects in the model, with time as an additional random effect. The lowest VWF:Ag level quartile (0.43 - 0.92 IU/mL) was associated with a minimal increase of 26 mL/h (95% CI 2 – 50 mL/h) in FVIII clearance when compared to the highest VWF:Ag level quartile of (1.70 – 3.84 IU/mL) (Table 3). In addition, sub analyses showed that FVIII clearance was not associated with mode of administration (bolus administration versus continuous infusion), when adding mode of administration in the model as an additional fixed effect.

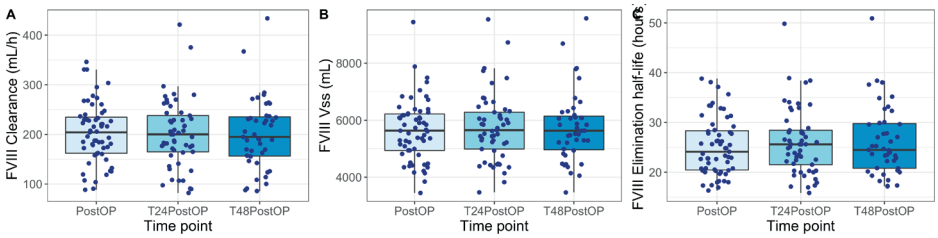


Figure 4. No differences between FVIII PK parameters at various time points in perioperative follow up period. For each boxplot, whiskers depict 2.5th and 97.5th percentile of the data, whereas the box depicts interquartile range. Median of the data is depicted by the black horizontal line inside the boxplot. A) Similar FVIII clearance; B) Similar volume of distribution in steady state (Vss) during the perioperative follow up period and; C) The elimination half-life of FVIII does not differ postoperative.

Perioperative bleeding

Both VWF:Ag and VWF:GPIbM were shown to increase postoperatively, suggesting an overall increase of procoagulant hemostatic factors . As some hemophilia A patients experience bleeding despite perioperative replacement therapy, associations between lower VWF:Ag and VWF:GPIbM and perioperative bleeding were investigated. Nine of the 59 study patients experienced bleeding, requiring additional FVIII concentrate treatment. In figure 5 results of two-way ANOVA are visualized which analyzes interactions between VWF:Ag and perioperative bleeding. No association between log(VWF:Ag), bleeding and perioperative time point was found F(4,227) = 0.54, p = 0.710) as patients with perioperative bleeding had similar log(VWF:Ag) levels when compared to patients without bleeding. Additionally, no association was found between log(VWF:GPIbM) of perioperative patients with and without surgical bleeding F(4,227) = 0.80, p = 0.525). Subanalysis showed that 4 of 24 (17%) patients had a VWFpp/VWF:Ag ratio >1.5 imme-

diately after surgery with a bleeding complication, while this was the case in 5 of 31 (16%) patients with a ratio <1.5. A Fisher exact test confirmed no statistically significant difference in risk between the subgroups (*p* value = 1.000).

Table 3. The association between VWF:Ag and FVIII concentrate clearance in the perioperative period. A linear mixed effects model was created with FVIII concentrate clearance as an outcome and time since start surgery and VWF:Ag as fixed effects. Time was set at t=0 at moment of first incision by the operating surgeon and was defined as a linear function. Time since start surgery was also set as a random effect. VWF:Ag was categorized according to quartiles. The reference category was the highest quartile with VWF:Ag levels between 1.70 – 3.84 IU/mL.

Fixed effects	Coefficient	95% Confidence interval	P-value
Intercept	183	161 – 205	0.000
Time since start surgery (hours)	0	0 – 0	0.647
VWF:Ag			
First quartile (0.43 – 0.92 IU/mL)	26	2 – 50	0.034
Second quartile (0.92 – 1.33 IU/mL)	23	0 – 46	0.056
Third quartile (1.33 – 1.70 IU/mL)	9	-10 – 28	0.367

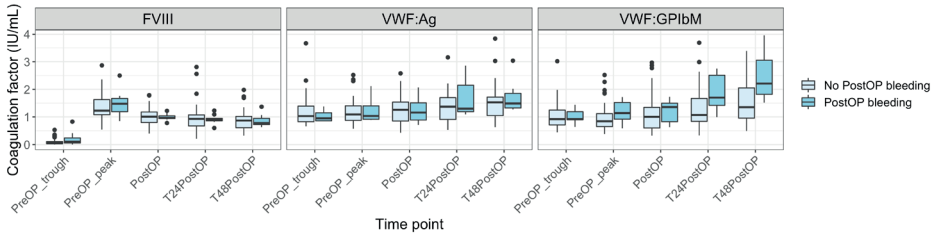


Figure 5. VWF:Ag or VWF:GPIbM are not associated with postoperative bleeding.
For each boxplot, whiskers depict 2.5th and 97.5th percentile of the data, whereas the box depicts the interquartile range. Median of the data is depicted by the black horizontal line inside the boxplot. Boxplots in light blue represent hemophilia A patients without postoperative bleeding, darker blue depicts patients with a postoperative bleeding. A) FVIII levels were similar between patients with and without postoperative bleeding. Of course, FVIII levels were low before surgery as all these patients received replacement therapy with FVIII concentrate. B) No association in VWF:Ag levels between patients with and without a bleeding; C) Finally, VWF:GPIbM levels were also similar between patients with and without a bleeding.

DISCUSSION

The present study was designed to investigate VWF and its influence on FVIII clearance in perioperative hemophilia A patients. Both VWF:Ag and VWF:GPIbM increased postoperatively but with large interpatient variability. Blood type non-O and medium surgical risk however were associated with higher perioperative VWF:Ag or VWF:GPIbM levels compared to blood type O and low surgical risk. Importantly, differences in VWF were associated with only minimal changes in FVIII concentrate clearance. Furthermore, VWF:Ag

and VWF:GPIbM levels were similar between patients with and without postoperative hemorrhage.

To the best of our knowledge, VWF levels in perioperative hemophilia A patients have not been studied in detail. Therefore, we are the first to describe VWF kinetics in hemophilia A patients undergoing elective surgery. Similar to results observed in mainly female patients without a bleeding disorder undergoing orthopedic surgery , VWF:Ag and VWF:GPIbM increased over time [6]. This can be explained by increased release of VWF from Weibel Palade bodies in the vascular endothelium due to adrenergic stress reactions and other related mechanisms causing endothelial activation such as blood flow turbulence, blood pressure variation, medication, hemostatic challenge due to surgery, and possibly increased VWF release to compensate for perioperative increase of VWF clearance [14,15]. In our study, besides a release of mature VWF (VWF:Ag), an increased release of VWFpp from Weibel Palade bodies was observed as VWFpp/VWF:Ag ratios increased significantly directly after surgery with a subsequent decrease. Normally in steady state, VWFpp and VWF are present in plasma with a molar ratio of 1:10 [16]. When acute release of both VWFpp and mature VWF (VWF:Ag) occurs, the molar ratio between VWFpp and VWF:Ag may increase up to four/five-fold [16]. However, VWFpp/VWF:Ag ratio is expressed in units, thereby set to one, and not expressed in molar amounts. An acute release of both VWFpp and VWF:Ag results in equal increases in molar amounts, but when interpreted as units, the increase of VWFpp will be much higher. Initially, during an acute release phase, VWFpp/VWF:Ag ratio increases due to increases of both VWFpp and VWF:Ag. However as VWFpp has a half-life of approximately two hours, and VWF:Ag a half-life of 8-12 hours, rapid VWFpp increase will also diminish within a short time period. Our findings support this hypothesis as VWFpp/VWF:Ag ratio was shown to normalize 48 hours after surgery. In addition, the VWFpp/VWF:Ag ratio will also normalize as a result of a probable increased consumption of VWF:Ag after surgery.

Acute phase VWF response may also be quantified by calculating VWF:Ag/VWF:GPIbM ratio. As VWF:Ag/VWF:GPIbM ratio decreased during subsequent postoperative days in our study, we hypothesized that this may be a consequence of the following pathophysiological mechanisms. Firstly, higher ADAMTS13 activity may be present due to surgery as a result of increased release of high molecular weight (HMW) VWF multimers. However, this is unlikely as Kahlon et al. has shown that ADAMTS13 actually decreases after surgery. Secondly, constitutive secretion of VWF from the endothelium may increase, resulting in more low molecular weight (LMW) VWF during surgery[17]. As strictly regulated VWF secretion from Weibel Palade bodies results in more HMW VWF multimers, more constitutive secretion with more LMW VWF may lead to a smaller VWF:Ag/VWF:GPIbM ratio. Thirdly, utilization of large HMW VWF multimers during surgical clot formation

may lead to decreasing postoperative VWF:Ag/VWF:GPIbM ratios, as HMW VWF multimers have the highest platelet binding activity.

Most likely, observed interpatient variability of VWF is multifactorial. However, in our linear mixed-effect model, blood type and surgical risk were shown to most relevant when predicting VWF:Ag fluctuations over time. As it is well known that VWF levels are on average 25% lower in patients with blood type O, it was not surprising to find blood type as an important risk factor for lower VWF levels [18]. Patients undergoing surgery with a medium surgical risk were associated with higher VWF:Ag levels compared to those with a low surgical risk, explained by greater physical adrenergic (shear) stress reactions as a consequence of more extensive surgery. Influence of VWF levels of age and BMI were unexpectedly small. Prior studies have identified age and BMI as important covariates when predicting VWF levels [19,20]. Therefore, exclusion of these variables was overruled. Unfortunately, extensive testing of VWF:Ag modifying factors was limited, due to small patient numbers.

Although the highest quartile VWF:Ag levels (VWF 1.70 – 3.84 IU/mL) was associated with a decrease of 26 mL/h (95% CI 2 – 50 mL/h) in FVIII clearance when compared to lowest VWF:Ag level quartile (0.43 – 0.92 IU/mL), VWF effects on FVIII clearance were only minimal and not as important as expected. However, a recent pilot study by Loomans et al. was also not able to show a decreased FVIII clearance with increased FVIII half-life after intravenous desmopressin infusion before FVIII concentrate administration. Study hypothesis was also that endogenous VWF increase after desmopressin would positively affect FVIII levels [21]. In our study, sufficiently high VWF levels, as is characteristic for the perioperative setting in non-VWD patients, may lead to a threshold effect and therefore not significantly affect FVIII clearance. Therefore, if patients have sufficiently high VWF levels, dosing of FVIII concentrate need not be adapted based on these VWF levels. However, it is important to realize thatPK parameters in our study were calculated with a perioperative population PK model without a time-dependent variable for clearance. This makes it more difficult to observe subtle changes of FVIII clearance over time [11]. Therefore, a limitation of our study is that the design may not be ideal to establish VWF effects on FVIII clearance during the perioperative time period. The novel perioperative population FVIII PK model under construction and enriched with prospectively collected VWF and FVIII levels from our randomized controlled OPTI-CLOT trial be will lead further elucidate FVIII clearance mechanisms.

Only a small number of study patients e.g. nine out of 59 (15%), experienced perioperative bleeding. Bleeding was not associated with VWF levels or VWFpp/VWF:Ag ratio. In addition, no statistically significant differences were observed in VWF:Ag or VWF:GPIbM

when comparing patients with surgical bleeding and patients without bleeding. We could not prove the hypothesis that VWFpp/VWF:Ag ratios higher than 1.5 were associated with perioperative bleeding as higher VWF clearance could potentially lead to an inadequate primary hemostasis. Capacity for statistical analyses were however limited due to small patient and complication numbers.

In conclusion, we are the first to report on VWF kinetics and FVIII clearance in perioperative hemophilia patients. VWF increased perioperatively in hemophilia A patients with blood type and surgical risk as most important predictors of VWF increase. VWF levels only showed a small effect on FVIII clearance and were not associated with perioperative hemorrhage. We recommend further investigation into VWF and its role in the perioperative period of hemophilia A patients by refinement of current population PK models with VWF data and ultimate population PK-pharmacodynamic modelling to further unravel pathophysiological mechanisms of the hemostatic system.

REFERENCES

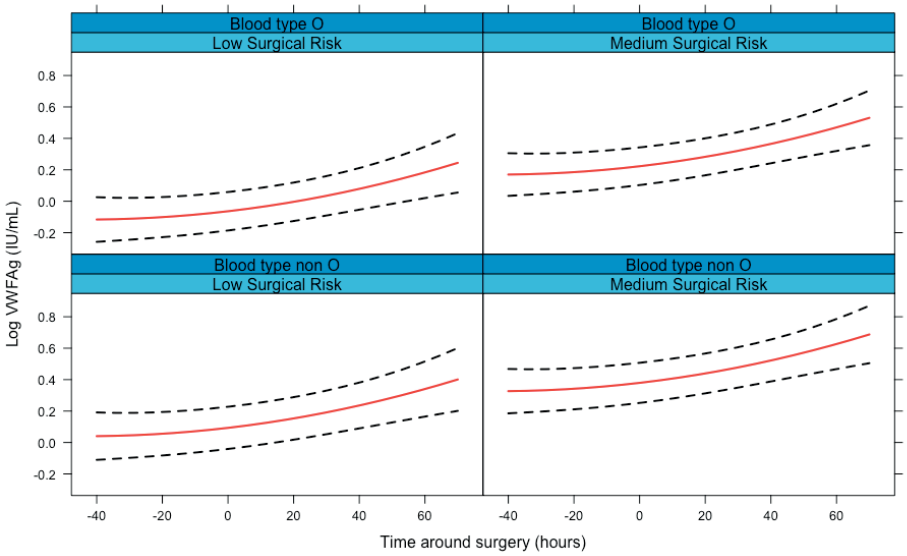
1. Srivastava A, Brewer AK, Mauser-Bunschoten EP, et al. Guidelines for the management of hemophilia. Haemophilia 2013;19:e1-47.
2. Hazendonk HC, Lock J, Mathot RA, et al. Perioperative treatment of hemophilia A patients: blood group O patients are at risk of bleeding complications. J Thromb Haemost 2016;14:468-78.
3. Leebeek FWG, Mauser-Bunschoten EP. Richtlijn diagnostiek en behandeling van hemofilie en aanverwante hemostase stoornissen. Utrecht: Van Zuiden Communications BV; 2009:1-197.
4. Pipe SW, Montgomery RR, Pratt KP, Lenting PJ, Lillicrap D. Life in the shadow of a dominant partner: the FVIII-VWF association and its clinical implications for hemophilia A. Blood 2016.
5. Fijnvandraat K, Peters M, ten Cate JW. Inter-individual variation in half-life of infused recombinant factor VIII is related to pre-infusion von Willebrand factor antigen levels. Br J Haematol 1995;91:474-6.
6. Kahlon A, Grabell J, Tuttle A, et al. Quantification of perioperative changes in von Willebrand factor and factor VIII during elective orthopaedic surgery in normal individuals. Haemophilia 2013;19:758-64.
7. Hazendonk HC, van Moort I, Fijnvandraat K, et al. The “OPTI-CLOT” trial. A randomised controlled trial on periOperative PharmacokineTIc-guided dosing of CLOTting factor concentrate in haemophilia A. Thromb Haemost 2015;114:639-44.
8. Koshy M, Weiner SJ, Miller ST, et al. Surgery and anesthesia in sickle cell disease. Cooperative Study of Sickle Cell Diseases. Blood 1995;86:3676-84.
9. Schulman S, Angeras U, Bergqvist D, et al. Definition of major bleeding in clinical investigations of antihemostatic medicinal products in surgical patients. J Thromb Haemost 2010;8:202-4.
10. Sanders YV, Groeneveld D, Meijer K, et al. von Willebrand factor propeptide and the phenotypic classification of von Willebrand disease. Blood 2015;125:3006-13.
11. Hazendonk H, Fijnvandraat K, Lock J, et al. A population pharmacokinetic model for perioperative dosing of factor VIII in hemophilia A patients. Haematologica 2016.
12. Jonsson EN, Karlsson MO. Xpose--an S-PLUS based population pharmacokinetic/pharmacodynamic model building aid for NONMEM. Comput Methods Programs Biomed 1999;58:51-64.
13. Hazendonk H, Fijnvandraat K, Lock J, et al. A population pharmacokinetic model for perioperative dosing of factor VIII in hemophilia A patients. Haematologica 2016;101:1159-69.
14. van Loon JE, Sonneveld MA, Praet SF, de Maat MP, Leebeek FW. Performance related factors are the main determinants of the von Willebrand factor response to exhaustive physical exercise. PLoS One 2014;9:e91687.
15. Claus RA, Bockmeyer CL, Sossdorf M, Losche W, Hilberg T. Physical stress as a model to study variations in ADAMTS-13 activity, von Willebrand factor level and platelet activation. J Thromb Haemost 2006;4:902-5.
16. Borchiellini A, Fijnvandraat K, ten Cate JW, et al. Quantitative analysis of von Willebrand factor propeptide release in vivo: effect of experimental endotoxemia and administration of 1-deamino-8-D-arginine vasopressin in humans. Blood 1996;88:2951-8.
17. Sporn LA, Marder VJ, Wagner DD. Inducible secretion of large, biologically potent von Willebrand factor multimers. Cell 1986;46:185-90.

18. Castaman G, Tosetto A, Eikenboom JC, Rodeghiero F. Blood group significantly influences von Willebrand factor increase and half-life after desmopressin in von Willebrand disease Vicenza. J Thromb Haemost 2010;8:2078-80.

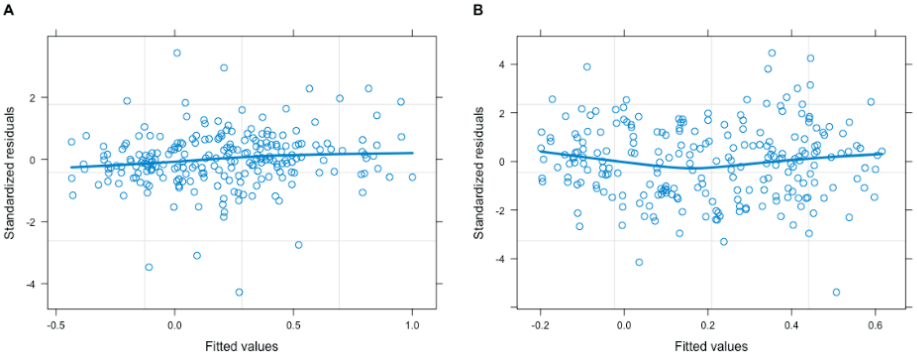
19. Sanders YV, Giezenaar MA, Laros-van Gorkom BA, et al. von Willebrand disease and aging: an evolving phenotype. J Thromb Haemost 2014;12:1066-75.

20. Atiq F, Fijnvandraat K, van Galen KPM, et al. BMI is an important determinant of VWF and FVIII levels and bleeding phenotype in patients with von Willebrand disease. Am J Hematol 2019;94:E201-E5.

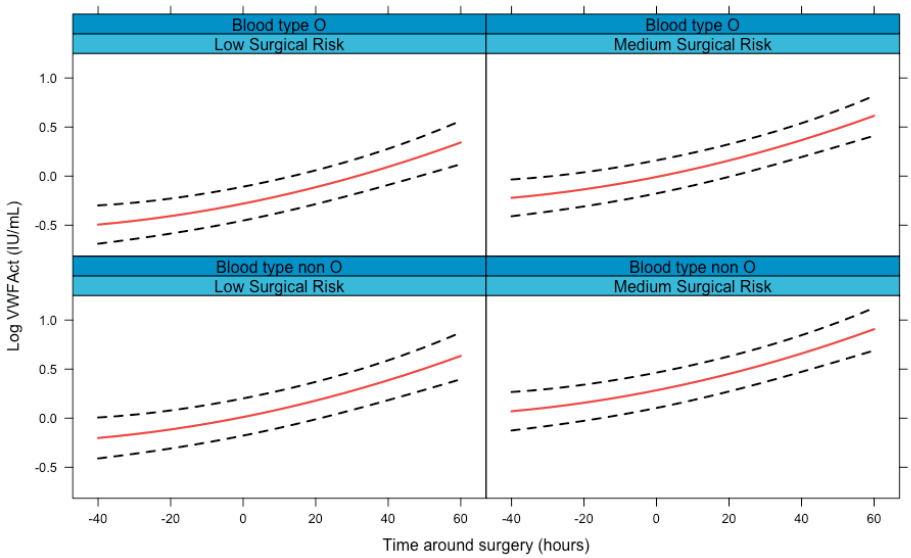
21. Loomans JI, Stokhuijzen E, Peters M, Fijnvandraat K. Administration of DDAVP did not improve the pharmacokinetics of FVIII concentrate in a clinically significant manner. J Clin Transl Res 2018;3:351-7.



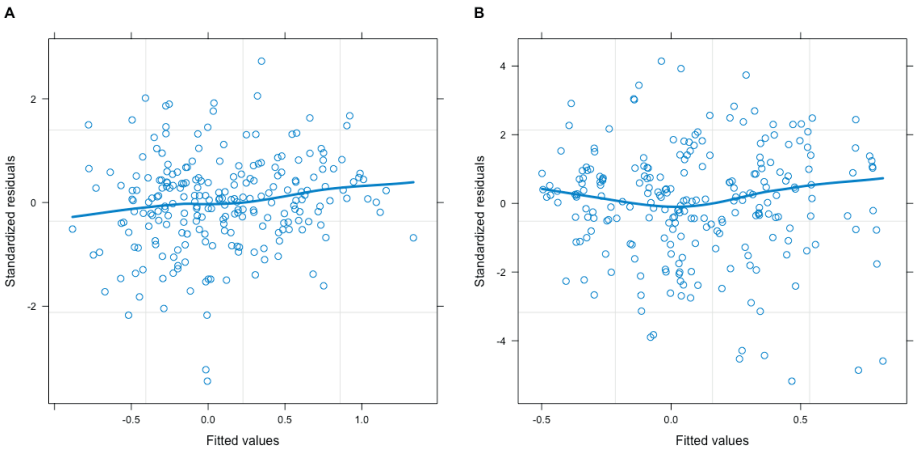
Supplementary Figure 1. Effect plot of linear mixed effect model describing log(VWF:Ag) in the perioperative period in blood type O versus non O patients and low risk versus medium risk surgical patients. Red line represents the linear mixed effects model of log(VWF:Ag) during the perioperative period for a mean patient of this cohort with a BMI of 26.4 kg/m^2 and age of 48.8 years. The dotted line represents the 95% confidence interval.



Supplementary Figure 2. Scatter plots of the residuals versus the fitted values to check model assumptions for the linear mixed effects model investigating associations between the determinants blood type, surgical risk, age, BMI and the outcome log(VWF:Ag). Scatter plots derived from the linear mixed effect model of log(VWF:Ag), showing no violation to the model assumptions. A) Standardized residuals versus fitted values; B) Marginal residuals versus fitted values.



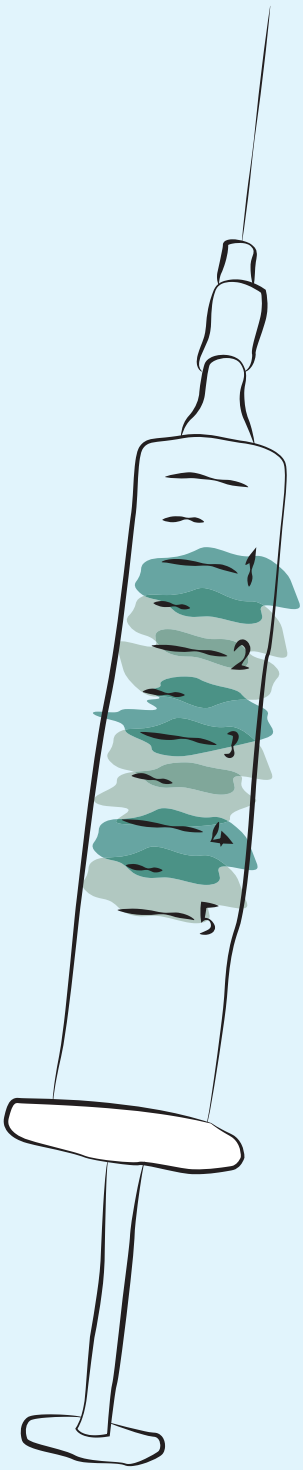
Supplementary Figure 3. Effect plot of linear mixed effect model describing log(VWF:GPIbM) in the perioperative period in blood type O versus non O patients and low risk versus medium risk surgical patients. Red line represents the linear mixed effects model of log(VWF:Ag) during the perioperative period for a mean patient of this cohort with a BMI of 26.4 kg/m^2 and age of 48.8 years. The dotted line represents the 95% confidence interval.



Supplementary Figure 4. Scatter plots of the residuals versus the fitted values to check model assumptions for the linear mixed effects model investigating associations between the determinants blood type, surgical risk, age, BMI and the outcome log(VWF:GPIbM). Scatter plots derived from the linear mixed effect model of log(VWF:GPIbM), showing no violation to the model assumptions. A) Standardized residuals versus fitted values; B) Marginal residuals versus fitted values.

Supplementary Table 1. Associations between the determinants blood type, surgical risk, age, BMI and the outcome log(VWF:GPIbM). Linear mixed-effects modeling was used to determine the associations with the outcome log(VWF:GPIbM). Time was set at t=0 at moment of first incision by the operating surgeon and was defined as a non-linear function. Especially blood type non-O and medium surgical risk were associated with higher VWF:GPIbM levels perioperatively.

Fixed effects	Coefficient	95% Confidence interval	P-value
Intercept	-0.351	-0.931 – 0.230	0.242
Time since start surgery (hours)	0.007	0.006 – 0.009	< 0.001
Time since start surgery^2 (hours)	0.000	0.000 – 0.000	< 0.001
Surgical Risk, medium risk	0.302	0.091 – 0.518	0.007
Blood group, type O	-0.291	-0.502 – -0.082	0.009
Age (years)	0.004	-0.003 – 0.010	0.300
BMI (kg/m^2)	0.008	-0.010 – 0.025	0.395



8

Dosing of factor VIII concentrate by ideal body weight is more accurate and seems safe in overweight and obese hemophilia A patients

van Moort I[*], Preijers T*, Hazendonk HCAM, Schutgens REG, Laros-van Gorkom BAP, Nieuwenhuizen L, van der Meer FJM, Fijnvandraat K, Leebeek FWG, Meijer K, Mathôt RAA[**], Cnossen MH[**], for the "OPTI-CLOT" study group.

Submitted

ABSTRACT

Aim Under-and especially overdosing of replacement therapy in hemophilia A patients may be prevented by application of other morphometric variables than body weight to dose factor VIII (FVIII) concentrates. Therefore, we aimed to investigate which morphometric variables best describe inter-individual variability (IIV) of FVIII concentrate pharmacokinetic (PK) parameters.

Methods PK profiling was performed by measuring three FVIII levels after a standardized dose of 50 $IUkg^{-1}$ FVIII concentrate. A population PK model was constructed, in which inter-individual variability (IIV) for clearance (CL) and central volume of distribution (V1) was quantified. Relationships between CL, V1 and five morphometric variables (body weight, ideal body weight IBW, lean body weight, adjusted body weight, and body mass index (BMI)) were evaluated in a cohort of normal weight (BMI <25 kgm^{-2}), overweight (BMI 25-30 kgm^{-2}) and obese hemophilia A patients (BMI >30 kgm^{-2}).

Results Fifty-seven hemophilia A patients (FVIII ≤0.05 $IUmL^{-1}$) were included with a median body weight of 83 kg (range: 53-133) and a median age of 48 years (range: 18-77). IBW best explained observed variability between patients, as IIV for CL and V1 was reduced from 45.1% to 37.6% and 26.8% to 14.1%, respectively. CL, V1, and half-life were similar in normal weight, overweight and obese patients. Simulated FVIII trough and peak levels in normal body weight patients were similar to those in obese patients when dosing was based on IBW, also in cases of dosing for life-threatening bleeds.

Conclusion Ideal body weight most accurately calculates FVIII concentrate doses in overweight and obese hemophilia A patients.

INTRODUCTION

Hemophilia A is an X-linked inherited bleeding disorder caused by a deficiency in coagulation factor VIII (FVIII). To prevent and treat bleeding in muscles and joints, hemophilia A patients are infused either prophylactically or on demand with intravenous replacement therapy consisting of recombinant or plasma derived FVIII concentrates [1,2]. Current FVIII concentrate dosing schedules are based on body weight. It is however well-known that a large inter-individual variability exists of observed FVIII levels after FVIII concentrate infusion which results in both under- and overdosing when specific FVIII ranges are targeted [3,4].

Dosing based on pharmacokinetics (PK) is able to reduce under-and overdosing. However, when applying this method, an individual's characteristics should be comparable to those individuals which have contributed data to the population PK model. In hemophilia A, this is especially important for age and body weight, as these characteristics have been shown to be closely associated with FVIII PK [4]. Recently, a meta-analysis in hemophilia patients demonstrated that the prevalence of overweight and obesity is steadily increasing with a pooled prevalence that has risen from 17% (95% CI: 15.0-19.3%) to 31% (95% CI: 26.8–36.2%) in the last decade [5]. This underlines the urgency to include representative numbers of overweight and obese patients in population PK dosing models for factor concentrates in hemophilia treatment.

More specifically, several authors report that obese patients defined as individuals with a body mass index (BMI) >30 kgm^{-2} have a higher in vivo recovery (IVR) of intravenously administered factor concentrates than patients with a normal BMI, defined as a BMI of 20-25 kgm^{-2} [6,7]. This is explained by the fact that obese patients receive higher weight-based doses that distribute over similar volumes of distribution to those measured in normal weight individuals, as the intravascular compartment does not increase with weight gain. This is however, mainly relevant for FVIII peak levels, as FVIII trough levels and steady state FVIII levels are more dependent on FVIII clearance parameters [8]. As parameters from a population PK model are generally scaled using body weight of the patient [3,4,9], other morphometric variables which also take body composition into account may have a higher predictive performance when describing FVIII PK parameters in overweight and obese hemophilia A patients [10,11]. Various population models describe PK variability observed after FVIII concentrate dosing in hemophilia A patients, incorporating different morphometric variables such as body weight, lean body mass or ideal body weight [3,4,12]. However, no study has yet investigated how different morphometric variables correlate with FVIII PK parameters using population PK modeling with real-

world data from hemophilia A patients treated with various FVIII concentrates. In addition, risk analyses for such an approach have not yet been reported.

Therefore, this study explores the extent to which morphometric variables other than body weight explain inter-individual variability of FVIII PK parameters in normal, overweight and obese hemophilia A patients. Our study is the first to apply population PK analysis using real-world patient data of which a representative number is overweight or obese. Moreover, with this real-world population PK model involved risks will be simulated to analyze results of this approach in critical circumstances when underdosing must be avoided.

METHODS

Patients

In this cross-sectional study, severe and moderate hemophilia A patients were included with endogenous baseline FVIII activity levels ≤ 0.05 $IUmL^{-1}$, aged older than or equal to 18 years, and without inhibitory FVIII antibodies (Bethesda Units (BU) < 0.2 IU). Patients were enrolled from six Academic Hemophilia Treatment Centers in the Netherlands (Erasmus University Medical Center Rotterdam, University Medical Center Groningen, University Medical Center Utrecht, Radboud university medical center Nijmegen / Maxima Medical Center, Veldhoven, Leiden University Medical Center/Haga Hospital, The Hague, Amsterdam University Medical Centers). This study was approved by the Medical Ethics Committee of the Erasmus University Medical Center and all patients gave written informed consent before enrollment according to the declaration of Helsinki. Although data mostly originates from patients enrolled in the perioperative OPTI-CLOT trial [13] (n=48) and consists of pre-operative individual PK profiling data, some patients (n=9) were included from a separate study investigating PK tools for FVIII dosing [14]. This study was not subject to the Medical Research Involving Human Subjects Act (WMO) and was approved by the Medical Ethics Committee of the Erasmus University Medical Center.

Blood sampling and analyses

A single intravenous dose of 50 $IUkg^{-1}$ FVIII concentrate was administered to each patient. Patients received the following recombinant FVIII concentrates (Kogenate FS®: Bayer, Berkeley, Ca, USA; Advate®: Baxter Bioscience, Thousand Oaks, CA, USA; Refacto AF®: Pfizer, New York, NY USA; NovoEight®: Novo Nordisk, Bagsværd, Denmark) or plasma-derived FVIII concentrates (Aafact®: Blood Transfusion council of the Netherlands Red Cross, Amsterdam, the Netherlands). In general, three FVIII level measurements were obtained at 4, 24 and 48 hours after FVIII bolus administration. In a minority of patients,

also a pre-infusion FVIII level was measured. The need for a washout period and baseline measurement was avoided by collecting time of dosing and doses of three previous FVIII concentrate infusions. FVIII plasma levels were measured locally in each treatment center, using a one-stage clotting assay.

Morphometric variables

For each patient, the following patient characteristics were collected: endogenous baseline FVIII level (IU/ml), inhibitor status, age (months), body weight (BW in kg) and height (cm). Lean body mass was determined using a bioelectrical impedance analyzer (Maltron BF-906, Maltron International, Rayleigh, United Kingdom). Using the body weight and height of the patient, the following morphometric variables were calculated: body mass index (BMI; kgm^{-2}) [15], ideal body weight (IBW; kg) [16], adjusted body weight (ABW; kg) [16], and calculated lean body mass (LBMc; kg) [17]. The equations used to calculate morphometric variables are presented in Supplementary Table S1. Patients were categorized into three BMI categories: normal weight patients (BMI < 25 kgm^{-2}), overweight patients (25 kgm^{-2} ≤ BMI ≤ 30 kgm^{-2}), and obese patients (BMI > 30 kgm^{-2}).

Pharmacokinetic modeling

A structural population PK model was constructed describing inter-individual variability of PK parameters. Models were compared using the objective function value (OFV). If the difference of the OFV between two models was larger than 3.84 it was considered to be statistically significant with a $p<0.05$.Subsequently, it was evaluated to what extent the various morphometric variables explained this variability (applied equations are described in Supplementary Table S1)..

The structural PK model was developed using nonlinear mixed-effects modeling software NONMEM v7.4 (ICON Development Solutions, Ellicott City, MD, USA) with the FVIII level data of all patients simultaneously. The endogenous baseline FVIII activity level for each patient, especially important in the moderate hemophilia patients (FVIII 0.01-0.05 IU/ml), at time of individual PK profiling was calculated by subtraction from predicted FVIII levels. Moreover, residual FVIII levels due to previous prophylactically administered FVIII concentrate doses were also taken into account.

The following PK parameters were estimated: clearance (CL), inter-compartmental clearance (Q), volume of distribution of the central (V1) and peripheral (V2) compartment. R software v3.4.1 (R Core Team; 2017) and Xpose v4.5.3 were used for data exploration and model diagnostics [18].

Evaluation of the morphometric variables

After establishing the structural population PK model, the ability of the morphometric variables to explain inter-individual variability of the obtained population PK parameters was evaluated. The difference between two values for OFV (dOFV) from two different models can be described by Chi-squared distribution in the case of nested models and, hence, a statistical significance can be calculated in terms of a *p*-value. However, the OFV does not consider the difference in number of parameters between two evaluated models. The Akaike information criterion (AIC) is based on the OFV and adds a penalty for the number of parameters from the corresponding model. For calculating AIC, the evaluated models do not necessarily have to be nested. As the model with the least number of parameters and the highest ability to describe measured FVIII levels is most favorable, AIC was used instead of OFV in this study evaluation. Besides the predictive ability of the model, reduction in inter-individual variability of CL and V1 was considered for model selection as well.

Allometric scaling was used to describe the correlation between morphometric variables and PK parameters according to the following equation:

$$\theta_{TV} = \theta_{Pop} x \left(\frac{MVi}{MV_{med}}\right)^{EXP_{allo}} \quad \text{(Eq. 1)}$$

in which θ_{TV} is the typical value for the population PK parameter (i.e. the median value), θ_{Pop} is the estimated population value for the population PK parameter, MV_i and MV_{med} are the individual value and median for the morphometric variable, respectively, and EXP_{allo} is the allometric exponent.

Allometric scaling was evaluated in two ways. Firstly, relationships between morphometric variables and PK parameters were evaluated with EXP_{allo} fixed to the values of 0.75 and 1 for the clearance parameters (CL and Q) and for the volume of distribution parameters (V1 and V2), respectively.[19] Secondly, EXP_{allo} values were estimated for clearance and volume of distribution parameters.

PK parameter evaluation

Individual (posthoc) PK parameters were estimated by *maximum a posteriori* (MAP) Bayesian analysis using the final model with inclusion of the morphometric variable. For each patient, terminal elimination half-life and *in vivo* recovery were calculated. The *in vivo* recovery was obtained by dividing body weight of the patients by the individual PK parameter estimate for V1. A Kruskall-Wallis test was used to compare the individual PK parameter estimates obtained for the patients from the three BMI categories, as the data was not normally distributed. *P*-values <0.01 were considered statistically significant.

Simulations of single dose and dosing of FVIII concentrate in case of a life-threatening bleed

For all patients in this cohort, peak and trough FVIII levels were simulated after a single dose of 50 $IUkg^{-1}$, and after treating a life-threatening bleed by infusing a loading dose of 50 $IUkg^{-1}$ followed by a twice daily dose of 25 $IUkg^{-1}$. The trough FVIII levels were obtained immediately before the 6^{th} dose, which corresponded with 72 hours after administration of the loading-dose. A Kruskall-Wallis test was used to compare the FVIII levels and *P*-values <0.01 were considered statistically significant.

RESULTS

Patient characteristics

In total, 57 severe and moderate hemophilia A patients were included with a median body weight of 83 kg (range: 53-133 kg) and a median age of 48 years (range: 18.4-76.9 years). In the three BMI categories, 26 patients (46%) had a normal body weight, 21 patients (37%) were overweight, and 10 patients (18%) were obese. General patient characteristics of the study population and medians and ranges of the morphometric variables are presented in Table 1. In this study, lean body mass was not measured for two patients (4%), due to logistical reasons. In these patients, median measured lean body mass of the population was used instead.

Pharmacokinetic modeling

A two-compartment model performed best as structural model (Table 2). Inter-individual variability could be estimated for both CL and V1. Inclusion of a correlation between the inter-individual variability of both parameters allowed a significantly better fit of the model with measured FVIII levels. In Supplementary Figure S2, the goodness-of-fit of the structural model is presented. Although a small deviation of population predictions of highest FVIII levels was observed, the main part of population predictions was distributed symmetrically around the line y=x demonstrating adequacy of the model to describe measured FVIII levels. When accounting for inter-individual variability by Bayesian analysis, the individual profiles were also well described, as practically all predictions were present on the line y=x (Figure S1-B). Furthermore, the visual predictive check (VPC) showed a good description of the data, and that the model was able to accurately predict the FVIII levels of each patient (Supplementary Figure S3).

Table 1. General characteristics of the study population

	Total population	BMI	BMI	BMI
		< 25 mg/kg^2	25 mg/kg^2 ≤ and < 30 mg/kg^2	≥ 30 mg/kg^2
	No. (%) or median [range]	No. (%) or median [range]	No. (%) or median [range]	No. (%) or median [range]
Patient characteristics				
Total no. of patients	57	26	21	10
Age (years)	48.0 [18.4 - 76.9]	36.0 [18.4 - 69.1]	53.4 [26.9 - 76.9]	48.7 [32.3 - 70.9]
Severe hemophilia A (<0.01 IU mL^{-1})	37 (65)	18 (69)	12 (57)	7 (70)
Blood group O†	31 (54)	14 (54)	12 (57)	5 (50)
Height (m)	1.78 [1.48 - 1.95]	1.77 [1.58 - 1.95]	1.81 [1.48 - 1.95]	1.76 [1.68 - 1.92]
Body weight (kg)	83.0 [53.0 - 133]	73.0 [53.0 - 92.0]	88.5 [60.8 - 113]	99.6 [93.0 - 133]
Adjusted body weight (kg)	77.5 [51.9 - 104]	73.0 [54.2 - 89.9]	81.9 [51.9 - 98.5]	83.5 [76.7 - 104]
Body Mass Index (kg/m^2)	26.3 [19.0 - 42.6]	23.3 [19.0 - 24.9]	27.9 [25.5 - 29.9]	32.4 [30.7 - 42.6]
Ideal body weight (kg)	73.1 [46.0 - 88.5]	72.2 [55.0 - 88.5]	75.9 [46.0 - 88.5]	71.3 [64.1 - 85.8]
Lean body mass measured (kg)	61.4 [39.4 - 83.5]	57.9 [39.4 - 76.5]	62.5 [40.6 - 80.8]	64.9 [57.7 - 83.5]
Lean body mass calculated (kg)	62.8 [43.6 - 85.2]	57.4 [43.6 - 71.6]	66.9 [44.4 - 80.1]	68.3 [63.2 - 85.2]
Type of clotting factor concentrate				
Advate®	34 (59.6)	15 (57.7)	13 (61.9)	6 (60.0)
Aafact®	1 (1.8)	0 (0.0)	1 (4.8)	0 (0.0)
Kogenate FS®	13 (22.8)	6 (23.1)	4 (19.0)	3 (30.0)
NovoEight®	5 (8.8)	3 (11.5)	1 (4.8)	1 (10.0)
Refacto AF®	4 (7.0)	2 (7.7)	2 (9.5)	0 0

Table 1. General characteristics of the study population (*continued*)

	Total population	BMI < 25 mg/kg^2	BMI 25 mg/kg^2 ≤ and < 30 mg/kg^2	BMI ≥ 30 mg/kg^2
	No. (%) or median [range]	No. (%) or median [range]	No. (%) or median [range]	No. (%) or median [range]
Pharmacokinetic (PK) data				
Total number of observations	173	80	63	30
No. of observations per individual	3 [2 - 4]	3 [3 - 4]	3 [2 - 4]	3 [3 - 3]
No. of prior doses per individual	3 [1 - 6]	3.5 [1 - 6]	2 [1 - 5]	4 [1 - 4]
No. of observations day 1 (> 0h – 12h)	63 (36)	30 (38)	23 (37)	10 (33)
No. of observations day 2 (>12h – 36h)	56 (32)	25 (31)	21 (33)	10 (33)
No. of observations day 3 (>36h – 72h)	54 (31)	25 (31)	19 (30)	10 (33)

BMI = body mass index; No. indicates number; kg, kilogram; and IUmL^{-1}; international units per milliliter. † Blood group available in 56 patients.

Table 2. Estimated population PK parameters for structural model and final model

	Structural model			Final model		
	Estimate	**RSE (%)**	**Shr. [%]**	**Estimate**	**RSE (%)**	**Shr. [%]**
Structural model						
Clearance (CL; mLh^{-1})	242	(6)		236	(5)	
Volume of central compartment (V1; mL)	2620	(19)		2840	(8)	
Distribution CL to compartment 2 (Q; mLh^{-1})	192	(62)		122	(24)	
Volume of compartment 2 (V2; mL)	1070	(37)		821	(47)	
Inter-individual variability						
IIV on CL (%)	45.1	(20)	[1]	37.6	(19)	[2]
IIV on V1 (%)	26.8	(62)	[20]	14.1	(95)	[37]
Correlation between CL and V1	66.4	(46)		45.6	(64)	
Residual variability						
Additive residual variability ($IUdL^{-1}$)	0.66	(47.1)		0.65	(35)	
Proportional residual variability (%)	13.8	(23)		13.7	(22)	
Covariate relations						
CL – allometric exponent	-			1.65	(21)	
V1 – allometric exponent	-			1.34	(19)	
Model characteristics						
Objective function value	-186.1			-217		
Condition number	332			99		

RSE: relative standard error. Shr.: shrinkage. IIV: inter-individual variability. The individual values for the clearance parameters (CL, Q) and the volume of distribution parameters (V1, V2) from the final model are described by the following equations:

$$CL_i = 236\ x\ \left(\frac{IBW}{IBW_{med}}\right)^{1.65} x\ e^{\eta_{CL,i}}$$

$$V1_i = 2840\ x\ \left(\frac{IBW}{IBW_{med}}\right)^{1.34} x\ e^{\eta_{V1,i}}$$

$$Q_i = 122\ x\ \left(\frac{IBW}{IBW_{med}}\right)^{1.65}$$

$$V2_i = 821\ x\ \left(\frac{IBW}{IBW_{med}}\right)^{1.34}$$

in which IBW is the value for the ideal body weight and IBW_{med} is the median of for the ideal body weight from the studied population.

Evaluation of the morphometric variables

The established structural model was used to evaluate the ability of morphometric variables to explain the inter-individual variability of CL and V1. In Table 3, a summary of all allometric scaling evaluations with the five morphometric variables is presented. Looking at the models in which the allometric exponents were fixed, scaling parameters with ideal body weight produced the lowest AIC and the greatest reduction of inter-individual variability from V1. Interestingly, allometric scaling using the body weight of the patient resulted in a worse fit as compared with the model without allometric scaling, which signifies the need for allometric scaling using an adequate predictor.

Table 3. Summary of the covariate relationship selection process

Model and parameter	Covariate	OFV	AIC	dAIC	IIV on CL	IIV on V1	Correlation between CL & V1
Comparator model		-186.1	-168.1		45.1	26.8	66.4
Allometric scaling with fixed exponents							
	WT	-176.2	-158.2	9.9	43.7	26.7	60.1
	LBM	-208.1	-190.1	-22.1	39.5	16.4	46.7
	IBW	-212.1	-194.1	-26.0	40.2	15.6	52.9
	BMI	-160.9	-142.9	25.2	47.7	34.1	69.3
	ABW	-200.7	-182.7	-14.6	41.2	18.9	55.7
	LBMc	-197.3	-179.3	-11.3	42.1	20.4	58.1
Allometric exponents estimated for CL & V1							
	WT	-190.6	-168.6	-0.5	43.9	23.8	64.2
	LBM	-212.6	-190.6	-22.5	37.3	15.7	46.2
	IBW	-217.2	-195.2	-27.2	37.6	14.1	45.6
	BMI	-186.8	-164.8	3.3	44.8	26.7	66.1
	ABW	-202.3	-180.3	-12.2	40.7	18.7	57.0
	LBMc	-198.8	-176.8	-8.7	41.9	20.2	60.1

OFV: objective function value. AIC: Akaike information criterion. dAIC: change in the AIC as compared to the AIC from the comparator model. IIV: inter-individual variability. CL: clearance of the central compartment. V1: Volume of distribution of the central compartment. WT: bodyweight. LBM: measured lean body mass. IBW: ideal body weight. BMI: body mass index. ABW: adjusted bodyweight. LBMc: lean body mass calculated.

Ideal body weight best explained the inter-patient variability in FVIII PK. Scaling PK parameters by ideal body weight reduced the inter-patient variability in CL and V1 from 45.1 to 37.6% and from 26.8% to 14.1%, respectively. However, the obtained inter-individual variability on CL using allometric scaling with lean body mass (37.3%) was similar to the inter-individual variability obtained using allometric scaling with ideal body weight (37.6%), whereas not for the inter-individual variability obtained for V1 (15.7%). Nevertheless, a significant difference was obtained in the ability of the model to describe the measured FVIII data favoring allometric scaling using ideal body weight.

PK parameter evaluation

In the final population model with parameters scaled for ideal body weight, the allometric exponents for CL and V1 were estimated (Table 2). As both estimated allometric exponents were above 1, the relation with ideal body weight allowed a more than proportional increase for the individual PK parameters CL and V1. The relationship between ideal body weight and the individual PK parameter estimates is shown in Figure 1. However, the eta-shrinkage of the IIV on V1 is quite large (37%). Nevertheless, the conclusion still stands that with increasing ideal body weight, the typical (median) values for CL and V1 increase as well.

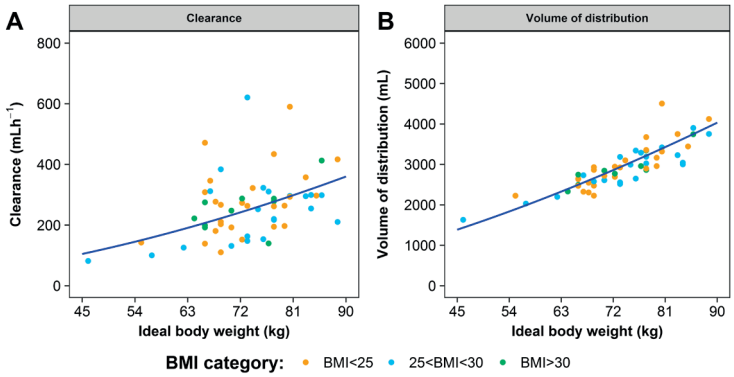


Figure 1. Clearance and volume of distribution increase when ideal body weight increases. (A) Clearance. (B) Volume of distribution of the central compartment. The individual PK parameter estimates were obtained by posthoc analysis using the final model. The blue line depicts the typical values, as calculated using the final model, versus ideal body weight. In both figures, an increase is demonstrated for the typical value with increasing ideal body weight.

In Figure 2, the individual posthoc PK parameter estimates for FVIII CL, V1, terminal half-life, and the calculated *in vivo* recovery are presented. For CL, V1, and terminal half-life, no significant differences were obtained for the values obtained within the three BMI

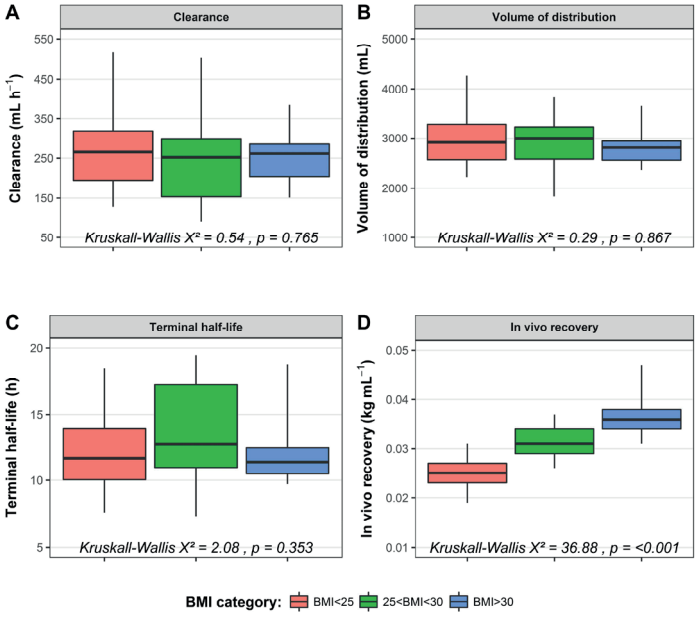


Figure 2. No differences in individual pharmacokinetic (PK) parameters (Clearance, Volume of distribution and terminal half-life) between BMI categories. (A) Clearance (B) Volume of distribution of the central compartment. (C) Terminal elimination half-life. (D) Calculated in vivo recovery. The in vivo recovery was calculated using the bodyweight of the patient divided by the individual PK parameters estimates for V1. For each boxplot, the whiskers depict the 2.5th and 97.5th percentile of the data, whereas the box depicts the interquartile range. The median of the data is depicted by the black horizontal line inside the boxplot.

categories. This shows that CL, V1 and terminal half-life were similar between normal weight, overweight and obese hemophilia A patients. However, the calculated *in vivo* recovery increased with increasing BMI and the values from the three BMI categories were significantly different ($X^2 = 28.8$, $p<0.001$).

Dosing in case of life-threatening bleed

PK simulations were performed using posthoc PK parameters from each patient included in this study. Body weight and ideal body weight were used to calculate the dose required in two clinical situations: a single dose of 50 IUkg^{-1} which may be a test-dose for PK profiling, and a dose required to treat a life threating bleed with a loading dose of 50 IUkg^{-1} followed by a twice daily dose of 25 IUkg^{-1}. In Figure 3, simulations are presented for one typical normal weight, one typical overweight and one typical obese patient. For the patient with a BMI <25 kgm^{-2}, no difference was observed between the FVIII levels calculated using body weight or ideal body weight. For the two other BMI categories, differences increased with increasing BMI for both achieved FVIII peak and trough levels. Importantly, dosing based on ideal body weight resulted in similar peak and trough levels for each BMI category.

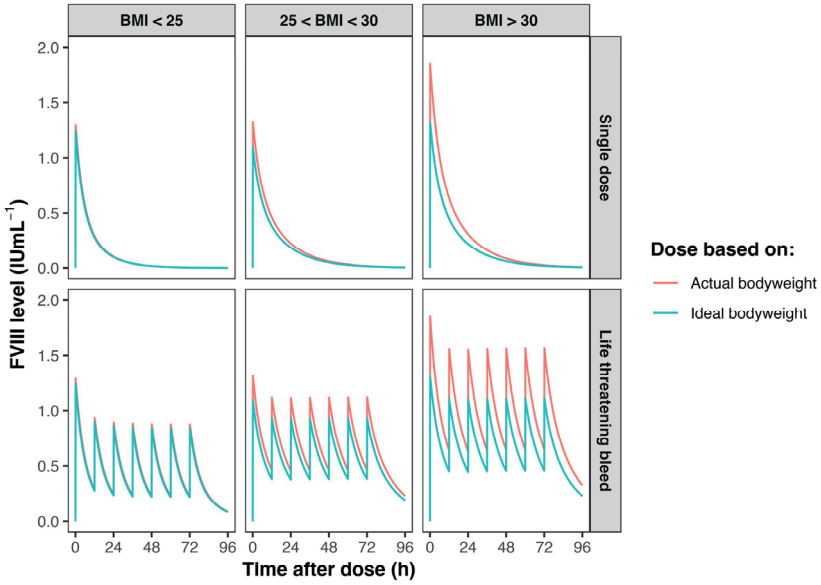


Figure 3. FVIII dosing based on body weight and ideal body weight in a normal weight, overweight and obese patient. The three examples were obtained by selecting typical patients from each body mass index (BMI: kgm^{-2}) category; normal weight (BMI <25 kgm^{-2}), overweight (BMI 25-30 kgm^{-2}) and obese (BMI >30 kgm^{-2}) patient. The lines from the plot depict the individual predicted FVIII levels after a simulated single-dose of 50 IUkg^{-1} (upper panels). In the lower panels, a simulated loading-dose was administered of 50 IUkg^{-1} followed by twice daily dosing of 25 IUkg^{-1} to treat a life-threatening bleed. The individual FVIII levels were estimated using the individual PK parameters from the corresponding example patients.

Figure 4 depicts the simulated FVIII trough and peak levels for all patients included in this cohort when treating a life-threatening bleed by a loading dose of 50 IUkg^{-1} followed by doses of 25 IUkg^{-1} twice daily. In all patients with dosing based on ideal body weight, no statistical differences were obtained for both FVIII peak (X^2 = 1.1, p=0.57) and trough (X^2 = 2, p=0.37) levels, whereas dosing based on body weight resulted in significant differences for both FVIII peak (X^2 = 33.5, p<0.001) and trough (X^2 = 9.43, p<0.009) levels. This demonstrates that dosing based on body weight results in unnecessary high peak levels in overweight/obese patients. Dosing based on ideal body weight however will prevent these high FVIII peak levels, while maintaining adequate FVIII trough levels to treat a life-threatening bleed.

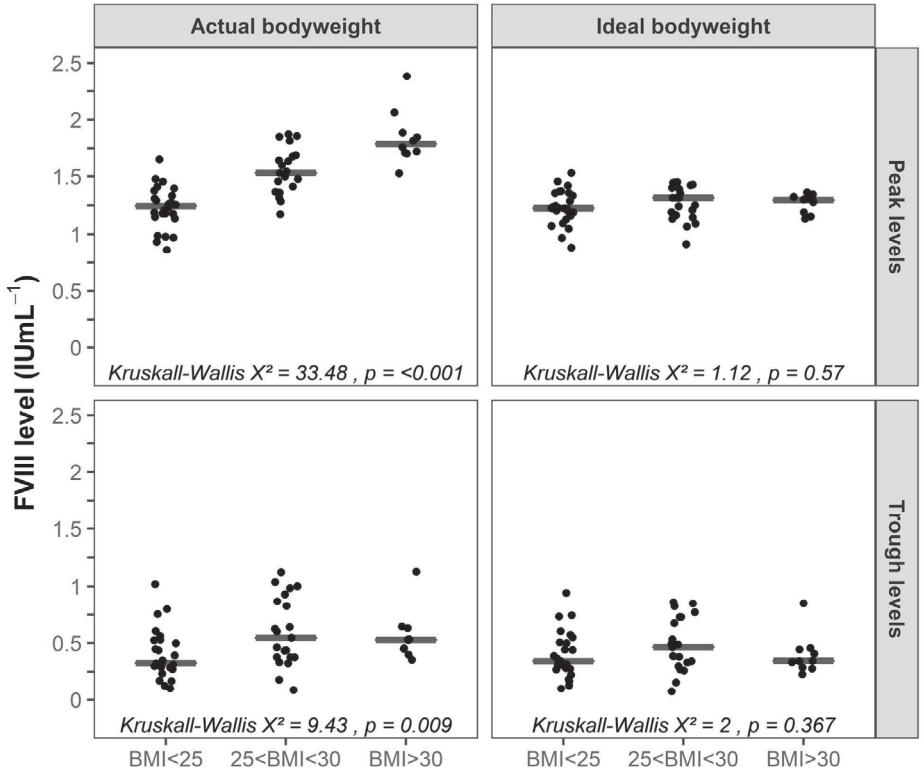


Figure 4. Dosing based on ideal body weight results in adequate FVIII peak and trough levels when treating a life-threatening bleed. The FVIII peak levels (upper panels) were obtained five minutes after the simulated loading-dose of 50 IUkg^{-1} that was followed by twice daily dosing of 25 IUkg^{-1} to treat a life-threatening bleed. The trough FVIII levels (lower panels) were obtained immediately before the 6th dose, which corresponded with 72 hours after administration of the loading-dose. The individual FVIII levels were simulated using the individual PK parameters from each included real-life patient of the studied population. The blue bar depicts the median FVIII level. To enhance the visibility for the number of FVIII levels in each category, the FVIII levels were scattered horizontally.

DISCUSSION

In this study, the relationship between five morphometric variables (e.g. ideal body weight, body mass index (BMI), lean body mass, adjusted body weight and lean body mass calculated) and the PK of FVIII concentrates was investigated in normal weight, overweight and obese hemophilia A patients. Ideal body weight best explained the inter-patient variability in FVIII PK. Scaling PK parameters by ideal body weight reduced the inter-patient variability in CL and V1 from 45.1 to 37.6% and from 26.8% to 14.1%, respectively. CL, V1, and FVIII half-life were similar between normal, overweight and obese patients while *in vivo* recovery increased with increasing BMI. This was also demonstrated in additional simulations of included study patients, in which it was observed that FVIII peak levels increased to unnecessary high values when dosing was based on body weight in the overweight/obese patients. Dosing based on ideal body weight however prevents these high FVIII peak levels, while generally maintaining adequate FVIII trough levels to adequately treat a life-threatening bleed by achievement of FVIII peak levels >0.80-1.0 $IUmL^{-1}$.

Allometric scaling can be applied to partly explain inter-individual variability for a population PK parameter. By inclusion of allometric scaling, the fit of the model to collected data may improve, resulting in more accurate estimation of individual PK parameters required to calculate individualized doses. Although body weight is generally used for allometric scaling, other morphometric variables can also be applied. Therefore, it was investigated which morphometric variable correlated best with FVIII PK parameters. It was demonstrated that a more adequate fit was obtained using allometric scaling with ideal body weight, reducing inter-individual variability of CL by 7.5% and V1 by 12.7%. If body weight was used, inter-individual variability of CL and V1 was only reduced with 1.2% and 3%, respectively. Therefore, we conclude that ideal body weight should be used for allometric scaling instead of body weight in overweight and obese hemophilia A patients.

No differences were found in the estimated individual PK parameters (CL, V1 and half-life) between normal weight, overweight and obese patients. Previous studies have demonstrated that increasing BMI results in higher von Willebrand factor (VWF) levels in both healthy individuals and hemophilia A patients [20-22]. This may be caused by increased shear stress upon the vessel wall caused by hypertension and atherosclerosis and subsequent secretion of VWF [23]. Furthermore, it has been shown that adipose tissue expresses VWF[24]. As VWF protects FVIII from proteolytic cleavage, it could be expected that FVIII CL may decrease with increasing BMI. However, the estimated individual PK

parameters showed no differences in FVIII CL, V1, and FVIII half-life between normal weight, overweight and obese hemophilia A patients.

Applying maximum *a posteriori* (MAP) Bayesian analysis using the established population PK model to calculate FVIII doses is only possible when the source data is generalizable to the population which you wish to dose. Due to rising prevalence of obesity, overweight and obese patients should be adequately represented in the population used to construct population PK models. This fact also emphasizes that other morphometric variables instead of body weight may be more adequate to calculate doses. The applied population PK model in this study was able to adequately fit FVIII levels obtained in all patients, as in the study population 36.8% of the patients were overweight and 17.5% were obese. Moreover, the population PK model was able to adequately fit FVIII levels obtained in all patients. Therefore, the established population PK model can be applied to the current hemophilia A patient population.

Simulations of the included real-life patients on the basis of this population PK model using real-world data, showed that dosing based on ideal body weight resulted in adequate FVIII peak and trough levels to treat or prevent a life-threatening bleed, a circumstance during which it is required to achieve FVIII peak and trough levels of respectively 0.80-1.0 $IUmL^{-1}$ and >0.50 $IUmL^{-1}$. To our knowledge, this is the first study that includes real-life patient data to verify the implications of ideal body weight dosing of FVIII concentrate. When dosing FVIII based on ideal body weight, inter-individual differences in FVIII PK however do still exist. Figure 4 illustrates that dosing based on ideal body weight leads to lower FVIII peak and trough levels than when dosing is based on body weight. However, the variation in FVIII peak and trough levels in obese patients were comparable to levels obtained for the non-obese patients. It is always important to realize that inter-individual variation in bleeding tendency e.g. bleeding phenotype is notably large and not always explained by the FVIII levels measured after FVIII concentrate infusion. Nevertheless, clinical guidelines advise specific FVIII levels to prevent or treat bleeding in certain settings, and in clinical practice these dosing strategies reduce this variability in response. It must also be realized and anticipated that clearance and volume of distribution may alter in a situation of a life threatening bleeding risk, which we have not been able to simulate in this study.

The potential use of alternative morphometric variables for allometric scaling of model parameters has been addressed in previous studies. Garmann et al. constructed a population PK model for recombinant FVIII (rFVIII) concentrate, in which scaling was investigated using only body weight and lean body mass [25]. This model was based on information from both children and adults with a median age of 22 years (range: 1-61

year). It is known that lean body mass should preferably be corrected when used in children [26]. However, the authors did not report whether they used different formulas to calculate lean body mass, while they did include lean body mass for allometric scaling of parameters in their final model [25]. In McEneny-King et al., this model was subsequently applied to perform a simulation study, in which alternative dosing strategies of one brand of rFVIII concentrate based on various morphometric variables were simulated in 1000 normal weight (BMI<29.6 kgm^{-2}) and 1000 overweight/ obese (BMI 29.6-40.0 kgm^{-2}) patients[12]. Although a different cut-off point (BMI: 29.6 kgm^{-2}) was applied to discriminate between normal and overweight/ obese patients than used in the present study, it was also concluded that ideal body weight demonstrates best predictive performance across all of the investigated dosing regimens. The present study using real-world data, substantiates the conclusion that ideal body weight is the best morphometric variable to dose hemophilia A patients with varying FVIII concentrates

In conclusion, this study investigated the relationship between five morphometric variables and FVIII PK in normal weight, overweight and obese hemophilia A patients. Scaling of the model parameters using ideal body weight best explained the inter-individual variability of PK parameters and provided the most optimal description of the measured FVIII levels. Although we still recommend FVIII monitoring of treatment, we carefully state that ideal body weight can be used safely to calculate FVIII concentrate dosing in overweight and obese hemophilia A patients in all circumstances including treatment for life-threatening bleeds.

REFERENCES

1. Srivastava A, Brewer AK, Mauser-Bunschoten EP, et al. Guidelines for the management of hemophilia. Haemophilia 2013;19:e1-47.
2. Fijnvandraat K, Cnossen MH, Leebeek FW, Peters M. Diagnosis and management of haemophilia. Bmj 2012;344:e2707.
3. Bjorkman S, Folkesson A, Jonsson S. Pharmacokinetics and dose requirements of factor VIII over the age range 3-74 years: a population analysis based on 50 patients with long-term prophylactic treatment for haemophilia A. Eur J Clin Pharmacol 2009;65:989-98.
4. Bjorkman S, Oh M, Spotts G, et al. Population pharmacokinetics of recombinant factor VIII: the relationships of pharmacokinetics to age and body weight. Blood 2012;119:612-8.
5. Wilding J, Zourikian N, Di Minno M, et al. Obesity in the global haemophilia population: prevalence, implications and expert opinions for weight management. Obes Rev 2018;19:1569-84.
6. Henrard S, Speybroeck N, Hermans C. Body weight and fat mass index as strong predictors of factor VIII in vivo recovery in adults with hemophilia A. J Thromb Haemost 2011;9:1784-90.
7. Henrard S, Hermans C. Impact of being overweight on factor VIII dosing in children with haemophilia A. Haemophilia 2016;22:361-7.
8. Feldschuh J, Enson Y. Prediction of the normal blood volume. Relation of blood volume to body habitus. Circulation 1977;56:605-12.
9. Mahmood I. Allometric extrapolation of factors VII, VIII and IX clearance in children from adults. J Thromb Haemost 2012;10:1609-13.
10. Hanley MJ, Abernethy DR, Greenblatt DJ. Effect of obesity on the pharmacokinetics of drugs in humans. Clinical pharmacokinetics 2010;49:71-87.
11. Green B, Duffull SB. What is the best size descriptor to use for pharmacokinetic studies in the obese? British journal of clinical pharmacology 2004;58:119-33.
12. McEneny-King A, Chelle P, Henrard S, Hermans C, Iorio A, Edginton AN. Modeling of Body Weight Metrics for Effective and Cost-Efficient Conventional Factor VIII Dosing in Hemophilia A Prophylaxis. Pharmaceutics 2017;9.
13. Hazendonk HC, van Moort I, Fijnvandraat K, et al. The “OPTI-CLOT” trial. A randomised controlled trial on periOperative PharmacokineTIc-guided dosing of CLOTting factor concentrate in haemophilia A. Thromb Haemost 2015;114:639-44.
14. Preijers T, van Moort I, Fijnvandraat K, et al. Cross-evaluation of Pharmacokinetic-Guided Dosing Tools for Factor VIII. Thromb Haemost 2018;118:514-25.
15. Garrow JS, Webster J. Quetelet’s index (W/H2) as a measure of fatness. Int J Obes 1985;9:147-53.
16. Devine BJ. Gentamicin therapy. Drug Intelligence and Clinical Pharmacy 1974;8:650-5.
17. Janmahasatian S, Duffull SB, Ash S, Ward LC, Byrne NM, Green B. Quantification of lean bodyweight. Clin Pharmacokinet 2005;44:1051-65.
18. Jonsson EN, Karlsson MO. Xpose--an S-PLUS based population pharmacokinetic/pharmacodynamic model building aid for NONMEM. Comput Methods Programs Biomed 1999;58:51-64.
19. Anderson BJ, Holford NH. Mechanistic basis of using body size and maturation to predict clearance in humans. Drug Metab Pharmacokinet 2009;24:25-36.

20. Mertens I, Van Gaal LF. Obesity, haemostasis and the fibrinolytic system. Obes Rev 2002;3:85-101.

21. Blann AD, Bushell D, Davies A, Faragher EB, Miller JP, McCollum CN. von Willebrand factor, the endothelium and obesity. Int J Obes Relat Metab Disord 1993;17:723-5.

22. Tuinenburg A, Biere-Rafi S, Peters M, et al. Obesity in haemophilia patients: effect on bleeding frequency, clotting factor concentrate usage, and haemostatic and fibrinolytic parameters. Haemophilia 2013;19:744-52.

23. Galbusera M, Zoja C, Donadelli R, et al. Fluid shear stress modulates von Willebrand factor release from human vascular endothelium. Blood 1997;90:1558-64.

24. Consortium GT. The Genotype-Tissue Expression (GTEx) project. Nat Genet 2013;45:580-5.

25. Garmann D, McLeay S, Shah A, Vis P, Maas Enriquez M, Ploeger BA. Population pharmacokinetic characterization of BAY 81-8973, a full-length recombinant factor VIII: lessons learned - importance of including samples with factor VIII levels below the quantitation limit. Haemophilia 2017;23:528-37.

26. Peters AM, Snelling HL, Glass DM, Bird NJ. Estimation of lean body mass in children. Br J Anaesth 2011;106:719-23

Table S1. Equations for the morphometric variables

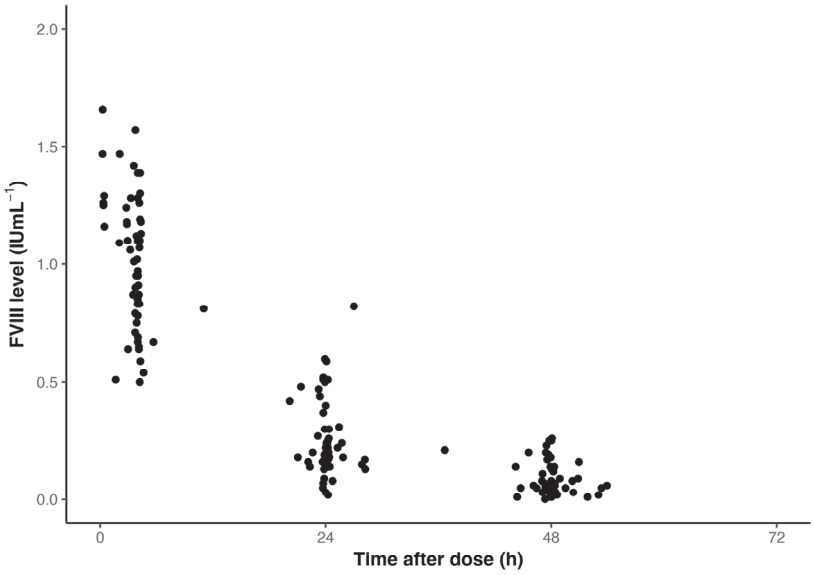
Morphometric variable	Dimension	Equation	Reference
Ideal body weight (IBW)	Kg	50 + 2.3 * (H * 0.394 - 60)	(Devine, 1974)
Adjusted body weight (ABW)	Kg	IBW + 0.4 (BW-IBW)	(Devine, 1974)
Body mass index	Kg m^{-2}	BW / (HT^2)	(Garrow et al., 1985)
Lean body weight	Kg	(9.27 *10^3 * BW)/(6.68*10^3+(216*BMI))	(Janmahasatian, 2005)

Kg= kilogram; H= height in centimeter; BW = body weight in kilograms; BMI= body mass index in kilogram per square meter; HT= height in meter.

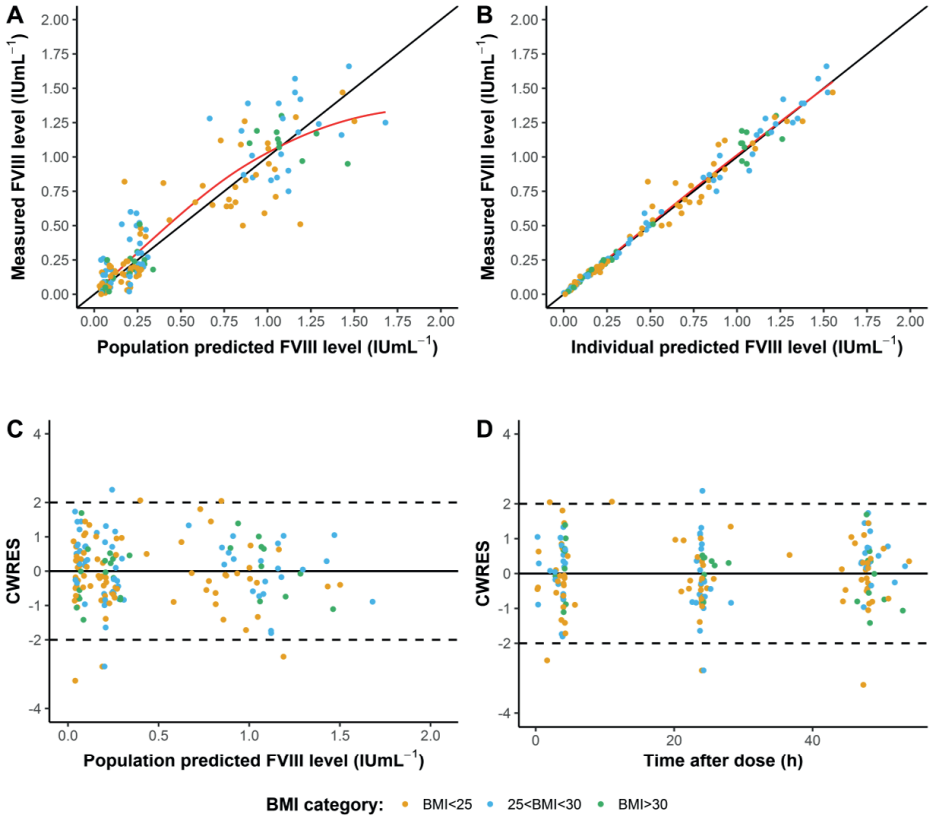
Table S2. Model building-steps for constructing the structural model for covariate analysis

		OFV	dOFV	No. of parameters
Structural one compartmental model				
1	Model with additive residual error	43.0	ND	3
2	Model with proportional residual error	59.6	+16.6	3
3	Model with mixed residual error	5.5	-37.6	4
4	Model 3 with IIV on CL	-127.5	-133	5
5	Model 3 with IIV on V1	-2.7	-8.2	5
6	Model 3 with IIV on CL and V1	-125.5	-131	6
7	Model 6 with eta-correlation between CL and V1	-138.4	-12.9	7
Structural two compartmental model				
10	Model with mixed residual error and IIV on CL and V1 with eta-correlation	-183.0	-44.6	9
11	Model 10 with IIV on Q*	-186.0	-2.97	10
12	Model 10 with IIV on V2*	-186.5	-3.5	10
13	Model 10 with correction for FVIII-BDD	-186.1	-3.1	9

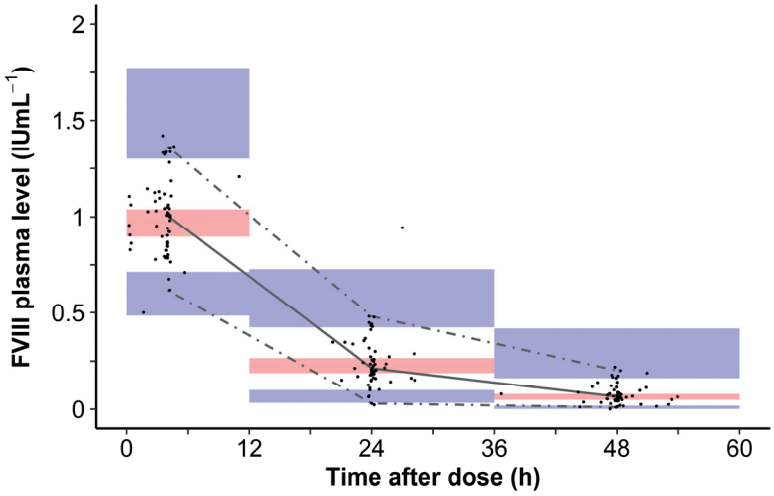
OFV indicates objective function value, as calculated by minus two times the logarithm of the likelihood (-2LL) of the model describing the data; ND: not determined. No., number; IIV: inter-individual variability. * Models 11 and 12 showed am eta-shrinkage for the newly introduced parameter of 54% and 60%, respectively. Therefore, these models was not chosen despite a significant drop in the OFV for model 12.



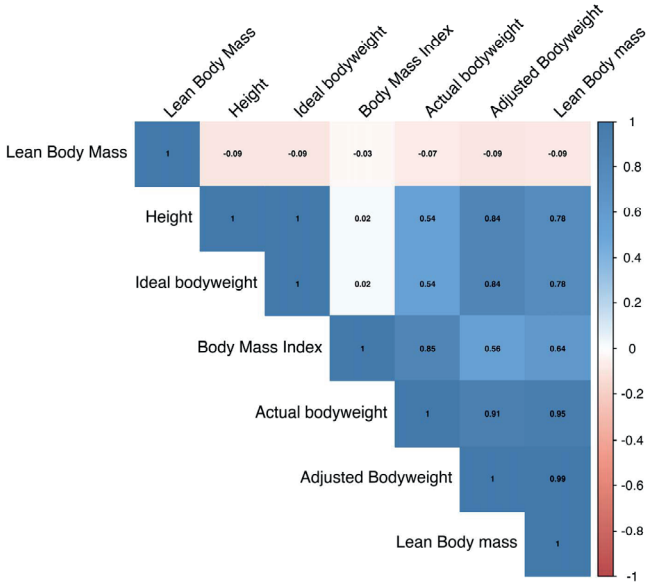
Supplementary figure 1. Observed FVIII concentrations versus time.



Supplementary figure 2. Goodness-of-fit plots for the final model. (A) Population predicted FVIII levels obtained using the structural model versus the measured FVIII levels. (B) Individual predicted FVIII levels obtained using the final model versus the measured FVIII levels. The individual FVIII levels were calculated using the individual PK parameters obtained using the final model with the estimated IIV. (C) Conditional weighted residuals (CWRES) versus the population predicted FVIII levels. (D) CWRES versus the time after dose administration. The black line depicts the line of identity (line y=x), whereas the red line depicts the local regressor (LOESS) line, following the densest part of the data.



Supplementary figure 3. Visual predictive check of the final model. Observed FVIII plasma level versus time for all patients who received 50 IU/kg FVIII concentrate. The black dots represent the measured FVIII for all patients. The solid black line represents the median of the observations and the dashed lines represent the 2.5th and 97.5th quantiles. The red and blue-shaded areas show the 95% prediction intervals for the predicted FVIII values, as obtained by Monte Carlo simulation (visual predictive check).



Supplementary figure 4. Correlation plot of all tested morphometric variables.

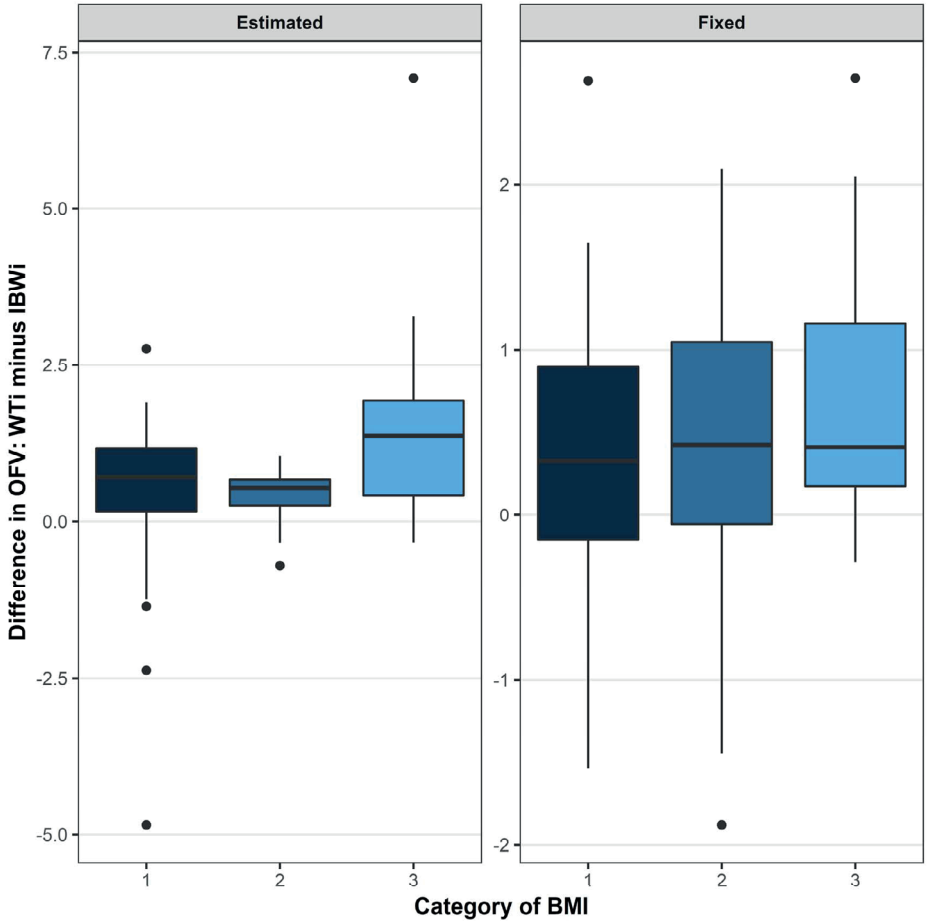
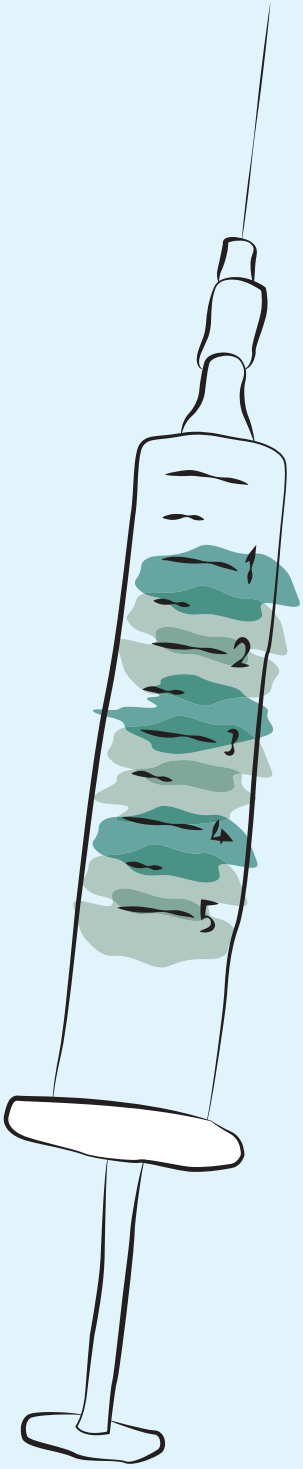


Figure S5. Difference in OFV between the models using allometric scaling with body weight and ideal body weight. No trend is visible both when estimating the allometric scaling and when the allometric scaling is fixed, indicating that no influential individuals drive the estimation of the covariate models. For each boxplot, the whiskers depict the 2.5th and 97.5th percentile of the data, whereas the box depicts the interquartile range. The median of the data is depicted by the black horizontal line inside the boxplot.



9

Impact of extreme weight loss on factor VIII concentrate pharmacokinetics in hemophilia

van Moort I, Bukkems LH, Nieuwenhuizen L, Leebeek FWG, Mathôt RAA & Cnossen MH for the OPTI-CLOT study group.

Submitted

SUMMARY

We explored the effects of extreme weight loss after gastric bypass surgery on FVIII concentrate pharmacokinetic (PK) parameters in a patient with hemophilia A. We present a 32 year old male with severe hemophilia A, with a body mass index (BMI) of 42.6 kg/m^2 who underwent laparoscopic sleeve gastrectomy. We showed that a population PK model with ideal body weight as morphometric variable instead of bodyweight led to an adequate description of the individual pharmacokinetics in this patient with a variable body mass index (BMI). Strikingly, no differences were observed in the individual PK parameters after extreme weight loss. Therefore, the resulting extreme weight loss after surgery did not lead to prophylactic dose changes in this severe hemophilia patient. We carefully conclude that population PK-pharmacodynamic (PD) models are still obligatory to give more insight into functional effects of significant weight loss on the hemostatic balance.

BACKGROUND

Hemophilia A is an X-linked inherited bleeding disorder caused by a deficiency in coagulation factor VIII (FVIII). To prevent spontaneous bleeding in muscles and joints, severe and some moderate hemophilia A patients receive FVIII prophylactic replacement therapy. In clinical practice, FVIII concentrate dosing is still mainly based on bodyweight.[1] As overweight and obesity are a growing global health problem with a current prevalence of 43.3% in the adult European and North American hemophilia population, appropriate dosing strategies for replacement therapy in this patient group are relevant to safeguard treatment costs without loss of quality of care.[2]

In several studies by Henrard et al., *in vivo* recovery (IVR) has been shown to be significantly higher in overweight and obese hemophilia patients than in normal weight patients.[3-5] In addition, weight-adjusted clearance decreases with age, whereas weight-adjusted volume of distribution does not.[6] The latter suggesting that weight-adjusted volume of distribution is constant over time.[3,4,6] We set out to further prove this assumption by describing the impact of extreme weight loss on FVIII PK parameters in hemophilia A, which has not been done earlier. Recently, a severe hemophilia A patient was reported who safely underwent a laparoscopic mini gastric bypass operation for weight reduction without details on FVIII PK parameters.[7] We are the first to describe such a surgical intervention in a hemophilia patient, including analyses of FVIII PK parameters.

CASE PRESENTATION

We present a 32 year old male with severe hemophilia A (FVIII< 0.01 IU/mL), with a bodyweight of 133.5 kg, and body mass index (BMI) of 42.6 kg/m^2. Patient was planned for laparoscopic sleeve gastrectomy after extensive clinical, laboratory and psychological testing and individual FVIII concentrate PK profiling. Consequently, a PK-guided perioperative loading dose and subsequent dosing regimen were calculated. Six months later surgery was performed. At the day of surgery, patient's bodyweight was 142.0 kg with a BMI of 45.3 kg/m^2. FVIII levels were monitored daily perioperatively and dosing was iteratively adjusted by application of *maximum a posteriori* (MAP) Bayesian analysis.[8] Surgery was performed without complications, more specifically without (peri)surgical bleeding. He was discharged from the hospital after four days and received additional FVIII doses until postoperative day 10, at which moment patient resumed FVIII prophylaxis.

INVESTIGATIONS

Preoperatively, a PK profile was obtained after infusion of 5000 IU (37.4 IU/kg) of recombinant FVIII (NovoEight®) (t=0). FVIII measurements were performed by one-stage assay at respectively t=4 hours, t=48 hours and t=52 hours after infusion.[9] The Sysmex® CS 5100 (Sysmex, Kobe, Japan) was used for the one-stage assay combined with following reagents all from Siemens® (Siemens Healthcare Diagnostics, Marburg, Germany): FVIII Actin FS, FVIII deficient plasma and Standard Human Plasma as a calibrator. A FVIII concentrate washout period or correction for the pre-administration FVIII levels was not necessary, as both timing and dose of three previous FVIII concentrate infusions were recorded. Individual PK parameters were calculated by MAP Bayesian analysis in NONMEM V.7.4.1 (ICON Development Solutions, Ellicott City, Maryland, USA) using our prophylactic population PK model including overweight and obese patients, with ideal body weight (IBW) as morphometric variable.[10,11] PK profiling was repeated six and twelve months after surgery to investigate impact of weight loss on patient's FVIII PK parameters.

OUTCOME AND FOLLOW-UP

Factor VIII concentrate PK parameters

Six months after surgery, bodyweight decreased with 31.6 kg, from 142.0 to 110.4 kg, with BMI decreasing to 35.3 kg/m^2. A PK profile was repeated to assess individual PK parameters. One year after surgery when final PK profiling was performed, patient weighed 106.4 kg with a BMI of 34.0 kg/m^2. Figure 1 shows individual PK curves at each time point with IBW (70.3 kg) as a morphometric variable. As depicted, measured FVIII levels follow predicted FVIII levels, confirming a good fit of the model to the data by MAP Bayesian analysis. The influence of weight loss on individual PK parameters is visualized in Figure 2. Figure 2D shows that IVR decreased significantly with decreasing bodyweight. Strikingly, FVIII clearance and volume of distribution remained similar over time (figure 2A + 2B), resulting in a similar half-life over time (figure 2C).

Factor VIII concentrate dosing and trough level simulations

As a prophylactic dose of 25-40 IU/kg is recommended by the World Federation of Hemophilia[1], time to trough of 0.01 IU/mL was calculated after a hypothetical prophylactic FVIII dose of 3500 IU (26 IU/kg before bariatric surgery). Simulations using the patient's individual PK parameters showed that time to 0.01 IU/mL was not subject to change (Figure 2E). After weight loss, a novel optimal prophylactic dosing schedule was calculated and the original prophylactic regimen of 750 IU (now 7 IU/kg) every other day remained adequate.

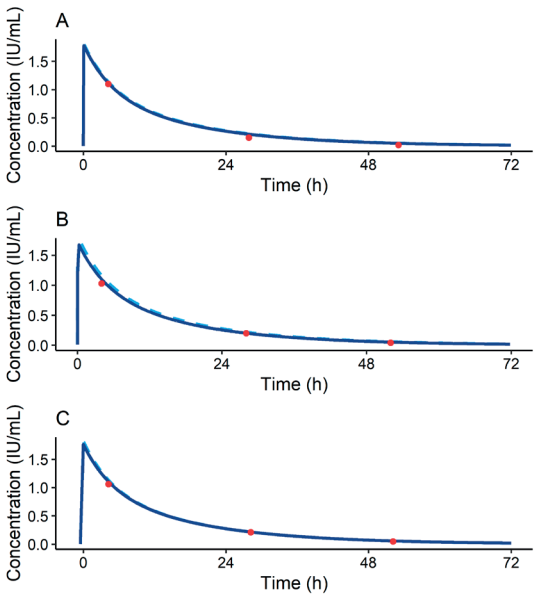
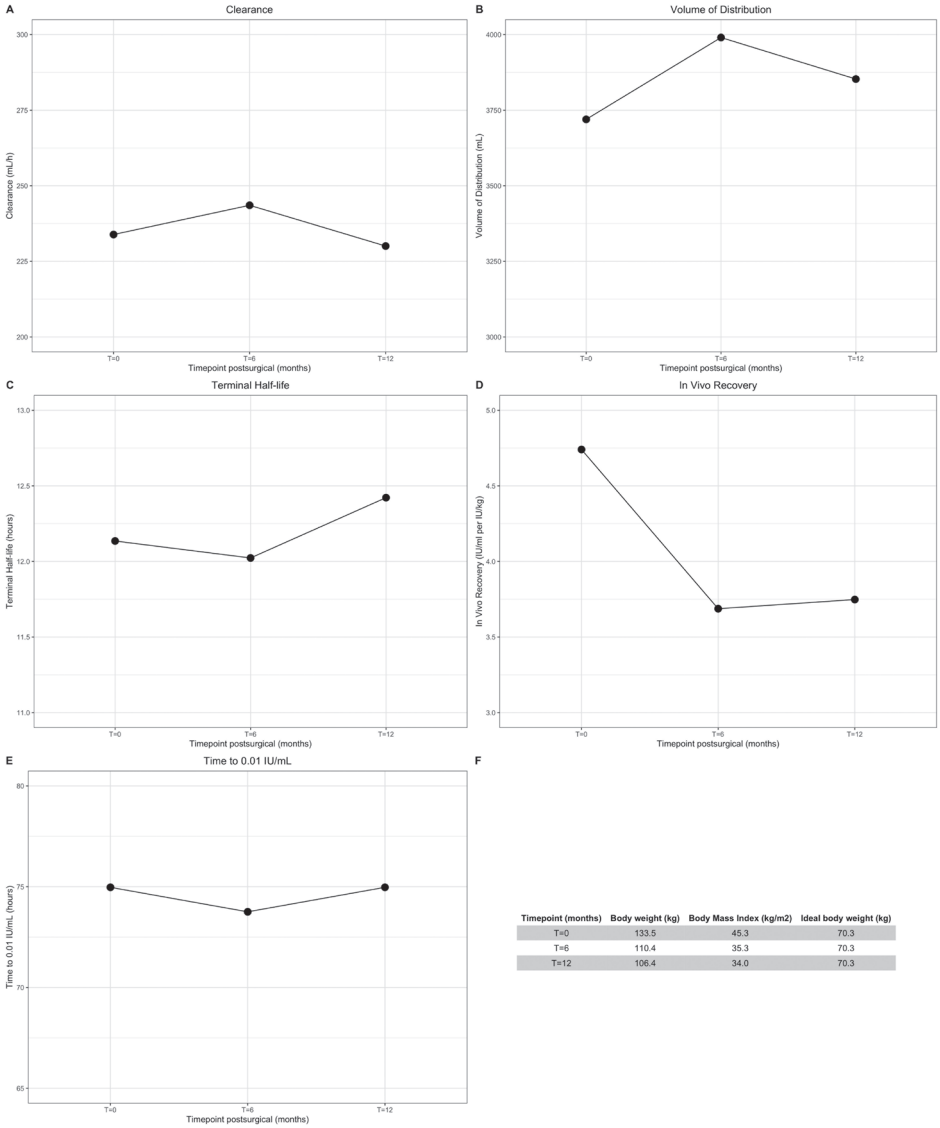


Figure 1. Individual pharmacokinetic (PK) profiles before, six months after and twelve months after bariatric surgery . The dark blue line depicts individually predicted FVIII activity levels after a dose of 5000 IU FVIII concentrate. The interrupted light blue line depicts the population values. The red dots are the measured FVIII levels. A) PK profile before surgery with a body weight of 133.5 kg (BMI 45.3 kg/m2); B) PK profile six months after the patient's surgery with a body weight of 110.4 kg (BMI 35.3 kg/m2); C) PK profile 12 months after the patient's surgery with a body weight of 106.4 (BMI 34.0 kg/m1).



Timepoint (months)	Body weight (kg)	Body Mass Index (kg/m2)	Ideal body weight (kg)
T=0	133.5	45.3	70.3
T=6	110.4	35.3	70.3
T=12	106.4	34.0	70.3

Figure 2. Individual pharmacokinetic (PK) parameters before surgery (t=0), six months after surgery (t=6 months) and 12 months after surgery (t=12 months). A) Clearance; B) Volume of Distribution; C) Terminal Half-life; D) In Vivo Recovery; E) Time to 0.01 IU/mL; F) Table summarizing the morphometric variables measured at each time point.

DISCUSSION

The present case was analyzed to determine impact of extreme weight loss on FVIII PK parameters. PK profiling before and after gastric bypass in a patient with severe hemophilia A strikingly did not differ with regard to calculated individual PK parameters and therefore did not lead to dose changes of prophylaxis.

Although extreme weight loss did not lead to alterations of individual FVIII PK parameters, it is important to realize that weight reduction may lead to shifts in hemostatic balance leading to clinically relevant presentations. Several reports have described changes in both procoagulant and anticoagulant factors. Overall, obese individuals are thought to be prothrombotic due to lower fibrinolytic potential caused by higher plasminogen inhibitor (PAI) levels, leading to decreased clot lysis and overall bleeding tendency may be lower.[12,13] Hypothetically after extreme weight loss, patients may experience more bleeding due to normalization of fibrinolysis, subsequently needing higher prophylactic FVIII concentrate doses due to increased bleeding. Contrastingly, it has also been reported that one year after gastric bypass surgery, anti-thrombotic protein levels are also lower.[14] Future PK-pharmacodynamic (PD) studies should evaluate influence of obesity and weight loss on hemostatic balance to establish its relevance.

In previous studies, it has been suggested that ideal body weight (IBW), as calculated according to Lorentz's formula including height and sex and not total body weight, should be applied to minimalize interindividual differences in FVIII PK.[10,11,15] In this case report, we additionally propose that IBW may be of value to compensate for intraindividual differences in FVIII PK when bodyweight is variable. Figure 1 shows the three individual PK profiles at consecutive time points with varying bodyweight, fitted with IBW as a morphometric variable to describe alterations in FVIII PK after weight loss. IBW estimates volume of distribution optimal, both before and after weight reduction and estimates FVIII peak levels accordingly. This can be explained physiologically as FVIII concentrate is infused into the vascular space. This is supported by the fact that volumes of distribution approximate plasma volume. Therefore, weight loss does not affect volume of distribution. Furthermore, FVIII clearance did not change over time, which we have also demonstrated in prior reports on interindividual variation in FVIII PK.[10] This case report shows that a population model with IBW as morphometric variable allows an adequate description of the individual pharmacokinetics in a patient with varying BMI.

In conclusion, obesity is a growing, global health care problem, also affecting hemophilia patients. Extreme weight loss does not result in altered individual PK parameters and there does not seem to necessitate adjustment of perioperative and prophylactic dosing regimens based on PK. However, monitoring of bleeding and ultimate construction of population PK-PD models are still obligatory to define effects of weight loss on hemostasis.

PATIENT'S PERSPECTIVE

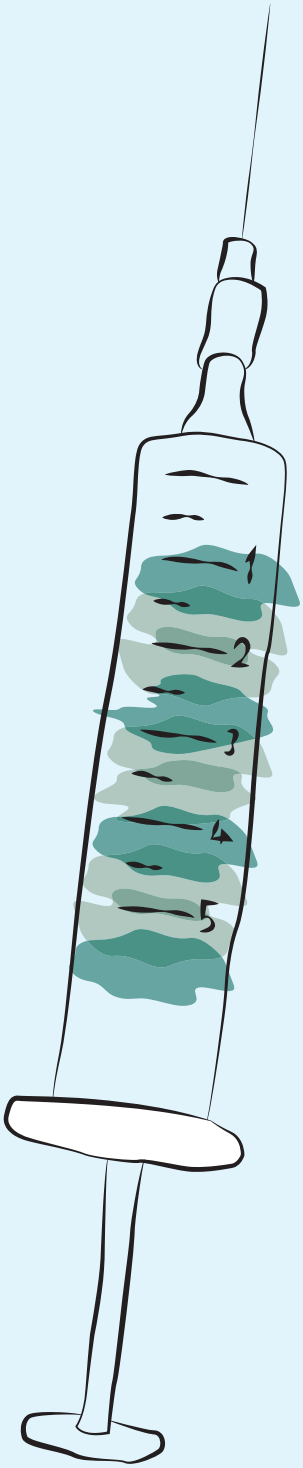
I was of course born with hemophilia A. Due to my overweight, which has further increased the last few years, I recently made the difficult decision with the hemophilia treatment team in the Erasmus MC to have bariatric surgery performed. The main reason for this decision were my concerns regarding my general health. I shared my anxiety for the operation due to my bleeding disorder with my doctor and the nurses. They were very supportive, and assured me that they would collaborate closely with the surgeon to organize the necessary replacement therapy to prevent any perioperative bleeding.

A few months before surgery, I was able to participate in a clinical research project e.g. the randomized controlled perioperative OPTI-CLOT trial in hemophilia A patients undergoing surgery. In this trial, standard dosing based on bodyweight is compared to an innovative strategy to individualize factor VIII concentrate dosing by looking at the velocity with which the factor concentrate disappears from the circulation, also called pharmacokinetic (PK)-guided dosing. This approach intrigued me and I decided that I very much wanted to participate. Later, when the research coordinator asked me to participate in a this small substudy, I gladly agreed. Understanding that bariatric surgery in a severe hemophilia A patient is rare and educative. The research team wanted to investigate the effect of extreme weight loss on the PK of the administered factor VIII concentrate. I experienced this as a unique chance to personalize my own treatment and to optimize therapy for other patients with a severe bleeding disorder. Especially as the number of overweight hemophilia patients is steadily increasing as I understood from the research coordinator. During the operation and after surgery, my factor VIII levels would be monitored extensively, which also made me feel safe that factor VIII levels would be sufficient to prevent bleeding.

One year after my bariatric surgery, I discussed the results of the study with the research coordinator. It was concluded that that the extreme weight loss I experienced was not of significant influence on the PK of the administered factor VIII concentrate. To be honest, I expected the contrary and I understood that the research team was also surprised by these results. I realize that this is exactly what the importance is of such studies as hypotheses can be tested and are sometimes shown to be untrue. I am glad to have been able to contribute to the ultimate aim of the study group to personalize treatment in patients with a bleeding disorders. I would be happy to participate in future research projects as I have found the whole escapade very special. I would like to very much thank the hemophilia and surgical team for all their care and organization!

REFERENCES

1. Srivastava A, Brewer AK, Mauser-Bunschoten EP, et al. Guidelines for the management of hemophilia. Haemophilia 2013;19:e1-47.
2. Wilding J, Zourikian N, Di Minno M, et al. Obesity in the global haemophilia population: prevalence, implications and expert opinions for weight management. Obes Rev 2018;19:1569-84.
3. Henrard S, Hermans C. Impact of being overweight on factor VIII dosing in children with haemophilia A. Haemophilia 2015.
4. Henrard S, Speybroeck N, Hermans C. Impact of being underweight or overweight on factor VIII dosing in hemophilia A patients. Haematologica 2013;98:1481-6.
5. Henrard S, Speybroeck N, Hermans C. Body weight and fat mass index as strong predictors of factor VIII in vivo recovery in adults with hemophilia A. J Thromb Haemost 2011;9:1784-90.
6. Bjorkman S, Oh M, Spotts G, et al. Population pharmacokinetics of recombinant factor VIII: the relationships of pharmacokinetics to age and body weight. Blood 2012;119:612-8.
7. Plamper A, Goldmann G, Lingohr P, et al. First Case of Laparoscopic Mini-Gastric Bypass for the Treatment of Morbid Obesity in Severe Haemophilia A. Hamostaseologie 2019;39:208-10.
8. Hazendonk H, Fijnvandraat K, Lock J, et al. A population pharmacokinetic model for perioperative dosing of factor VIII in hemophilia A patients. Haematologica 2016;101:1159-69.
9. Bjorkman S. Limited blood sampling for pharmacokinetic dose tailoring of FVIII in the prophylactic treatment of haemophilia A. Haemophilia 2010;16:597-605.
10. van Moort I, Preijers T, Hazendonk HCAM, et al. Ideal Body Weight Is Proven Most Reliable to Dose Factor VIII Concentrate in Overweight and Obese Hemophilia A Patients. Research and Practice in Thrombosis and Hemostasis 2019;3:1-891.
11. Devine B. Gentamicin therapy. Drug Intell Clin Pharm 1974;8:5.
12. Stolberg CR, Mundbjerg LH, Funch-Jensen P, Gram B, Juhl CB, Bladbjerg EM. Effects of gastric bypass followed by a randomized study of physical training on markers of coagulation activation, fibrin clot properties, and fibrinolysis. Surg Obes Relat Dis 2018;14:918-26.
13. Tuinenburg A, Biere-Rafi S, Peters M, et al. Obesity in haemophilia patients: effect on bleeding frequency, clotting factor concentrate usage, and haemostatic and fibrinolytic parameters. Haemophilia 2013;19:744-52.
14. Rega-Kaun G, Kaun C, Ebenbauer B, et al. Bariatric surgery in morbidly obese individuals affects plasma levels of protein C and thrombomodulin. J Thromb Thrombolysis 2019;47:51-6.
15. McEneny-King A, Chelle P, Henrard S, Hermans C, Iorio A, Edginton AN. Modeling of Body Weight Metrics for Effective and Cost-Efficient Conventional Factor VIII Dosing in Hemophilia A Prophylaxis. Pharmaceutics 2017;9.



10

Cross-validation of pharmacokinetic–guided dosing tools for factor VIII concentrate dosing

van Moort I*, Preijers T*, Fijnvandraat K, Leebeek FWG, Cnossen MH, Mathôt RAA.

Thromb Haemost. 2018 Mar;118(3):514-525.

ABSTRACT

Background Patients with severe and moderate hemophilia A are treated prophylactically with factor VIII (FVIII) concentrate. Individualization of prophylaxis can be achieved by pharmacokinetic (PK)-guided dosing.

Aim In this study, the performance of three PK tools (myPKFiT™, WAPPS and NONMEM®) is compared.

Methods In 39 patients, with severe or moderate hemophilia A, blood samples were collected 4, 24 and 48 hours after administration of 50 $IUkg^{-1}$ of recombinant FVIII (Advate® (n=30) or Kogenate® (n=9)). FVIII dose, FVIII activity and patient characteristics were entered into the three PK tools. Obtained PK parameters and dosing advises were compared.

Results MyPKFiT™ provided PK parameters for 24 of 30 patients receiving Advate®, whereas WAPPS and NONMEM® provided estimates for all patients. Half-life was different between the three methods: medians were 12.6h (n=24), 11.2h (n=30) and 13.0h (n=30) for myPKFiT™, WAPPS and NONMEM® (p<0.001), respectively. To maintain a FVIII trough level of 0.01 $IUmL^{-1}$ after 48h, doses for myPKFiT™ and NONMEM® were 15.1 and 11.0 $IUkg^{-1}$ (p<0.01, n=11) and for WAPPS and NONMEM® were 9.0 and 8.0 $IUkg^{-1}$ (p<0.01, n=23), respectively. In nine patients receiving Kogenate®, WAPPS and NONMEM® produced different PK-parameter estimates; half-life was 15.0h and 12.3h and time to 0.05 $IUmL^{-1}$ was 69.2h and 60.8h, respectively (p<0.01, p<0.01, n=9). However, recommended doses to obtain these levels were not different.

Conclusions The three evaluated PK tools produced different PK parameters and doses for recombinant FVIII. Hematologists should be aware that recommended doses may be influenced by the choice of PK tool.

INTRODUCTION

Severe and moderate hemophilia A patients often receive coagulation factor VIII (FVIII) concentrates prophylactically. The aim of prophylactic treatment is to prevent spontaneous bleeding in joints and muscles by achieving a FVIII activity >0.01 $IUmL^{-1}$ [1]. After administration of a dose, the concentration-time profile of FVIII is determined by the individual's pharmacokinetic (PK) parameters [2]. For several FVIII concentrates, these parameters have been reported to exhibit considerable inter-patient variability [3–8], resulting in large differences in half-life [9]. As a result, dosing of FVIII can be optimized by assessment of the individual PK parameters and adjustment of the dose accordingly [10,11].

Bayesian techniques are frequently used for patient-tailored dosing [12,13]. In Bayesian analysis, individual PK parameters estimates are obtained by combining the information from the individual patient (administered dose, observed concentrations, specific characteristics such as age and body weight) with information from a specific cohort of patients (population). After Bayesian analysis, the individual PK parameters can be used to evaluate several different dosing regimens and to choose the most appropriate dosing regimen for a specific patient.

Recently, two PK dosing tools (PK tools) for FVIII concentrates have become available for hemophilia healthcare practitioners, myPKFiT™ and Web-Accessible Population Pharmacokinetic Service-Hemophilia portal (WAPPS) [14,15]. Both tools use Bayesian techniques. MyPKFiT™ is designed to estimate individual PK parameters and dose regimen for a single brand of recombinant FVIII (Advate®), whereas for WAPPS individual PK parameters can be estimated for other FVIII brands as well.

Individual PK parameters, provided by Bayesian techniques, are influenced by the population parameters from population PK models used in the analysis. Ideally, these population PK models should be validated [16]. For both myPKFiT™ and WAPPS, the exact population PK parameters are unknown. Moreover, results of validation studies have not been published. Therefore, in this study, individual PK parameters and recommended doses for recombinant FVIII in severe and moderate hemophilia A patients were estimated by myPKFiT™ and WAPPS and compared to estimates from NONMEM® using population PK models from literature.

METHODS

Population data

Patients with severe and moderate hemophilia A were enrolled in this study. The Medical Ethical Committees in all participating centers approved the study; all participants gave informed consent. This study was conducted in accordance with the Declaration of Helsinki. For each patient, age, body weight, blood group, a record of neutralizing antibodies (in the past), and the (lowest) endogenous baseline level were collected. Furthermore, if a patient was on prophylaxis, information for at most six previously administered doses was collected. In each patient, blood samples were collected 4, 24 and 48 hours after administration of 50 $IUkg^{-1}$ Advate® (Shire) or Kogenate® (Bayer), as proposed by *Björkman et al.* [13,17]. For patients using prophylaxis, no washout period was taken into account. FVIII was measured using a one-stage aPTT-based clotting assay [18]. A flowchart of which patients were analyzed by a specific PK tool is shown in *Supplementary Figure 1*.

Pharmacokinetic tools

Two online PK tools were used to obtain estimates of individual PK parameters and individual dose regimens: myPKFiT™ (Baxalta (now part of Shire), www.myPKFiT.com, version 2.0) and Web-Accessible Population Pharmacokinetic Service for Hemophilia (WAPPS, www.wapps-hemo.org, version 3.0) [14,15]. MyPKFiT™ has been developed for PK analysis of Advate® solely, whereas WAPPS provided individual PK parameters for other brands of FVIII as well.

MyPKFiT™

In myPKFiT™, the following information was entered: date of birth and body weight of the patient, endogenous baseline level, administered dose with one possible previous dose and observed FVIII. The following PK parameters were produced for patients that received Advate®: clearance (CL), distribution volume in steady-state (Vss), terminal elimination half-life ($t_{½}$) and time to 0.01 $IUmL^{-1}$. The latter parameter is defined as a FVIII activity of 0.01 $IUmL^{-1}$ above the endogenous baseline level.

Besides estimation of PK parameters, myPKFiT™ also provided the dose to achieve a target FVIII within a specified interval (24, 48 or 72 hours after dose administration). Herewith, the target FVIII was defined as the FVIII above the endogenous baseline level, i.e. for a patient having an endogenous baseline level of 0.01 $IUmL^{-1}$, a target FVIII of 0.03 $IUmL^{-1}$ is achieved when a FVIII of 0.04 $IUmL^{-1}$ is observed. Doses were obtained for target FVIII levels of 0.01 $IUmL^{-1}$, 0.03 $IUmL^{-1}$ and 0.05 $IUmL^{-1}$ within the 48 and 72 hours interval.

In myPKFiT™, individual PK parameters for patients with a body weight outside the range 5 - 120 kg were not provided by the PK-tool, as the implemented population PK model was apparently not constructed using data from patients having body weights outside this range. Furthermore, dose regimens were only provided by myPKFiT™, if these were in accordance with the Summary of Product Characteristics (SmPC) of Advate® [19]. Therefore, only doses between 10 and 100 $IUkg^{-1}$ could be obtained.

Furthermore, it was possible to indicate whether a wash-out had been performed. In this case, a pre-infusion level could be specified or, if this pre-infusion level was unknown, the dose prior to the loading-dose could be entered. In the latter case, the pre-infusion level was given as output by myPKFiT™. This pre-infusion level was compared to estimates for the pre-infusion FVIII obtained using NONMEM®.

WAPPS

In WAPPS, the following information was specified: date of birth and body weight of the patient, endogenous baseline level, administered dose, observed FVIII, type of coagulation test used to measure FVIII and the brand of the administered product. One pre-infusion FVIII could be specified (taken shortly prior to the loading-dose). However, pre-infusion FVIII measurements were not measured in this study.

WAPPS provided PK parameters estimates for both Advate® and Kogenate®. Estimates for the following parameters were reported: $t_{½}$, time to 0.01 $IUmL^{-1}$, time to 0.02 $IUmL^{-1}$ and time to 0.05 $IUmL^{-1}$. For calculating the time to a specific target FVIII, the endogenous baseline level was taken into account. Therefore, the time to a specific target FVIII was only provided by WAPPS, if the endogenous baseline level was lower than this target level.

Furthermore, WAPPS provided dose recommendations of FVIII trough levels 0.01 $IUmL^{-1}$, 0.03 $IUmL^{-1}$ and 0.05 $IUmL^{-1}$ within the 48 and 72 hours interval, which were obtained until the 24th of July 2017. As similar to the time to target FVIII, dose regimen were not provided if the target FVIII was equal to or below the endogenous baseline level.

NONMEM®

Bayesian analysis was performed in NONMEM® using population PK models specific for Advate® and Kogenate® (*Supplementary Table 1*), as reported by *Björkman et al.* [6,7]. In both population models, PK were described using two-compartment models in terms of clearance (CL), volume of distribution of the first and second-compartment (V1, V2) and inter-compartmental clearance (Q). Data, obtained from severe and moderate hemophilia A patients, was used to construct both population PK models [6,7]. The

population PK model described by *Björkman et al.* [7], as applied for the Kogenate® data, contained inter-patient variability as well as inter-occasion variability for both CL and V1, respectively. Both variabilities were taken into account using an exponential model, as specified by Karlsson and Sheiner (1993) [20].

In both reported population PK models, endogenous baseline levels were considered using different methods. In this study, the endogenous (lowest) baseline level from each patient was taken into account, as recorded in the patient file. An endogenous baseline level can be accurately estimated by nonlinear mixed-effect modelling or by Bayesian analysis only if well-timed sampling data are present, for instance after a long wash-out period. Since this data was not available in the present study, the measured endogenous (lowest) baseline level for each patient was used to obtain individual PK parameter estimates instead of using an estimated baseline value, as comprised in the population PK model from Björkman *et al.* [7]. Hereby, the (lowest) endogenous baseline level was subtracted from all measured FVIII levels, prior to the estimation of the individual PK parameters by Bayesian analysis.

After programming the models in NONMEM®, PK parameters estimates were obtained for each patient by Bayesian analysis. The following information was used as input: the time of dose administration and measurement of FVIII levels, and the age and body weight.

For imputation of terminal elimination half-life, time to target FVIII and the dose, necessary to keep FVIII above a target level of 0.01 $IUmL^{-1}$, 0.03 $IUmL^{-1}$ and 0.05 $IUmL^{-1}$ during an interval of 48 and 72 hours, analytical equations were derived using the Mathematical Expressions library from *Dubois et al.* [21] (see *Supplementary Table 3*). Vss was imputed by the summation of V1 and V2. As mentioned above, myPKFiT™ and WAPPS used different approaches to calculate the time to target concentration. Both approaches were implemented in R-software (R Core Team (2017), version 3.4.1) [22], allowing comparison of the different output generated by the PK tools. For the calculation of time to 0.01 $IUmL^{-1}$, 0.02 $IUmL^{-1}$ and 0.05 $IUmL^{-1}$, the estimated maximum achieved FVIII after dose administration was used.

Comparison of pharmacokinetic tools

All statistical comparisons were performed using paired Wilcoxon signed-rank tests in SPSS® version 24.0 (IBM Corp., Armonk, N.Y., USA). Values were summarized as medians and corresponding ranges (min – max), and a *p*-value below 0.05 was used to define a significant difference.

Validation of population PK models

The availability of an independent dataset, i.e. a dataset that was not used to construct the model, allows the (external) validation of a population PK model [16]. As population PK parameters implemented in myPKFiT™ and WAPPS were not available, only the population models from literature were validated. Population predicted concentrations (PRED) were obtained using typical values of the population PK parameters, corrected for age and body weight. Individual predicted concentrations (IPRED) were obtained by using the individual PK parameters produced after Bayesian analysis. Both PRED and IPRED were imputed at the same time points as compared to the observed FVIII. The validity of the applied population PK models was evaluated by constructing plots of PRED and IPRED versus the observed FVIII (goodness-of-fit plots).

Furthermore, the models were validated by construction of prediction-corrected visual predictive checks (VPCs) [23]. These plots are created by Monte Carlo simulation and show whether the applied population PK model adequately describes the median observed concentrations as well as the inter-patient variability [24]. In this study, for each patient, 1000 Monte Carlo simulations were performed in NONMEM®. Prediction-corrected VPCs were constructed using PsN [25] and a R-software package developed by *Keizer et al.* [26].

RESULTS

Population data

In total, PK assessments were collected for 39 patients. Thirty patients received Advate®, of which 23 had severe and 7 had moderate hemophilia A having endogenous baseline levels of less than 0.01 $IUmL^{-1}$ and between 0.01 and 0.05 $IUmL^{-1}$, respectively (*Table 1*). Nine patients (4 severe and 5 moderate) received Kogenate®. No patients with inhibitors were included, although three patients (8%) had a history with neutralizing antibodies (*Table 1*). The observed FVIII activity after a bolus infusion of 50 $IUkg^{-1}$ FVIII concentrate is shown in *Figure 1*.

Advate®: NONMEM® versus MyPKFiT™

MyPKFiT™ provided individual PK parameter estimates for 24 patients receiving Advate® (*Table 2*). In the other six patients receiving Advate®, PK parameter estimates were not provided due to an ‘unacceptable goodness-of-fit’ error. The latter error indicated that the FVIII measurements of the individual patient were outside the limits of prediction for the population model.

Table 1. General characteristics of the study population

	Total cohort		Adults		Children	
	No. (%); or median [min-max]					
Patient characteristics						
No. of patients	39		33		6	
Age (years)	40.2	[7.6 - 76.7]	48.3	[18.4 - 76.7]	14.1	[7.6 - 17.6]
Body weight (kg)	82.0	[28.0 - 105.0]	85.7	[60.8 - 105.0]	64.0	[28.0 - 75.0]
Height (cm)	177.5	[135.0 - 192.0]	179.5	[148.0 - 192.0]	170.0	[135.0 - 185.0]
Severe hemophilia A (<0.01 $IUmL^{-1}$)	27	(69)	22	(67)	5	(83)
On prophylaxis	28	(72)	23	(70)	5	(83)
Blood group O[a]	24	(62)	19	(58)	5	(83)
Neutralizing antibodies (historically)[b]	3	(8)	3	(9)	-	
Replacement therapy with factor concentrate						
Product						
Patients using Advate®	30	(77)	25	(76)	5	(83)
Patients using Kogenate®	9	(23)	8	(24)	1	(17)
Availability of output after PK analysis						
PK-tool						
MyPKFiT™	24	(62)	21	(64)	3	(50)
WAPPS	39	(100)	33	(100)	6	(100)
Time of blood-sampling						
First measurement (h)	4.0	[2.1 - 4.6]	4.0	[2.1 - 4.6]	3.9	[3.3 - 4.1]
Second measurement (h)	24.0	[20.2 - 28.1]	24.0	[20.2 - 25.9]	26.4	[23.8 - 28.1]
Third measurement (h)	48.0	[44.2 - 52.1]	48.0	[44.2 - 50.9]	48.4	[45.6 - 52.1]

No. = number; kg = kilogram; cm = centimeter; IUmL-1 = international units per milliliter. [a] for one patient blood-group was not assessed. [b] maximum measured inhibitor concentration was 0.9 BU.

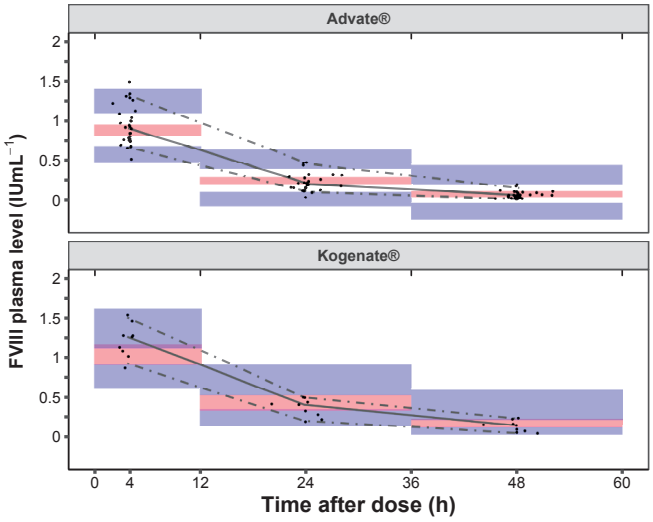


Figure 1. Observed FVIII plasma level versus time for all patients that received 50 $IUkg^{-1}$ Advate® (n=30) or Kogenate® (n=9). The black dots represent the measured FVIII for all patients. The solid black line represents the median of the obeservations and the dashed-lines represent the 2.5th and 97.5th quantiles. The red and blue-shaded areas show the 95% prediction intervals for the predicted FVIII values, as obtained by Monte Carlo simulation (visual predictive check).

For the 24 patients, myPKFiT™ produced estimates for clearance that were higher than those obtained from NONMEM®: medians were 3.1 $mLh^{-1}kg^{-1}$ versus 3.04 $mLh^{-1}kg^{-1}$ (p<0.001, n=24), respectively. Moreover, $t_{½}$ as estimated by myPKFiT™ was shorter than the $t_{½}$ obtained by NONMEM® (*Figure 2A*); medians were 12.6 h and 13.0 h (p<0.001, n=24), respectively. Consequently, the reported time to 0.01 $IUmL^{-1}$ was shorter for myPKFiT™ than for NONMEM®: 77.5 h versus 81.1 h (p<0.001, n=24), see *Figure 2B*. Estimates for Vss were not different (p=0.60, n=24).

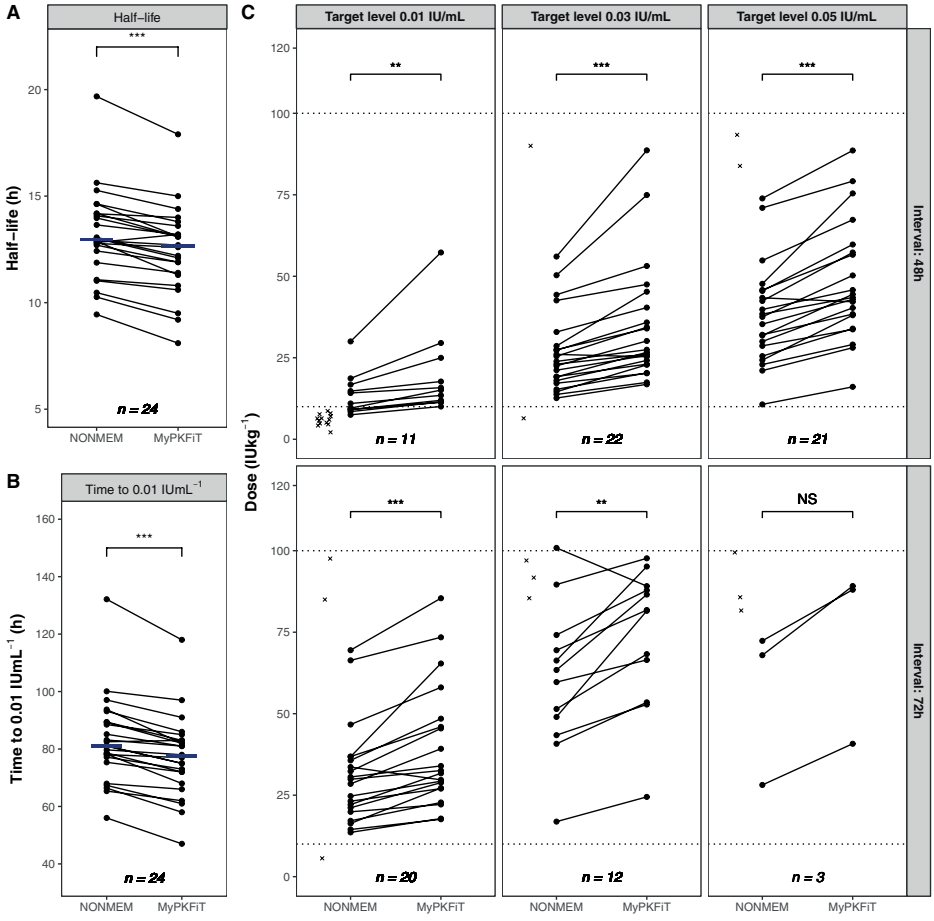


Figure 2. Comparison of the elimination half-life (panel A), time to 0.01 $IUmL^{-1}$ (panel B) and recommended doses (panel C) for Advate® produced by NONMEM® and myPKFiT™. The time to 0.01 $IUmL^{-1}$ is defined as the time to target value of 0.01 $IUmL^{-1}$ above the endogenous baseline level. MyPKFiT™ did not support dose recommendation below 10 $IUkg^{-1}$ and above 100 $IUkg^{-1}$. These thresholds are depicted by the dashed horizontal lines. Doses obtained using NONMEM® for which no dose recommendation was available from myPKFiT™ are depicted by stars on the left. NS: not significant, *: p-value<0.05, **: p-value<0.01, ***: p-value<0.001.

MyPKFiT™ produced dosage regimens for the target trough levels of 0.01 IUmL^{-1}, 0.03 IUmL^{-1} and 0.05 IUmL^{-1}, above the endogenous baseline level for dosing intervals of 48 and 72 hours (*Table 2*). These dose regimens are only reported when they are within the range of 10 to 100 IUkg^{-1}, which is in accordance with the Summary of Product Characteristics (SmPC) of Advate® [19]. For the 48 hour dosing interval, doses targeting at trough levels of 0.01 IUmL^{-1}, 0.03 IUmL^{-1} and 0.05 IUmL^{-1} were obtained for 11, 22 and 21 of the 24 patients (*Figure 2C*), respectively. For the 72 hour interval, doses were obtained in 20, 12 and 3 patients, respectively. In general, doses recommended by myPKFiT™ were higher than those obtained using NONMEM®. For the 48 hour interval, median doses targeting at 0.01 IUmL^{-1} were 15.1 IUkg^{-1} and 11.0 IUkg^{-1} for myPKFiT™ and NONMEM®, respectively (p<0.01, n=11). Corresponding doses for the 0.03 IUmL^{-1} target were 27.1 IUkg^{-1} and 23.5 IUkg^{-1} (p<0.001, n=22) and 43.5 IUkg^{-1} and 37.5 IUkg^{-1} (p<0.001, n=21) for the 0.05 IUmL^{-1} target. Doses, targeting at 0.01 IUmL^{-1} and 0.03 IUmL^{-1} and administered every 72 hours, were also higher for myPKFiT™ compared with NONMEM® (see *Table 2*).

Table 2. Individual PK parameter estimates from NONMEM® versus myPKFiT™ (Advate® only)

		NONMEM®		myPKFiT™		
	N[a]	Median	[min-max]	Median	[min-max]	*p*-value[b]
Parameter						
Half-life (h)	24	13.0	[9.4 - 19.7]	12.6	[8.1 - 17.9]	<0.001
Clearance (mLh^{-1}kg^{-1})	24	3.04	[1.54 – 5.44]	3.1	[1.7 – 6.0]	<0.001
Distribution volume in steady-state (mLkg^{-1})	24	50.3	[37.9 – 64.6]	50	[40 - 60]	0.60
Time to 0.01 IUmL^{-1} above baseline level (h)	24	81.0	[56.0 - 132.1]	77.5	[47.0 - 118.0]	<0.001
Recommended dose regimen (IUkg^{-1})						
Trough 0.01 IUmL^{-1}, per 48 hours	11	11.0	[7.5 - 30.0]	15.1	[10.0 - 57.3]	<0.01
Trough 0.01 IUmL^{-1}, per 72 hours	20	29.2	[13.6 - 69.5]	32.1	[17.6 - 85.4]	<0.001
Trough 0.03 IUmL^{-1}, per 48 hours	22	23.5	[12.6 - 56.0]	27.1	[16.8 - 88.6]	<0.001
Trough 0.03 IUmL^{-1}, per 72 hours	12	61.5	[16.9 - 100.9]	81.7	[24.5 - 97.7]	<0.01
Trough 0.05 IUmL^{-1}, per 48 hours	21	37.5	[10.7 - 73.9]	43.5	[16.1 - 88.6]	<0.001
Trough 0.05 IUmL^{-1}, per 72 hours	3	67.9	[28.1 - 72.4]	88.0	[40.8 - 89.2]	0.25

[a] Number of estimates used for comparison. [b] *p*-value is obtained using a paired Wilcoxon signed rank test.

Advate®: NONMEM® versus WAPPS

WAPPS produced individual PK parameters for all patients receiving Advate® (*Figure 3A and 3B*). Half-life estimated by WAPPS was shorter than the $t_{½}$ estimated by NONMEM®; respective medians were 11.2 h and 13.0 h (p<0.001, n=30). Despite the difference in $t_{½}$ produced by WAPPS and NONMEM®, a difference was observed only for the time to 0.05 IUmL^{-1} (*Table 3*); respective medians were 51.0 h and 52.7 h (p<0.001, n=29). The time to 0.01 IUmL^{-1} could only be imputed for 23 patients, as 7 patients had an endogenous

baseline level higher than 0.01 IUmL^{-1} No differences were obtained for the time to 0.01 IUmL^{-1} and 0.02 IUmL^{-1}: medians were 79.8 h versus 81.0 h (p=0.60, n=23) and 65.0 h versus 67.9 h (p=0.15, n=29), respectively (*Table 4*).

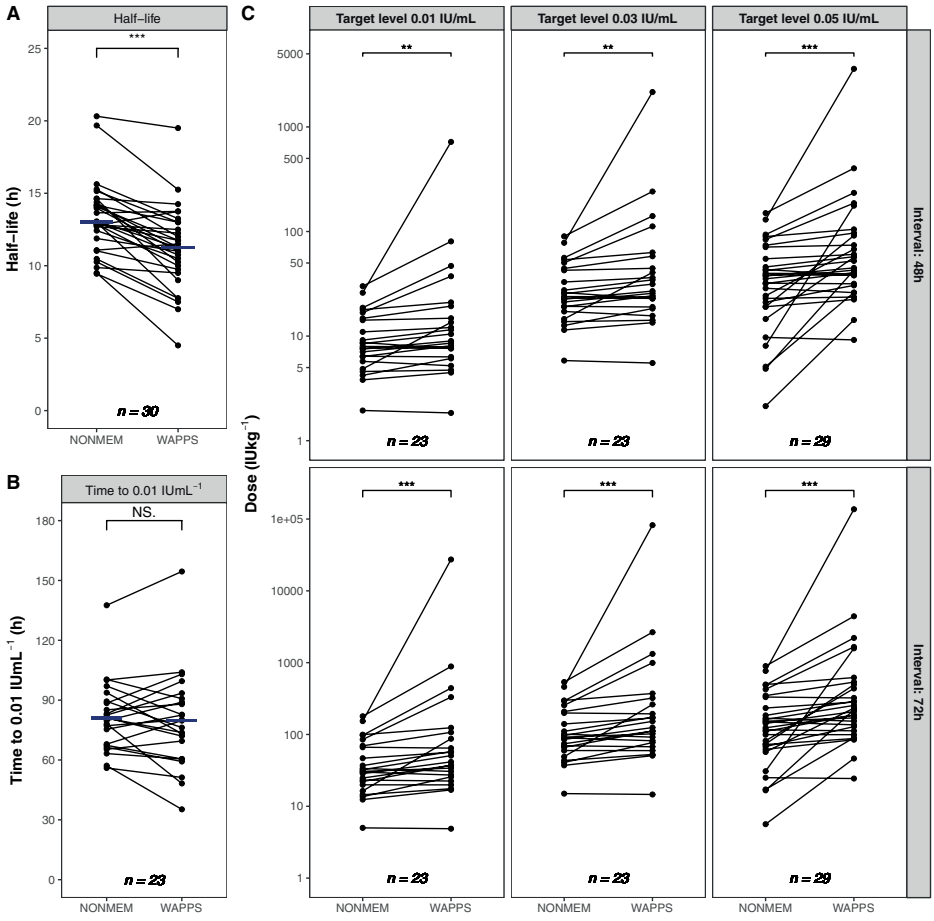


Figure 3. Comparison of the elimination half-life (panel A), time to 0.01 IUmL^{-1} (panel B) and recommended doses (panel C) for Advate® produced by NONMEM® and WAPPS. Time to 0.01 IUmL^{-1} was only provided by WAPPS if the endogenous baseline level was below this target value. Moreover, WAPPS did not provided a dose recommendation, if the target trough value was equal to or below the endogenous baseline level of a patient. For instance, seven patients receiving Advate® had an endogenous baseline of 0.01 IUmL^{-1} or higher. Therefore, WAPPS provided dose recommended only for 23 of 30 patients receiving Advate®. NS: not significant, *: p-value<0.05, **: p-value<0.01, ***: p-value<0.001.

WAPPS produced dosage regimens for intervals of 48 and 72 hours and target FVIII of 0.01 IUmL^{-1}, 0.03 IUmL^{-1} and 0.05 IUmL^{-1} if the endogenous baseline level was not higher than the target FVIII itself (*Table 3*). For both the 48 and 72 hour interval, doses targeting at trough levels of 0.01 IUmL^{-1}, 0.03 IUmL^{-1} and 0.05 IUmL^{-1} were obtained for 23, 23 and 29 of the 30 patients (*Figure 3C*), respectively. In general, doses recommended by WAPPS

were higher than those obtained from NONMEM®. For the 48 hour interval, median doses targeting at 0.01 IUmL^{-1} were 9.0 IUkg^{-1} and 8.0 IUkg^{-1} for WAPPS and NONMEM®, respectively (p<0.01, n=23). Corresponding doses for the 0.03 IUmL^{-1} target were 26.9 IUkg^{-1} and 23.9 IUkg^{-1} (p<0.01, n=23) and 44.9 IUkg^{-1} and 35.3 IUkg^{-1} (p<0.001, n=29) for the 0.05 IUmL^{-1} target. Strikingly, doses targeting at 0.01 IUmL^{-1}, 0.03 IUmL^{-1} and 0.05 IUmL^{-1}, and administered every 72 hours were much higher for WAPPS as compared with NONMEM®, whereas the maximum doses recommended for this interval by WAPPS were extremely high; values were 27366 IUkg^{-1}, 82098 IUkg^{-1} and 136831 IUkg^{-1}, respectively.

Table 3. Individual PK parameter estimates from NONMEM® versus WAPPS (Advate® only)

		NONMEM®		WAPPS		
	N[a]	Median	[min-max]	Median	[min-max]	*p*-value[b]
Parameter						
Half-life (h)	30	13.0	[9.4 - 20.3]	11.2	[4.5 - 19.5]	<0.001
Time to 0.01 IUmL^{-1} (h)	23	81.0	[56.0 - 137.6]	79.8	[35.2 - 154.5]	0.60
Time to 0.03 IUmL^{-1} (h)	23	67.9	[46.5 - 117.2]	65.0	[28.0 - 123.5]	0.15
Time to 0.05 IUmL^{-1} (h)	29	52.7	[34.1 - 132.1]	51.0	[20.8 - 108.0]	<0.01
Recommended dose regimen (IUkg^{-1})						
Trough 0.01 IUmL^{-1}, per 48 hours	23	8.0	[1.9 - 30.0]	9.0	[1.8 - 718.9]	<0.01
Trough 0.01 IUmL^{-1}, per 72 hours	23	30.6	[5.0 - 179.1]	41.5	[4.9 - 27366.1]	<0.001
Trough 0.03 IUmL^{-1}, per 48 hours	23	23.9	[5.8 - 90.0]	26.9	[5.5 - 2156.6]	<0.01
Trough 0.03 IUmL^{-1}, per 72 hours	23	91.7	[15.0 - 537.2]	124.6	[14.6 - 82098.3]	<0.001
Trough 0.05 IUmL^{-1}, per 48 hours	29	35.3	[2.1 - 150.0]	44.9	[9.2 - 3594.3]	<0.001
Trough 0.05 IUmL^{-1}, per 72 hours	29	123.6	[5.6 - 895.3]	220.4	[120.4 - 1e+05]	<0.001

[a] Number of estimates used for comparison. [b] *p*-value is obtained using a paired Wilcoxon signed rank test.

Kogenate®: NONMEM® versus WAPPS

In *Figure 4A and 4B*, a comparison between estimates for individual PK parameters for Kogenate® obtained using WAPPS and NONMEM® is shown. Estimates of $t_{½}$, produced by WAPPS, were longer as compared to estimates of $t_{½}$ produced by NONMEM®; respective medians were 15.0 h and 12.3 h (p<0.01, n=9). Moreover, differences were observed for the time to 0.02 IUmL^{-1} and 0.05 IUmL^{-1}; corresponding medians were 84.9 h versus 81.5 h (p<0.05, n=6) and 69.2 h versus 60.8 h (p<0.01, n=9), respectively.

For dosing every 48 and 72 hours, no differences were found between the dosage regimen as produced by WAPPS and NONMEM® for patients receiving Kogenate® (*Table 4*).

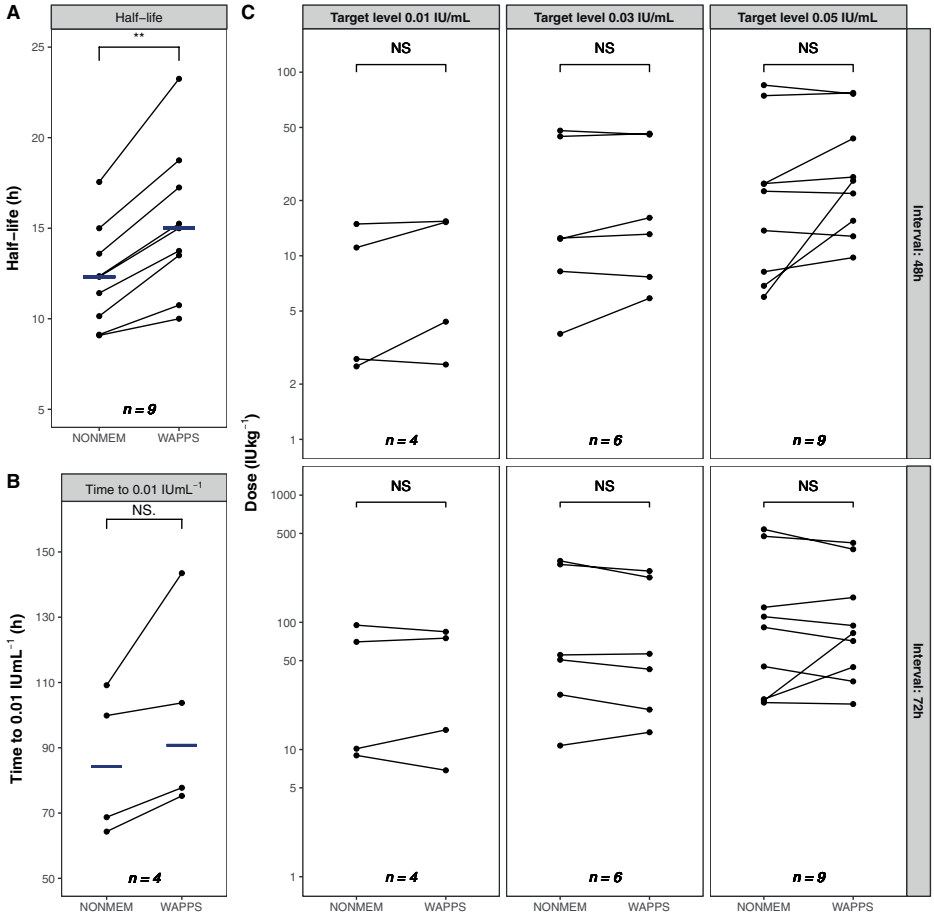


Figure 4. Comparison of the elimination half-life (panel A), time to 0.01 IUmL^{-1} (panel B) and recommended doses (panel C) for Kogenate® produced by NONMEM® and WAPPS. Time to 0.01 IUmL^{-1} was only provided by WAPPS if the endogenous baseline level was below this target value. Moreover, WAPPS did not provided a dose recommendation, if the target trough value was equal to or below the endogenous baseline level of a patient. For instance, five patients receiving Kogenate® had an endogenous baseline of 0.01 IUmL^{-1} or higher. Therefore, WAPPS provided dose recommended only for 4 of 9 patients receiving Kogenate®. NS: not significant, *: p-value<0.05, **: p-value<0.01, ***: p-value<0.001.

Validity of the NONMEM® population PK models

The availability of an independent dataset allows the validation of the two population PK models programmed in NONMEM®. As the individual predicted FVIII were calculated using individual PK parameters, it is expected that values were similar to the measured FVIII for a specific time-point. Moreover, for both population PK models, the imprecision of the individual PK parameter estimates was less than 20%, which is generally regarded as an 'accurate' estimate (supplementary material *Table 4*). In this case, all individual predicted FVIII should be on the line x=y in the goodness-of-fit plots. In plots of observed

FVIII versus individual predicted FVIII, only minor deviations were found indicating the general adequacy of the individual PK fits (*Supplementary Figure 2* - upper panels). Nevertheless, the highest FVIII at sampling time T=4 were slightly underpredicted.

Population predicted FVIII are calculated using the population (median) PK parameters values and individual values for body weight and age (*Supplementary Figure 2* - lower panels). Population predicted FVIII activity measurements will therefore deviate from the line x=y due to inter-patient variability. Although, it is expected that population predicted FVIII values are distributed symmetrically around the line x=y, i.e. do not display a trend. As in the plots of observed versus individual predicted concentrations, a minor trend for underprediction of the highest measured FVIII was seen for both Advate® and Kogenate®.

In *Figure 1*, prediction-corrected VPCs are shown for the population PK models used to describe the measured FVIII, obtained after administration of Advate® or Kogenate®. Black dots depict the measured FVIII, from which black solid-lines show the observed 50th quantile (median) and the black dashed-lines the 5th and 95th quantile of the observed FVIII. The red and blue-shaded areas show the 95% prediction interval for the predicted FVIII values, as obtained by Monte Carlo simulation. If the red and blue-shaded areas cover the corresponding quantiles from the measured FVIII, this demonstrates that the model is able to adequately describe the measured FVIII. In this case, the predicted concentrations (red and blue-shaded area) generally predicted the measured FVIII well, indicating the appropriateness of the models. Nevertheless, for Kogenate®, a minor underprediction of the measured FVIII (4 hours after administration) was seen. Furthermore, for the FVIII measured 24 hours after administration of Kogenate®, a minor overprediction was seen.

DISCUSSION

In this study, a cross-evaluation of three PK tools (myPKFiT™, WAPPS and NONMEM®) that perform Bayesian analysis was conducted, using data from hemophilia A patients receiving Advate® or Kogenate®. In general, significant differences between the three PK tools were found for CL, $t_{½}$ and time to target FVIII. Moreover, clinical relevant differences were found between the doses, as recommended by myPKFiT™, WAPPS and NONMEM®.

In Bayesian analysis, individual PK parameter estimation is influenced by the population PK model parameters used in the analysis. A population PK model is comprised of both population parameters (medians) and their corresponding inter-patient variability.

For instance, the population parameter value for CL serves as *a priori* information and is used as the initial value for estimating an individual value for CL. The inter-patient variability, associated with CL, determines the extent to which CL may be adjusted by the individual's clinical data. Moreover, if there is no variability associated to a population parameter, as is the case for Q and V2 from the models applied in NONMEM®, the individual PK parameter estimates equals the population value. Furthermore, when performing Bayesian analysis, the extent of residual variability also affects the estimation of individual PK parameters. The residual variability determines the weighting of a single observation being taken into account for estimating individual parameters. For instance, the fitted concentration-time profile will generally be closer to the observed concentrations with low residual variability (high weighting) as compared to high residual variability (low weighting). In the latter case, observed concentrations may deviate more from the predicted concentration-time profile than in the former case. Therefore, using similar observations, different population PK models may produce different individual PK parameter estimates when Bayesian analysis is performed.

The results of the present study demonstrate how differences in population PK parameters influence individual PK parameter estimates. For both Advate® and Kogenate®, different individual PK parameters are obtained when different PK tools are applied. These differences are clinically relevant, as doses for Advate® recommended to obtain a certain target trough level by myPKFiT™ are 10% to 37% (3 to 20 $IUkg^{-1}$) higher than those recommend by NONMEM®. For Advate®, WAPPS recommended 13% to 78% (1 to 96.8 $IUkg^{-1}$) higher doses than NONMEM®. These differences are caused by the fact that individual values for the primary PK parameters CL, V1, Q and V2 are not similar for the three evaluated PK tools. WAPPS does not provide primary PK parameters, whereas myPKFiT™ provides only values for clearance and volume of distribution. However, both PK tools provide individual estimates for $t_{½}$, a secondary PK parameter of which the value is determined by all primary parameters. The shorter $t_{½}$ provided by both myPKFiT™ and WAPPS may be explained by higher values for clearance and/or lower values for the volume of distribution in comparison with NONMEM®. Clearly, shorter $t_{½}$ correspond to higher recommended doses. For Kogenate®, however, this association was not found. WAPPS produced a longer $t_{½}$ than NONMEM®; recommended doses were however not significantly different. This may be explained by the fact that data was available from only 4 to 9 patients (*Table 4*).

In clinical practice, doses are rounded up or down to a full vial of FVIII concentrate (250 IU). The decision to round up or round down a recommended FVIII dose to a full vial depends on the following reasons: personal preferences of the treating physician, amount

of FVIII concentrate available in the country and bleeding phenotype of the patient. Thus, rounding up or down of the FVIII dose to a full vial is performed subjectively.

In this study, none of the PK tools is the gold standard. A PK tool can be used as a gold standard for the estimation of individual PK parameters when it has been validated for this purpose. Validation of PK tools is relatively simple [16]. Following a loading dose, 10 samples are obtained and individual PK parameters are estimated by fitting the data to a two-compartment model. Subsequently, a limited number (2-3) of samples is selected to estimate individual PK parameters by Bayesian analysis and are compared to the values obtained from the fit obtained using all data. This method has been applied by *Björkman et al.* [13]. The population PK model used in the Bayesian analysis should be developed on data different from the data used in the validation procedure (independent datasets) [27]. In the present study, the described validation procedure could not be applied since only sparse data was available for each patient. Nevertheless, the availability of sparse (independent) data still allows the validation of the applied population models. Validation consisted of constructing plots of observed versus predicted FVIII ("goodness-of-fit" plots) and/or visual predictive checks (VPCs). These techniques are used commonly in pharmacometric analyses and are advocated by the EMA and FDA to validate population PK models [28,29]. In the present study, these validation procedures were applied to the models programmed in NONMEM®, as the population values of myPKFiT™ and WAPPS were not available. The goodness-of-fit plots and VPCs both demonstrated the adequacy of the applied models (*Supplementary Figure 2*). However, the minor underprediction on T=4 for FVIII from Kogenate® may cause an overprediction of the volume of distribution and, consequently, an overprediction of $t_{½}$. These differences may contribute to the shorter $t_{½}$ as estimated using NONMEM®. Nevertheless, validation of the population PK models, implemented in myPKFiT™ and WAPPS, should be performed before the cause of the observed differences can be identified.

In this study, pre-infusion levels were taken into account for estimation of individual PK parameters, using myPKFiT™ and NONMEM® by specifying (at most six) doses administered prior to the investigational dose. In myPKFiT™, an estimate of the pre-infusion level was produced when a dose administered prior to the investigational dose was entered. No prior doses can be entered in WAPPS. As an alternative, measured pre-infusion levels may be specified in both myPKFiT™ and WAPPS. In the present study, pre-infusion levels were not measured. Therefore, Bayesian estimates produced by WAPPS may be biased in patients that have received considerable FVIII doses before administration of the investigational FVIII dose. For Advate®, median (min-max) pre-infusion FVIII were 0.016 $IUmL^{-1}$ (0 – 0.159) and 0.039 $IUmL^{-1}$ (0 – 0.140) for myPKFiT™ and NONMEM®, re-

spectively (*p*-value=0.59). As a result, it is expected that, particularly in patients with high pre-infusion levels, parameter estimates produced by WAPPS will be biased.

When the pre-infusion level is not taken into account, the distribution volume of the first compartment (V1) may be underpredicted and/or CL may be overpredicted, resulting in an underprediction of $t_{½}$. In the present study, it is not clear to what extent this phenomena contributed to the estimating of $t_{½}$ by WAPPS. Nevertheless, when using WAPPS for Bayesian analysis in a patient with significant pre-infusion levels, a sample should be taken before the administration of the dose, allowing this value to be entered.

The patients that will be selected for dose individualization will most likely be patients that are still suffering from bleeding events despite their current prophylactic dose regimen or patients that are using high doses, which may be attenuated. The former type of patient could be 'deviant' from the population, from which the data was derived to construct the population PK model. For instance, a patient could have a much higher clearance or distribution volume as compared to the model population and, consequently, dose estimations will also be higher. myPKFiT™ did not support doses outside the range 10 to 100 $IUkg^{-1}$. Moreover, if a recommended dose leads to an estimated FVIII higher than 2.5 $IUmL^{-1}$, WAPPS warns that clinical judgment with regard to dose should be exercised. Nevertheless, doses for Advate® within the 72 hours interval were obtained which are not clinically applicable (*Table 3*). Therefore, PK tools should be validated for application of dose individualization in the population, which is likely to have patient-tailored dosing.

CONCLUSIONS

Online PK-guided dosing tools are new technologies, which may contribute to support clinical decision making and optimize the individualization of clotting factor dosing. This could be beneficial for the patients by achieving adequate FVIII levels, but may also be more cost-effective by preventing overdosing of patients. In this study, significant differences among three PK tools were found for estimated individual PK parameters as well as recommended dose regimen. Therefore, hematologists should be aware of the fact that estimates for individual PK parameters and dose recommendations may differ, depending on their choice for a PK tool. Moreover, PK tools that perform Bayesian analysis using population PK models should be validated for this purpose.

REFERENCES

1 Blanchette VS. Prophylaxis in the haemophilia population. *Haemophilia* 2010; 16: 181–188.

2 Björkman S, Berntorp E. Pharmacokinetics of coagulation factors: clinical relevance for patients with haemophilia. *Clin Pharmacokinet* 2001; 40: 815–32.

3 Garmann D, McLeay S, Shah A, Vis P, Maas Enriquez M, Ploeger BA. Population pharmacokinetic characterization of BAY 81-8973, a full-length recombinant factor VIII: lessons learned - importance of including samples with factor VIII levels below the quantitation limit. *Haemoph Off J World Fed Hemoph* 2017; 23: 528–37.

4 Shah A, Solms A, Garmann D, Katterle Y, Avramova V, Simeonov S, Lissitchkov T. Improved Pharmacokinetics with BAY 81-8973 Versus Antihemophilic Factor (Recombinant) Plasma/Albumin-Free Method: A Randomized Pharmacokinetic Study in Patients with Severe Hemophilia A. *Clin Pharmacokinet* 2016; Open access.

5 Jiménez-Yuste V, Lejniece S, Klamroth R, Suzuki T, Santagostino E, Karim FA, Saugstrup T, Møss J. The pharmacokinetics of a B-domain truncated recombinant factor VIII, turoctocog alfa (NovoEight®), in patients with hemophilia A. *J Thromb Haemost* 2015; 13: 370–9.

6 Bjorkman S, Oh M, Spotts G, Schroth P, Fritsch S, Ewenstein BM, Casey K, Fischer K, Blanchette VS, Collins PW. Population pharmacokinetics of recombinant factor VIII: the relationships of pharmacokinetics to age and body weight. *Blood* 2012; 119: 612–8.

7 Björkman S, Folkesson A, Jönsson S. Pharmacokinetics and dose requirements of factor VIII over the age range 3–74 years: A population analysis based on 50 patients with long-term prophylactic treatment for haemophilia A. *Eur J Clin Pharmacol* 2009; 65: 989–98.

8 Oh M, Björkman S, Schroth P, Fritsch S, Collins P, Fischer K, Blanchette VS, Casey KM, Spotts G, Ewenstein BM. Population Pharmacokinetic Model of ADVATE in Pediatric and Adult Patients with Hemophilia A. *Blood* 2009; 114: 3492–3492.

9 Collins PW, Fischer K, Morfini M, Blanchette VS, Björkman S. Implications of coagulation factor VIII and IX pharmacokinetics in the prophylactic treatment of haemophilia: Pharmacokinetcs and Prophylaxis. *Haemophilia* 2011; 17: 2–10.

10 Valentino LA. Considerations in individualizing prophylaxis in patients with haemophilia A. *Haemophilia* 2014; 20: 607–15.

11 Petrini P, Valentino LA, Gringeri A, Re WM, Ewenstein B. Individualizing prophylaxis in hemophilia: a review. *Expert Rev Hematol* 2015; 8: 237–46.

12 Iorio A, Marcucci M. Clinical trials and haemophilia: does the Bayesian approach make the ideal and desirable good friends? *Haemoph Off J World Fed Hemoph* 2009; 15: 900–3.

13 Björkman S. Limited blood sampling for pharmacokinetic dose tailoring of FVIII in the prophylactic treatment of haemophilia A. *Haemoph Off J World Fed Hemoph* 2010; 16: 597–605.

14 Iorio A, Keepanasseril A, Foster G, Navarro-Ruan T, McEneny-King A, Edginton AN, Thabane L, WAPPS-Hemo co-investigator network. Development of a Web-Accessible Population Pharmacokinetic Service-Hemophilia (WAPPS-Hemo): Study Protocol. *JMIR Res Protoc* 2016; 5: e239.

15 Álvarez-Román MT, Fernandez-Bello I, de la Corte-Rodríguez H, Hernández-Moreno AL, Martín-Salces M, Butta-Coll N, Rivas-Pollmar MI, Rivas-Muñoz S, Jiménez-Yuste V. Experience of tailoring

prophylaxis using factor VIII pharmacokinetic parameters estimated with myPKFiT(®) in patients with severe haemophilia A without inhibitors. *Haemoph Off J World Fed Hemoph* 2017; 23: e50–4.

16 Sherwin CMT, Kiang TKL, Spigarelli MG, Ensom MHH. Fundamentals of population pharmacokinetic modelling: validation methods. *Clin Pharmacokinet* 2012; 51: 573–90.

17 Björkman S, Collins P, for the Project on Factor VIII/Factor IX Pharmacokinetics of the Factor VIII/Factor IX Scientific and Standardization Committee of the ISTH. Measurement of factor VIII pharmacokinetics in routine clinical practice: Factor VIII pharmacokinetics. *J Thromb Haemost* 2013; 11: 180–2.

18 Castellone DD, Adcock DM. Factor VIII Activity and Inhibitor Assays in the Diagnosis and Treatment of Hemophilia A. *Semin Thromb Hemost* 2017; 43: 320–30.

19 Baxter, now part of Shire. Advate® - Summary of Product Characterstics. 2017.

20 Karlsson MO, Sheiner LB. The importance of modeling interoccasion variability in population pharmacokinetic analyses. *J Pharmacokinet Pharmacodyn* 1993; 21: 735–750.

21 Dubios A, Bertrand J, Mentré F. Mathematical Expressions of the Pharmacokinetic and Pharmacodynamic Models implemented in the PFIM software. 2011.

22 R Core Team (2017). R: A language and environment for statistical computing. Vienna, Austria; 2016.

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

24 Bonate PL. A brief introduction to Monte Carlo simulation. *Clin Pharmacokinet* 2001; 40: 15–22.

25 Lindbom L, Pihlgren P, Jonsson EN, Jonsson N. PsN-Toolkit--a collection of computer intensive statistical methods for non-linear mixed effect modeling using NONMEM. *Comput Methods Programs Biomed* 2005; 79: 241–57.

26 Keizer R, Pastoor D, Savic R. New open source R libraries for simulation and visualization: "PKPDsim" and "vpc." Hersonissos, Crete, Greece: PAGE; 2015.

27 Brendel K, Dartois C, Comets E, Lemenuel-Diot A, Laveille C, Tranchand B, Girard P, Laffont CM, Mentré F. Are population pharmacokinetic and/or pharmacodynamic models adequately evaluated? A survey of the literature from 2002 to 2004. *Clin Pharmacokinet* 2007; 46: 221–34.

28 U.S. Department of Health and Human Services - Food and Drug Administration. Guidance for Industry - Population Pharmacokinetics. FDA; 1999.

29 Committee for Medicinal Products for Human Use (CHMP). Guideline on reporting the results of population pharmacokinetic analyses. EMA; 2007.

Supplementary Table 1. Population PK models for Advate® and Kogenate®

	Model for Advate® [6]		Model for Kogenate® [7]	
	Estimate	**(%RSE)**	**Estimate**	**(%RSE)**
Structural model				
Clearance (CL; mLh^{-1})	193	2.7	222	5
Volume of central compartment (V1; mL)	2220	1.9	3520	3
Distribution CL to compartment 2 (Q; mLh^{-1})	147	8.6	256	30
Volume of compartment 2 (V2; mL)	730	7.8	241	14
Baseline of endogenous FVIII (BL; $IUmL^{-1}$)	-		0.012	17
Inter-individual variability (%CV)				
IIV on CL	30	15	28	33
IIV on V1	21	14	17	37
Correlation between CL and V1 (%)	45	22	64	44
IIV on Baseline	-		31	110
Inter-occasion variability (%CV)				
IOV CL	-		13	31
IOV V1	-		10	36
Residual variability				
Additive residual error (SD; $IUmL^{-1}$)	0.089	3.5	0.012	17
Proportional residual error (%CV)	-		8.5	6.7
Covariate relations				
CL – Allometric exponent	0.80	5.9	-	
V1 – Allometric exponent	0.95	2.8	-	
V2 – Allometric exponent	0.76	18	-	
CL – (% change with age different from 22 years)	-0.45	41	-	
CL – (% change with age different from 24 years)	-		-0.7	16
CL – (% difference if preparation = full-length recombinant)	-		-20	15
V1 – (% difference if preparation = full-length recombinant)	-		-20	15

IIV: Inter-individual variability; RSE: relative standard error; CV: coefficient of variation. SD: standard deviation.

Supplementary Table 2. Equations for the estimation of individual PK parameters

Model for Advate

$$CL_i(mL/h) = 193 \times \left(\frac{BW_i}{56}\right)^{0.80} \times \left(1 - 0.0045 \times (Age_i - 22)\right) \times \exp^{\left(\eta_i^{CL}\right)} \quad (1a)$$

$$V1_i(mL) = 2220 \times \left(\frac{BW_i}{56}\right)^{0.95} \times \exp^{\left(\eta_i^{V1}\right)} \quad (1b)$$

$$Q_i(mL/h) = 147 \quad (1c)$$

$$V2_i(mL) = 730 \times \left(\frac{BW_i}{56}\right)^{0.76} \quad (1d)$$

Model for Kogenate

$$CL_i(mL/h) = 222 \times \left(\frac{BW_i}{68}\right)^{0.75} \times \left(1 - 0.00696 \times (Age_i - 24)\right) \times (1 - 0.201)^{FLRP} \times \exp^{\left(\eta_i^{CL} + \pi_\kappa^{CL}\right)} \quad (1e)$$

$$V1_i(mL) = 3520 \times \left(\frac{BW_i}{68}\right) \times \exp^{\left(\eta_i^{V1} + \pi_\kappa^{V1}\right)} \quad (1f)$$

$$Q_i(mL/h) = 256 \times \left(\frac{BW_i}{68}\right)^{0.75} \times (1 - 0.201)^{FLRP} \quad (1g)$$

$$V2_i(mL) = 241 \times \left(\frac{BW_i}{68}\right) \quad (1h)$$

Equations for the estimation of the individual PK parameters: CL = clearance, V1 = volume of distribution of the compartment 1, Q = inter-compartmental clearance, V2 = volume of distribution of compartment 2, BW = body weight. η is the individual (Bayesian) estimate of deviance from the population typical value of a PK parameter. Π is the individual estimate of the deviance from the population typical value in each (dosing) occasion. The latter adds extra intra-patient variability to the estimation of individual PK parameter, i.e. this variability describes to what extent the individual PK estimates can differ each occasion. FLRP is 1 if a full-length recombinant product has been administered, otherwise, its value is 0. Both models are from literature [6,7].

Supplementary Table 3. Equations for calculated dose, terminal half-life and time to target plasma level

$$\beta = \frac{1}{2}\left(\frac{Q}{V_1} + \frac{Q}{V_2} + \frac{CL}{V_1} - \sqrt{\left(\frac{Q}{V_1} + \frac{Q}{V_2} + \frac{CL}{V_1}\right)^2 - 4\frac{Q}{V_2}\frac{CL}{V_1}}\right) \tag{1a}$$

$$\alpha = \frac{\frac{Q}{V_2}\frac{CL}{V_1}}{\beta} \tag{1b}$$

$$A = \frac{1}{V_1}\frac{\alpha - \frac{Q}{V_2}}{\alpha - \beta} \tag{1c}$$

$$B = \frac{1}{V_1}\frac{\beta - \frac{Q}{V_2}}{\beta - \alpha} \tag{1d}$$

$$Dose = \frac{C_{trough}(t)}{\left(\frac{Ae^{(-\alpha t)}}{1-e^{-\alpha\tau}} + \frac{Be^{(-\beta t)}}{1-e^{-\beta\tau}}\right)} \tag{1e}$$

$$t_{1/2} = \frac{\ln(2)}{\beta} \tag{1f}$$

$$\tag{1g}$$

$$MyPKFiT:\ Time\ to\ C_{target} = \frac{\left(\ln(C_{target}) - \ln(C_{max}) - \ln(B)\right)}{-\beta} \tag{1h}$$

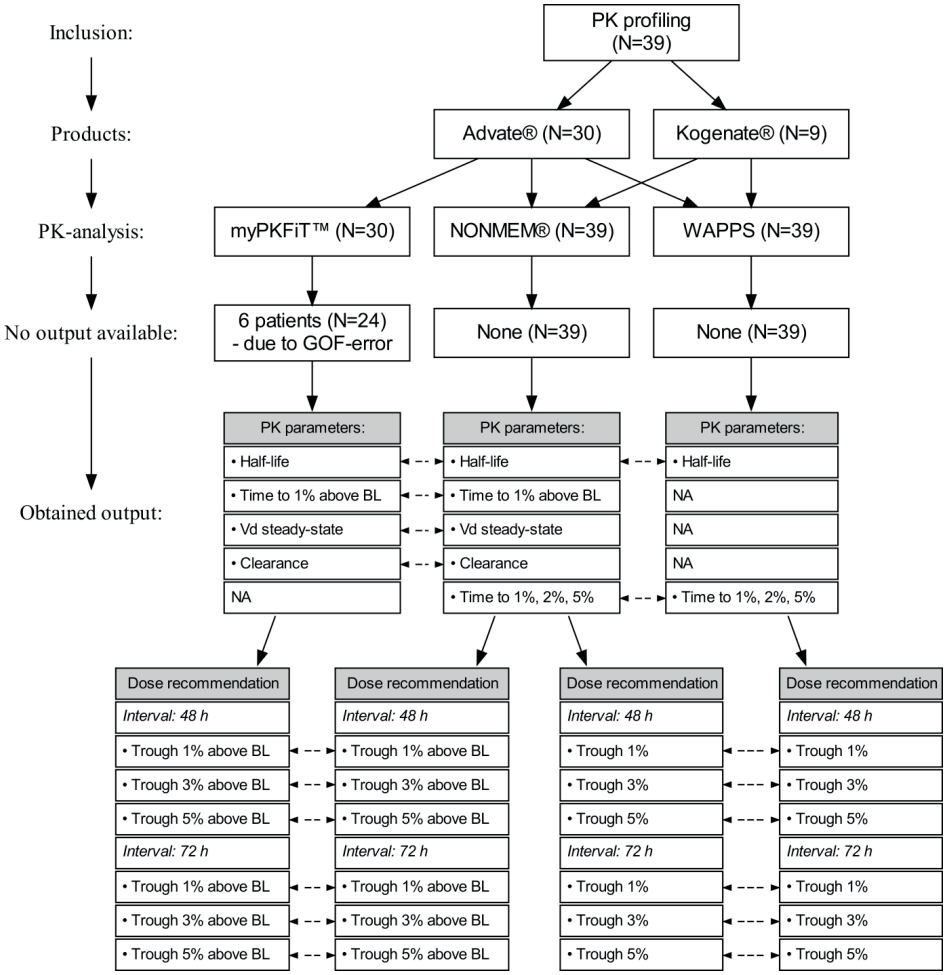
$$WAPPS:\ Time\ to\ C_{target} = \frac{\left(\ln(C_{target} - C_{baseline}) - \ln(C_{max}) - \ln(B)\right)}{-\beta} \iff C_{target} > C_{baseline} \tag{1i}$$

Equations were derived using the Mathematical Equations library from *Dubois et al.* [21].

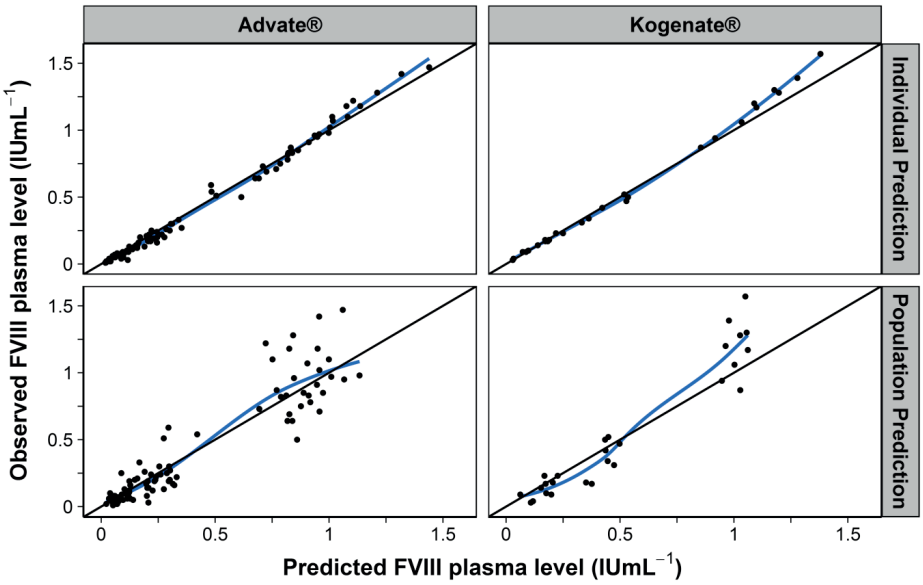
Supplementary Table 4. Relative standard errors of individual PK parameter estimates as derived from Bayesian analysis.

		Clearance (CL)		Distribution volume (V1)		Terminal half-life	
	No. of patients	Median (%)	[min-max]	Median (%)	[min-max]	Median (%)	[min-max]
Applied population PK model							
Advate®[a]	30	15.3	[10.7 - 18.5]	13.2	[7.5 - 17.5]	15.3	[12.3 - 18.1]
Kogenate®[b]	9	5.6	[5.1 - 6.3]	9.2	[8.4 - 9.4]	6.2	[5.9 - 7]

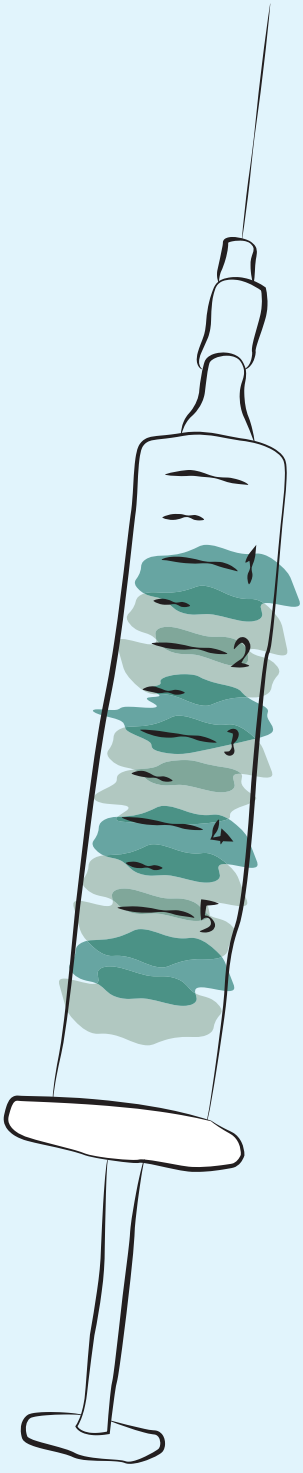
[A] Björkman *et al.*, 2011 [6]. [B] Bjorkman et al., 2009 [7].



Supplementary Figure 1. Flowchart of the study data. In this flowchart, solid black lines describe the data analyses for Advate® as well as Kogenate®. The black dashed lines depict the paired comparisons, which are performed using a paired Wilcoxon sign rank test. GOF: goodness-of-fit, BL: (endogenous) baseline level, Vd: volume of distribution.



Supplementary Figure 2. Observed versus predicted FVIII plasma level. Observed FVIII plasma levels for Advate® and Kogenate® versus individual and population FVIII plasma levels predicted using NONMEM®. Values for predicted FVIII are similar to measured FVIII, when depicted close to the line x=y. The blue line depicts a LOWESS curve, which is a smoothed line that follows the most dense part of the data. Individual predictions are expected to be close to the line x=y, whereas population predictions are expected to be distributed symmetrically around this line.



11

General discussion

STUDY OBJECTIVES AND MAIN FINDINGS

In this thesis, we have presented the conditions, strengths and limitations but also potential applications of PK-guided dosing of FVIII concentrate in hemophilia A patients. In part 1 we evaluated *Current diagnostics and treatment monitoring*. Based on a case history of two young boys with hemophilia, we showed that variations in FVIII measurements may result in misclassification or delay in treatment of hemophilia A patients if treating physicians are not aware of this phenomenon. This shows that quality, e.g. accuracy and reproducibility, of FVIII measurements are important conditions for the diagnosis of hemophilia and monitoring of factor replacement therapy, especially when PK-guided dosing is applied. In part 2, entitled *Implementation of PK-guided dosing of factor VIII concentrate in hemophilia A*, several examples of PK-guided dosing in clinical practice are reported. Importantly, as the first research group, we designed and performed a randomized controlled trial which compared perioperative PK-guided dosing of FVIII concentrate with standard dosing based on bodyweight in severe and moderate hemophilia A patients, the "OPTI-CLOT" trial. This trial showed that perioperative consumption of factor concentrate was similar between the two dosing strategies. However, PK-guided dosing resulted in more accurate targeting of pre-specified FVIII ranges and therefore improved FVIII dosing. We also observed that von Willebrand factor (VWF) increased perioperatively, but that VWF levels only minimally affected FVIII clearance. In two studies, we showed that ideal body weight best explains the interindividual variability of FVIII concentrate PK in obese patients. Finally, we demonstrated that systematic differences exist in currently available PK-guided dosing tools resulting in different dosing advices. In the following section, our main findings will be placed into a broader perspective, weighing up opportunities and obstacles, ultimately concluding that implementation of PK-guided dosing is an important innovation for daily clinical practice in hemophilia.

CONDITIONS NECESSARY FOR IMPLEMENTATION OF PK GUIDED-DOSING

Accuracy of documentation of factor concentrate dosing and timing

PK guided-dosing can be applied by Bayesian forecasting. This technique requires a limited number of observations by blood sampling in the individual and availability of a model describing the PK in a large population. In a population PK model typical values of PK parameters e.g. clearance and volume of distribution are supplied with corresponding interpatient and intrapatient variability. Oftenly, these models also describe relationships between a PK parameter and a specific patient characteristic e.g. weight

and VWF level.[1] Bayesian forecasting can only be applied successfully when information of the individual and the population is accurate and of high quality. Accuracy of PK data is determined by the reliable data collection of patient-related factors, precise amount of drug administered, and exact information on timing of dosing and blood sampling.[1] It is not surprising that data collection therefore requires special training and persistently remains challenging , especially around surgery and bleeding events, and when administered prophylactically by the patients themselves, unsupervised in the home setting. However, in the Netherlands, this information is generally documented in patients logs and a condition for distribution of factor concentrates in all Hemophilia Treatment Centers. The current Dutch HemoNed registry, which aims to register all patients with a bleeding disorder, was recently provided with a mobile phone application which facilitates dosing documentation by patient and/or family members.[2]

PK tools, such as the PK tools described in chapter 10, are an important option to provide dosing advice in a user-friendly manner. Furthermore, these tools may be used to collect data which can subsequently be used to create and enrich existing population PK models. However, it is important to realize that online tools can also be filled in inadequately, which will subsequently affect data quality and lead to less accurate estimations of individual PK parameters and predictions of best doses to acquire factor target levels. This aspect should therefore always be a realized and safeguarded when using data through e-health modules.

Population PK modeling necessitates accurate FVIII levels

Measurement of FVIII levels in itself is not challenging, however, generating accurate FVIII levels necessitates constant attention and diligence. Prior discussion on assay types has focused on the relevance of chromogenic assays (CSA) in non-severe hemophilia A, and which assay to use when predicting bleeding phenotype and indication for prophylactic treatment, as well as consequences of B-domain depletion in some FVIII concentrates and effect on FVIII assay reproducibility.[3-6] When considering implications, it is important to realize that one-stage assay (OSA) and CSA are tests which fundamentally measure FVIII activity differently. The OSA is based on the activated partial thromboplastin time (aPTT).[7] In the OSA, the incubation period is short and endpoint is determined as part of the steep part of an S-shaped curve. In CSA setting, the coagulation cascade is triggered resulting in factor Xa generation (FXa).[8,9] This generated FXa cleaves a chromogenic substrate resulting in a color change, of which overall absorbance reflects the amount of FVIII. During CSA, the incubation period is longer than in the OSA and the endpoint or extinction, is set at a plateau phase. Furthermore, the CSA contains supraphysiological concentrations of thrombin and factor IX. As a result of different test methods and endpoints, it is not surprising that both assays may lead to different FVIII results in he-

mophilia patients with varying *F8* mutations.[4] Previous studies have also demonstrated that B-domain deleted FVIII concentrates lead to FVIII measurements that have been reported to be 20% lower in OSA, when compared to CSA results.[10,11] Interestingly, this discrepancy was not observed in a FVIII concentrate of which 22 aminoacids of FVIII B-domain were still present.[12] Due to these reports, the Scientific and Standardization Committee (SSC) for FVIII and FIX (Toronto 2015) recommends to measure FVIII by both OSA and CSA in all hemophilia A patients. Two FVIII measurements give the opportunity to compare values and to diagnose and initiate treatment based on lowest FVIII value measured, ensuring patient safety.

The discussion above has become more important with the introduction of extended half-life concentrates, which are increasingly used. Reports show that choice of assay is extremely relevant for accurate measurement of factor levels after administration of extended half-life concentrates.[13] In chapter 3, we have shown that considerable variation is present when measuring endogenous FVIII levels, and that this variation is partly explained by the reagents used. When measuring FVIII activity in samples with (extended half-life) FVIII concentrate, the variation in the measurements will increase, especially if guidelines and information from ongoing publications on assay choice are not followed.[13] As a result, major under-or overestimations of FVIII measurements will occur, most importantly with consequences for clinical care but also with regard to PK-guided dosing. As most hemostasis laboratories are required to be able to perform validated measurements of plasma samples for various factor concentrates, it is challenging to safeguard quality and availability of diagnostic procedures 24/7, as is obligatory in majority of Hemophilia Treatment Centers. In addition to assay type, choice of activator and plasma standard are also of importance as they may influence assay outcomes, further complicating standardization of assays and uniformity of laboratory results. Discrepancies between the OSA and CSA are not only observed after infusing factor concentrates, but also when measuring the efficacy of gene therapy. A recent multiyear follow-up study showed that values of OSA were higher than CSA in patients treated with adeno-associated virus (AAV)–mediated gene therapy.[14] Education of (pediatric) hematologists, treating physicians and laboratory personnel on these aspects and limitations is vital to guarantee best treatment for each hemophilia patient. Moreover, the predictive performance of population PK models should be improved. This may be accomplished by enriching the models with data from “real-world” patients, typically exhibiting more variability than patients participating in registration trials. Furthermore, extensive information on potential covariates should be implemented in the model by incorporation of PK data of large heterogeneous population groups. Lastly, population PK modes should also be specific for the type of assay and reagents used.

Hemostasis tests should be associated with bleeding tendency

Novel global hemostatic tests should be able to predict bleeding tendency both reliably and reproducibly, as well as rapidly in both whole blood and (frozen) plasma samples. The largest advantage of global hemostatic tests is that they take all coagulation factors into account, instead of only measuring FVIII or FIX activity. Attempts to create such a global hemostatic test include thrombin generation tests, thromboelastography, and thromboelastometry (TEG® and ROTEM®).

For the thrombin generation test, dose response curves have been created by FVIII concentrate spiking. Reports show that endogenous thrombin potential (ETP) and initial rate of thrombin generation correlate well with the amount of FVIII in plasma.[15] Additional observations show that associations can be found between clinical bleeding phenotype, as Santagostino et al. reported lower ETP in patients with a mild bleeding phenotype (annual bleeding rate or ABR ≤ 2 and factor concentrate consumption ≤ 500 IU/kg/year) compared to patients with a more severe bleeding phenotype (ABR ≥ 2 and factor concentrate consumption ≥ 500 IU/kg/year).[16] However, thrombin generation testing contains several limitations regarding pre-analytical variables. Firstly, some blood-drawing systems cause overestimations of ETP which amount to up to 29.5%, probably as a result of an *in vitro* activation of the coagulation system.[17] Secondly, pneumatic tube transport is regularly used in large hospitals. This transport increases thrombin generation significantly when compared to hand-carrier transport.[18] Thirdly, samples need to be processed preferably within one hour, which is an important practical issue.[19] Besides these pre-analytical challenges, the assay is also very sensitive to source and concentration of tissue factor, used to initiate thrombin generation.[20] Finally, despite the fact that large improvements have been made to decrease inter-and intra-assay variability, interpatient variability is still demonstrated and overall does not seem to correlate with bleeding phenotype.[16,21] More specifically, large differences in bleeding phenotype have been confirmed by thrombin generation tests, but clinically relevant subtle variations in lower range of FVIII levels of patient *in vivo* samples, cannot be adequately assessed by current thrombin generation test results.[16] In conclusion, although thrombin generation testing may be a suitable candidate to measure hemostatic potential, these tests still require optimization before practically applicable in hemophilia care. However, the Scientific and Standardization Committee of the International Society of Thrombosis and Hemostasis has published a guideline which helps to uniform and standardize thrombin generation tests.[22]

Viscoelastic testing in TEG® or ROTEM® was originally developed as promising in small pioneering studies in hemophilia A patients.[23,24] As in thrombin generation testing, large differences in factor levels and bleeding phenotype could be quantified by TEG® but

interindividually the more subtle changes in levels and bleeding phenotype, such as the difference between mild hemophilia and normal controls, could not be detected.[25,26] Similar to the thrombin generation test, viscoelastic testing is also sensitive to (pre-) analytical variables, such as needle size and time between blood collection and laboratory analysis to minimize platelet activation. In addition, TEG® coefficient of variation is variable and may increase up to 19% for the TEG 5000®.[27] However, intra-laboratory variation was reported acceptable compared to a high inter-laboratory variation, which was stated to rise up to 49% for TEG 5000® and 83% for the ROTEM delta®.[28] There is ongoing discussion if viscoelasticity testing remains a promising global hemostatic test in hemophilia due to its disadvantages.

Alternative tests that predict overall bleeding tendency are also important with regard to upcoming non-replacement therapies such as bispecific antibodies that mimic FVIII or drugs that inhibit anticoagulant pathways. Nevertheless, it will always remain questionable if an *in vitro* test is able to measure *in vivo* hemostasis in the context of patients treated with various hemostatic agents. Hopefully, future studies focusing on development of novel global hemostatic tests will reveal more knowledge on this aspect, bringing us closer to the ultimate goal of actually assessing an individual's bleeding tendency. This may improve treatment significantly, as it can be based on clinical phenotype instead of residual factor levels.

STRENGTHS AND LIMITATIONS OF PK-GUIDED DOSING

Improved targeting of pre-specified FVIII levels

Previous studies have demonstrated that standard perioperative dosing of factor concentrates based on bodyweight leads to factor levels ≥0.20 IU/mL below or above predefined target ranges[29-34], resulting in either underdosing with increased risk of bleeding or overdosing associated with high treatment costs.[35-38] This can be at least partially explained by well-known interindividual differences in PK of factor concentrates.[39,40] PK-guided dosing using Bayesian forecasting takes the interindividual differences in PK parameters into account and generates the individualized dose that will produce the target activity level thereby increasing efficacy and patient safety.

We have shown that PK-guided dosing has the potential to improve targeting of pre-specified FVIII levels both perioperatively and in the prophylactic setting in several varying circumstances.[41-43] Furthermore, the OPTI-CLOT trial is the first randomized controlled trial that underlines the superiority of factor level targeting in the perioperative setting. Although, alternative treatments with non-factor replacement therapy are upcoming,

factor replacement therapy is still considered treatment of choice. Individualization of prophylaxis does not only consist of adjusting of dosing according to individual PK parameters, but also according to developmental phase and lifestyle, taking patient age, bleeding phenotype, most practical dosing intervals, joint status, physical activity and sporting activities into account, and should become standard clinical practice in our opinion. As it improves quality of care and regulates costs of treatment, taking cost and benefit into account.[44-46] As experience with PK-guided dosing of extended half-life products is increasing and dosing is still mainly based on population PK models derived from data of drug trials in homogeneous selected patient populations, further enriching of population PK models is indicated and is currently ongoing in the OPTI-CLOT TARGET study.

Expensive treatment under PK guidance may lead to cost reduction

The OPTI-CLOT trial was designed to detect a difference in perioperative FVIII concentrate consumption between PK-guided and standard dosing based on bodyweight. This endpoint was chosen to investigate cost-effectiveness of this approach, but also to be able to power the study adequately as endpoint perioperative bleeding was calculated not be feasible due to low bleeding risk and scarcity of hemophilia A patients undergoing surgery. Nevertheless, PK-guided dosing did not result in lower perioperative FVIII concentrate consumption. Based on our retrospective perioperative study, we calculated that potential savings of FVIII consumption could amount to 44%, if all FVIII levels were kept within target ranges.[32] Interestingly, we did not demonstrate such a reduction in factor concentrate consumption. There are a number of factors that may explain this unexpected outcome. Firstly, more optimal and efficient targeting in the first 24 hours after surgery by PK-guided dosing leading to higher factor VIII concentrate doses may have compensated a decrease in consumption >120 hours after surgery. Importantly, if this is the case this will increase patient safety as bleeding and thrombosis risk may decrease. Unfortunately lack of factor VIII measurements at end of the perioperative period with a median number of 6.5 factor VIII measurements per patient in the total study, corresponding with a time point approximately 96 - 120 hours after surgery, hampers statistical analyses to prove this. Secondly, median hospitalization period in the retrospective study was 9.0 (IQR 5.0 – 12.0) versus 3.5 (IQR: 0.0 – 9.0) days in the OPTI-CLOT trial. This does not coincide with severity of surgery but seems related to changes in clinical practice, leading to shorter hospital admissions as no differences in severity of surgery were demonstrated in treatment arms. Post hoc analyses were performed to see if differences were due to earlier discharge and switch to bolus infusion and thus higher consumption in the home treatment setting, this could not be demonstrated.

Additional studies in OPTI-CLOT data will investigate actual cost-benefit ratio of PK-guided dosing in clinical practice in more detail, both perioperatively as well as prophylactically. Already in 1997, Carlsson et al. showed that PK-guided dosing was feasible and resulted in decreased consumption of FVIII concentrate in the prophylactic setting.[46] However, in this study only 14 of 21 participants completed the study protocol. Another small prospective trial by Lindvall et al. showed that daily dosing of FVIII concentrate may substantially reduce factor concentrate consumption, with higher patient burden due to intensive infusion schedule. Such cost-effectiveness studies should not only calculate amount of factor concentrate but also take other miscellaneous costs into account. These miscellaneous costs should include team efforts to perform iterative PK-guided dosing by clinical pharmacologist, (pediatric) hematologist, nurse, laboratory technician as formulated in guidelines for economic evaluation of new healthcare interventions by Hakkaart-van Roijen.[47] In the near future, costs for PK-guided dosing may decline as more detailed population PK models will minimize PK profiling to one blood sample, decreasing costs associated with laboratory measurements, and logistics performed by hemophilia team. Furthermore, implementation of web-based PK tools using validated population PK models, may also further decrease costs of treatment as efforts by clinical pharmacologist will concomitantly decline.

Unknown association between factor levels and bleeding

Dutch guidelines prescribe target factor ranges perioperatively, but it is well known that the relationship between these target ranges and bleeding is unclear and not evidence based. It is unethical to lower perioperative target factor ranges to test cut off levels defining bleeding risk. Therefore, prospective studies on factor levels and perioperative bleeding are difficult to perform. Nevertheless, future studies should merge datasets from perioperative and prophylactic hemophilia A and hemophilia B patients and bleeding events to unravel these associations. In addition, these combined datasets are an opportunity to construct PK-pharmacodynamic (PD) models, correlating predicted factor levels with bleeding events and other relevant factors influencing bleeding risk.

Phenotypic heterogeneity in bleeding risk is intriguing in hemophilia,[48,49] as two patients with similar baseline FVIII levels may differ considerably in bleeding phenotype. In this thesis, we have reviewed several factors (chapter 4) that influence bleeding phenotype, such as developmental phase, age, bodyweight, blood type and von Willebrand factor (VWF), life style, and sporting activities among others.[50] Besides these known and measurable factors there remain unknown modifying factors that are yet to be investigated. Importantly, the endothelial compartment is known to play a role in this interindividual variation as it produces, stores and secretes VWF. VWF is carrier protein for FVIII, protecting it from proteolytic cleavage, premature activation and clearance from the circula-

tion. Therefore, VWF behavior should be analyzed to better understand FVIII clearance long term. Factors that delay or inhibit production and secretion cause lower plasma VWF levels and thereby less VWF to protect FVIII.[51]

Another component influencing bleeding phenotype may be the presence and mobilization of patient's own, endogenous FVIII. In non-severe hemophilia A patients, this may play an important modulating role. Desmopressin administration may mimic activation of the hemostatic system during bleeding, trauma or stress. Therefore desmopressin administration, may serve as a model to study interindividual variation of bleeding phenotype.[52] Such a model may be used to analyze patients with atypical bleeding phenotypes with regard to their proteomic plasma profiles present before and after desmopressin administration. Research into proteomic profiling, construction of induced pluripotent stem cell (iPSC) lines and endothelial cell models, forms an important part of the novel Dutch multicenter, interdisciplinary research consortium SYMPHONY. Biomarkers uncovering underlying mechanisms leading to interindividual variation in bleeding phenotype, are expected to be found in these studies and can be integrated into future population PK-PD models.

In the prophylactic setting, mainstay of prophylaxis has been that factor concentrate trough levels should be kept equal to or above 0.01 IU/mL by regular factor concentrate dosing. However, it remains to be elucidated if this minimal trough level is sufficient to prevent all bleeding and accomplishing an annualized bleeding rate (ABR) of zero. Several research groups have attempted to associate PK and PD, with PD defined as bleeding in hemophilia A.[53-55] Nevertheless, a recent prospective randomized controlled trial comparing two different FVIII trough target ranges e.g. 0.01-0.03 versus 0.08-0.12 IU/mL showed that despite trough levels of 0.08 – 0.12 IU/mL, zero bleeds was not achieved. Additionally, it was observed that many patients (15/58) withdrew from the study, the majority of which were randomized into the higher trough level arm, creating attrition bias and causing difficulties to generalize study results to the general hemophilia population.[56] Study withdrawal was most probably due to frequent dosing, obligatory to achieve the high troughs indicated. Importantly, it should also be realized that not only trough levels are meaningful. Moreover, importance of FVIII peak levels should not be underestimated. Valentino et al. have already reported that higher peak FVIII levels may create more effective protection from bleeding.[57]

Furthermore not unimportantly, increased knowledge on PK and PD in general, acquired through studies on factor replacement therapy will also promote application in novel upcoming non-factor replacement therapies, and gene therapy.

Sufficient representation of specific patient groups in population PK models

Bayesian adaptive dosing is only possible when PK models are representative of the individual patient and his or her specific clinical setting or circumstances. Therefore, special attention should be paid to include all patient populations and settings in constructed models. Most population PK models available did not include or report the amount number of overweight or obese patients included in constructed models.[39,40] Due to rising prevalence of obesity in the global population, we developed a prophylactic population PK model applicable for multiple standard half-life FVIII concentrates with a representative number of patients who were overweight or obese. As FVIII concentrate remains mainly intravascular, it was not surprising that the morphometric variable ideal bodyweight (IBW), that is generally seen as a reflection of the intravascular compartment, described inter- and intraindividual variation in FVIIII PK best.[42] Our study substantiated the study described by McEneny-King et al.[58] In this simulation study, 1000 normal weight (BMI<29.6 kg/m^2) and 1000 overweight/obese (BMI 29.6-40.0 kg/m^2) patients were simulated for use of a one specific FVIII concentrate. Although a different cut-off point (BMI: 29.6 kg/m^2) was applied to distinguish between normal weight and overweight or obese patients, our results are in line with this study. Another often underrepresented group is the pediatric hemophilia population. As FVIII clearance decreases with age, children have a more rapid FVIII clearance (expressed per kilogram) than adults, while volume of distribution per kilogram remains stable.[39,40,59] This results in shorter factor concentrate half-lives, and therefore need of higher and more frequent dosing of FVIII concentrate in children. Furthermore, as children grow, an individual PK profile may change and is therefore only valid for a short period of time.[40] Extensive data collection in pediatric hemophilia A patients on timing and amount of dosing is therefore recommended to create more accurate population PK models also applicable in children.

Finally, especially older hemophilia A patients may have abnormal liver function tests due to hepatitis B or C (HCV) infections after contamination of plasma derived factor concentrates or due to the actual medication to treat these viral liver diseases. Previous studies have shown that liver disease affects coagulation factor levels as FVIII is synthesized in sinusoidal cells in the liver.[60] Hemophilia A patients with liver disease may demonstrate lower FVIII clearance, most probably due to increased VWF levels. To our knowledge, only one study in a small patient sample has been performed without appropriate estimation of clearance.[61] Therefore, further studies investigating this relationship are needed but difficult to perform due to strongly decreasing numbers of affected patients.

Continuing importance of factor replacement therapy

In an era with many new treatment options including non- factor replacement therapy and gene therapy, the question arises if the hemophilia community should still rely on factor replacement therapy as mainstay of treatment? We believe that it is a safe and effective option to prevent and treat bleeding with low thrombosis risk. For the novel treatment modalities, safety profiles and risk factors are still to be determined and in many cases treatment in case of bleeding or during surgery is still under investigation. Furthermore, for gene therapy existing antibodies against viral vectors may limit gene therapy options.[62] Recently, a multiyear follow-up showed that FVIII expression levels of approximately 0.20 IU/mL decreased slightly over time.[14,63] Although clinically relevant, these factor levels do not rule out bleeding altogether. Moreover, gene therapy may be a once only option as re-treatment with the same vector is not yet possible. Finally, accurate monitoring by hemostasis laboratories for novel products or gene therapy are still under development.

Moreover, developing countries may finally be able to afford treatment if factor replacement therapy becomes less expensive and therefore more widely available. Currently, the World Federation of Hemophilia estimates that 70-75% of all hemophilia patients worldwide do not receive any form of appropriate treatment.[64] Simulations show that low dose frequent prophylaxis decreases bleeding risk compared to on demand treatment, but saves 75% of costs associated with high dose prophylaxis schedules.[65] The concept of PK-guided dosing, achieving best results with minimal but more frequent dosing is therefore of great relevance for countries with limited health care resources. In conclusion, in our opinion factor replacement therapy will not disappear and innovations in factor replacement therapy for hemophilia are still extremely relevant.

APPLICATION OF PK-GUIDED DOSING IN CLINICAL PRACTICE

User friendly PK guidance web portals and measurement of patient reported outcome measures

The development of a digital interactive shared-decision-making tool will further facilitate implementation of PK-guided dosing into clinical practice. Before developing such a tool, focus groups should reveal most important patient-related factors e.g. frequency of treatment, efficacy, safety, and costs. These factors should be incorporated and discussed with patients to ensure most suitable dosing strategy for each patient. Implementation of PK-guided dosing into clinical practice also needs a user friendly PK tool. Currently, two web-based PK tools are available, myPKFiT and WAPPS. Both tools perform Bayesian forecasting, but the precise population PK models behind these tools

are unknown. In chapter 10 we demonstrate that different population models produce different individual PK parameters and as a consequence different dose recommendations.[66,67] Therefore, new PK tools should 1) be transparent with respect to the incorporated population model ; 2) be able to calculate dosing advices for every available concentrate (standard half-life and extended half-life); 3) be applicable in different settings (prophylactic and perioperative); and 4) be representative for all patient populations. Last but not least, it is essential to include patient-reported outcome measures (PROMs) to evaluate the impact of PK-guided dosing and more importantly of other upcoming novel treatment modalities on patients. PROMs incorporate patients' perspective and positive coping capacities. Furthermore, they provide valuable information and facilitate implementation of value based health care.[68,69] A combination of a shared decision making tool, PROMs and PK tool will facilitate implementation of PK-guided dosing. But will also set the stage for obligatory choices by governmental health care institutions and health care payers in the upcoming era of novel treatment modalities, with regard to which treatment is best for each patient, in which phase of life at what societal cost.

Personal approach and good communication

As mentioned previously by Jameson and Longo in the New England Journal of Medicine, "Precision medicine is personalized, problematic and promising".[70] Personalized treatment does not only consist of accurate targeting of factor level ranges, taking troughs and peak levels into account during activities and other risk factors, but also consists of close collaboration with patients to accommodate treatment according to developmental phase, lifestyle, practical and emotional preferences.

For PK-guided dosing, our research group has shown that both patients and health care professionals are willing to cooperate intensively to personalize treatment accordingly.[71] Good communication between patients and family members, nurses, (pediatric) hematologists and clinical pharmacologists is crucial to achieve optimal results. Current and future more refined PK tools will facilitate implementation and lower time investments by hemophilia treatment team and clinical pharmacologist.

In conclusion, we believe that taking necessary conditions, strength and limitations of PK-guided dosing into account as well as potential applications which may facilitate implementation of PK-guided dosing into daily clinical practice, that PK–guided dosing is an important improvement of factor replacement therapy leading to personalization of hemophilia treatment.

REFERENCES

1. Mould DR, Upton RN. Basic concepts in population modeling, simulation, and model-based drug development. CPT Pharmacometrics Syst Pharmacol 2012;1:e6.
2. Goedhart-De Wolf G, Van Der Meer F, Driessens M, Van Beurden K, Fischer K. Towards evaluation of hemophilia therapies in the Netherlands: A nationwide patient registry and digital infusion log. Haemophilia 2019;25:92.
3. Hubbard AR, Dodt J, Lee T, et al. Recommendations on the potency labelling of factor VIII and factor IX concentrates. J Thromb Haemost 2013;11:988-9.
4. Pavlova A, Delev D, Pezeshkpoor B, Muller J, Oldenburg J. Haemophilia A mutations in patients with non-severe phenotype associated with a discrepancy between one-stage and chromogenic factor VIII activity assays. Thromb Haemost 2014;111:851-61.
5. Pipe SW, Saenko EL, Eickhorst AN, Kemball-Cook G, Kaufman RJ. Hemophilia A mutations associated with 1-stage/2-stage activity discrepancy disrupt protein-protein interactions within the triplicated A domains of thrombin-activated factor VIIIa. Blood 2001;97:685-91.
6. Rudzki Z, Duncan EM, Casey GJ, Neumann M, Favaloro EJ, Lloyd JV. Mutations in a subgroup of patients with mild haemophilia A and a familial discrepancy between the one-stage and two-stage factor VIII:C methods. Br J Haematol 1996;94:400-6.
7. Over J. Methodology of the one-stage assay of Factor VIII (VIII:C). Scand J Haematol Suppl 1984;41:13-24.
8. Rosén S FP, Andersson M, Vinazzer H. A new chromogenic assay for determination of human factor VIII:C activity1986.
9. Barrowcliffe TW. Methodology of the two-stage assay of Factor VIII (VIII:C). Scand J Haematol Suppl 1984;41:25-38.
10. Barrowcliffe TW, Hubbard AR, Kitchen S. Standards and monitoring treatment. Haemophilia 2012;18 Suppl 4:61-5.
11. Hubbard AR, Sands D, Sandberg E, Seitz R, Barrowcliffe TW. A multi-centre collaborative study on the potency estimation of ReFacto. Thromb Haemost 2003;90:1088-93.
12. Pahl S SR, Oldenburg J, Herbiniaux U, Aburubaiha Z. Characterisation of FVIII variants of the B-domain regarding their biological activity. Haemophilia 2010;16:95.
13. Young GA, Perry DJ, International Prophylaxis Study G. Laboratory assay measurement of modified clotting factor concentrates: a review of the literature and recommendations for practice. J Thromb Haemost 2019;17:567-73.
14. Pasi KJ, Rangarajan S, Mitchell N, et al. Multiyear Follow-up of AAV5-hFVIII-SQ Gene Therapy for Hemophilia A. N Engl J Med 2020;382:29-40.
15. Lewis SJ, Stephens E, Florou G, et al. Measurement of global haemostasis in severe haemophilia A following factor VIII infusion. Br J Haematol 2007;138:775-82.
16. Santagostino E, Mancuso ME, Tripodi A, et al. Severe hemophilia with mild bleeding phenotype: molecular characterization and global coagulation profile. J Thromb Haemost 2010;8:737-43.
17. Dargaud Y, Negrier C. Thrombin generation testing in haemophilia comprehensive care centres. Haemophilia 2010;16:223-30.

18. Le Quellec S, Paris M, Nougier C, et al. Pre-analytical effects of pneumatic tube system transport on routine haematology and coagulation tests, global coagulation assays and platelet function assays. Thromb Res 2017;153:7-13.

19. Adcock DM, Favaloro EJ, Lippi G. Critical pre-examination variables in the hemostasis laboratory and their quality indicators. Clin Biochem 2016;49:1315-20.

20. Dargaud Y, Luddington R, Gray E, et al. Effect of standardization and normalization on imprecision of calibrated automated thrombography: an international multicentre study. Br J Haematol 2007;139:303-9.

21. Trossaert M, Lienhart A, Nougier C, et al. Diagnosis and management challenges in patients with mild haemophilia A and discrepant FVIII measurements. Haemophilia 2014;20:550-8.

22. Dargaud Y, Wolberg AS, Gray E, et al. Proposal for standardized preanalytical and analytical conditions for measuring thrombin generation in hemophilia: communication from the SSC of the ISTH. J Thromb Haemost 2017;15:1704-7.

23. Sorensen B, Ingerslev J. Whole blood clot formation phenotypes in hemophilia A and rare coagulation disorders. Patterns of response to recombinant factor VIIa. J Thromb Haemost 2004;2:102-10.

24. Chitlur M, Warrier I, Rajpurkar M, et al. Thromboelastography in children with coagulation factor deficiencies. Br J Haematol 2008;142:250-6.

25. Bowyer AE, Van Veen JJ, Goodeve AC, Kitchen S, Makris M. Specific and global coagulation assays in the diagnosis of discrepant mild hemophilia A. Haematologica 2013;98:1980-7.

26. van Veen JJ, Gatt A, Bowyer AE, Cooper PC, Kitchen S, Makris M. Calibrated automated thrombin generation and modified thromboelastometry in haemophilia A. Thromb Res 2009;123:895-901.

27. Anderson L, Quasim I, Steven M, et al. Interoperator and intraoperator variability of whole blood coagulation assays: a comparison of thromboelastography and rotational thromboelastometry. J Cardiothorac Vasc Anesth 2014;28:1550-7.

28. Kitchen DP, Kitchen S, Jennings I, Woods T, Walker I. Quality assurance and quality control of thrombelastography and rotational Thromboelastometry: the UK NEQAS for blood coagulation experience. Semin Thromb Hemost 2010;36:757-63.

29. Batorova A, Martinowitz U. Intermittent injections vs. continuous infusion of factor VIII in haemophilia patients undergoing major surgery. Br J Haematol 2000;110:715-20.

30. Dingli D, Gastineau DA, Gilchrist GS, Nichols WL, Wilke JL. Continuous factor VIII infusion therapy in patients with haemophilia A undergoing surgical procedures with plasma-derived or recombinant factor VIII concentrates. Haemophilia 2002;8:629-34.

31. Bidlingmaier C, Deml MM, Kurnik K. Continuous infusion of factor concentrates in children with haemophilia A in comparison with bolus injections. Haemophilia 2006;12:212-7.

32. Hazendonk HC, Lock J, Mathot RA, et al. Perioperative treatment of hemophilia A patients: blood group O patients are at risk of bleeding complications. J Thromb Haemost 2016;14:468-78.

33. Hazendonk H, Heijdra JM, de Jager NCB, et al. Analysis of current perioperative management with Haemate((R)) P/Humate P((R)) in von Willebrand disease: Identifying the need for personalized treatment. Haemophilia 2018.

34. Hazendonk H, Preijers T, Liesner R, et al. Perioperative replacement therapy in haemophilia B: An appeal to “B” more precise. Haemophilia 2018;24:611-8.

35. Leebeek FWG, Mauser-Bunschoten EP. Richtlijn diagnostiek en behandeling van hemofilie en aanverwante hemostase stoornissen. Utrecht: Van Zuiden Communications BV; 2009:1-197.

36. Johnson KA, Zhou ZY. Costs of care in hemophilia and possible implications of health care reform. Hematology Am Soc Hematol Educ Program 2011;2011:413-8.

37. Shrestha A, Eldar-Lissai A, Hou N, Lakdawalla DN, Batt K. Real-world resource use and costs of haemophilia A-related bleeding. Haemophilia 2017.

38. Zhou ZY, Koerper MA, Johnson KA, et al. Burden of illness: direct and indirect costs among persons with hemophilia A in the United States. J Med Econ 2015;18:457-65.

39. Bjorkman S, Folkesson A, Jonsson S. Pharmacokinetics and dose requirements of factor VIII over the age range 3-74 years: a population analysis based on 50 patients with long-term prophylactic treatment for haemophilia A. Eur J Clin Pharmacol 2009;65:989-98.

40. Bjorkman S, Oh M, Spotts G, et al. Population pharmacokinetics of recombinant factor VIII: the relationships of pharmacokinetics to age and body weight. Blood 2012;119:612-8.

41. Hazendonk HC, van Moort I, Fijnvandraat K, et al. The "OPTI-CLOT" trial. A randomised controlled trial on periOperative PharmacokineTIc-guided dosing of CLOTting factor concentrate in haemophilia A. Thromb Haemost 2015;114:639-44.

42. van Moort I, Preijers T, Hazendonk HCAM, et al. Ideal Body Weight Is Proven Most Reliable to Dose Factor VIII Concentrate in Overweight and Obese Hemophilia A Patients. Research and Practice in Thrombosis and Hemostasis 2019;3:1-891.

43. Preijers T, van Moort I, Fijnvandraat K, et al. Cross-evaluation of Pharmacokinetic-Guided Dosing Tools for Factor VIII. Thromb Haemost 2018;118:514-25.

44. Iannazzo S, Cortesi PA, Crea R, Steinitz K, Mantovani LG, Gringeri A. Cost-effectiveness analysis of pharmacokinetic-driven prophylaxis vs. standard prophylaxis in patients with severe haemophilia A. Blood Coagul Fibrinolysis 2016.

45. Collins PW, Fischer K, Morfini M, Blanchette VS, Bjorkman S, Group IPSGPEW. Implications of coagulation factor VIII and IX pharmacokinetics in the prophylactic treatment of haemophilia. Haemophilia 2011;17:2-10.

46. Carlsson MB, E; Björkman, S; Lethagen, S; Ljung,R. Improved cost-effectiveness by pharmacokinetic dosing of factor VIII in prophylactic treatment of haemophilia A. Haemophilia 1997;3:96-101.

47. Hakkaart-van Roijen L, van der Linden N, Bouwmans C, Kanters T, Swan Tan S. Handleiding voor kostenonderzoek: Methodologie van kostenonderzoek en referentieprijzen voor economische evaluaties in de gezondheidszorg. Diemen: Zorginstituut Nederland; 2010:1-120.

48. Pavlova A, Oldenburg J. Defining severity of hemophilia: more than factor levels. Semin Thromb Hemost 2013;39:702-10.

49. van den Berg HM, De Groot PH, Fischer K. Phenotypic heterogeneity in severe hemophilia. J Thromb Haemost 2007;5 Suppl 1:151-6.

50. Hazendonk H, van Moort I, Mathot RAA, et al. Setting the stage for individualized therapy in hemophilia: What role can pharmacokinetics play? Blood Rev 2018;32:265-71.

51. Schillemans M, Karampini E, Kat M, Bierings R. Exocytosis of Weibel-Palade bodies: how to unpack a vascular emergency kit. J Thromb Haemost 2019;17:6-18.

52. Candy V, Whitworth H, Grabell J, et al. A decreased and less sustained desmopressin response in hemophilia A carriers contributes to bleeding. Blood Adv 2018;2:2629-36.

53. Abrantes JA, Solms A, Garmann D, Nielsen EI, Jonsson S, Karlsson MO. Bayesian Forecasting Utilizing Bleeding Information to Support Dose Individualization of Factor VIII. CPT Pharmacometrics Syst Pharmacol 2019.

54. Abrantes JA, Solms A, Garmann D, Nielsen EI, Jonsson S, Karlsson MO. Relationship between factor VIII activity, bleeds and individual characteristics in severe hemophilia A patients. Haematologica 2019.

55. Klamroth R, Windyga J, Radulescu V, et al. Results from a phase 3, randomized, multicenter study of rurioctocog alfa pegol PK-guided prophylaxis targeting 2 FVIII trough levels in patients with severe hemophilia A (propel study). Haemophilia 2019;25:162.

56. Klamroth R, Windyga J, Vlad R, et al. Results of a phase 3, randomized, multicenter study of RURIOCTOCOG ALFA PEGOL PK-guided prophylaxis targeting 2 FVIII trough levels in patients with severe Hemophilia A (propel study). European Association of Haematology and Allied Disorders (EAHAD); 2020; The Hague.

57. Valentino LA, Pipe SW, Collins PW, et al. Association of peak factor VIII levels and area under the curve with bleeding in patients with haemophilia A on every third day pharmacokinetic-guided prophylaxis. Haemophilia 2016;22:514-20.

58. McEneny-King A, Chelle P, Foster G, Keepanasseril A, Iorio A, Edginton AN. Development and evaluation of a generic population pharmacokinetic model for standard half-life factor VIII for use in dose individualization. J Pharmacokinet Pharmacodyn 2019.

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

60. Lisman T, Leebeek FW, de Groot PG. Haemostatic abnormalities in patients with liver disease. J Hepatol 2002;37:280-7.

61. Suzuki N, Hirakawa A, Kishimoto M, et al. Retrospective analysis of in vivo recovery and clearance during continuous infusion of recombinant factor VIII products: a single-institution study. Haemophilia 2017;23:215-21.

62. Herzog RW. Complexity of immune responses to AAV transgene products - Example of factor IX. Cell Immunol 2019;342:103658.

63. Rangarajan S, Walsh L, Lester W, et al. AAV5-Factor VIII Gene Transfer in Severe Hemophilia A. N Engl J Med 2017;377:2519-30.

64. The Lancet H. Bittersweet progress for haemophilia A. Lancet Haematol 2018;5:e127.

65. Brekkan A, Degerman J, Jonsson S. Model-based evaluation of low-dose factor VIII prophylaxis in haemophilia A. Haemophilia 2019.

66. Iorio A, Keepanasseril A, Foster G, et al. Development of a Web-Accessible Population Pharmacokinetic Service-Hemophilia (WAPPS-Hemo): Study Protocol. JMIR Res Protoc 2016;5:e239.

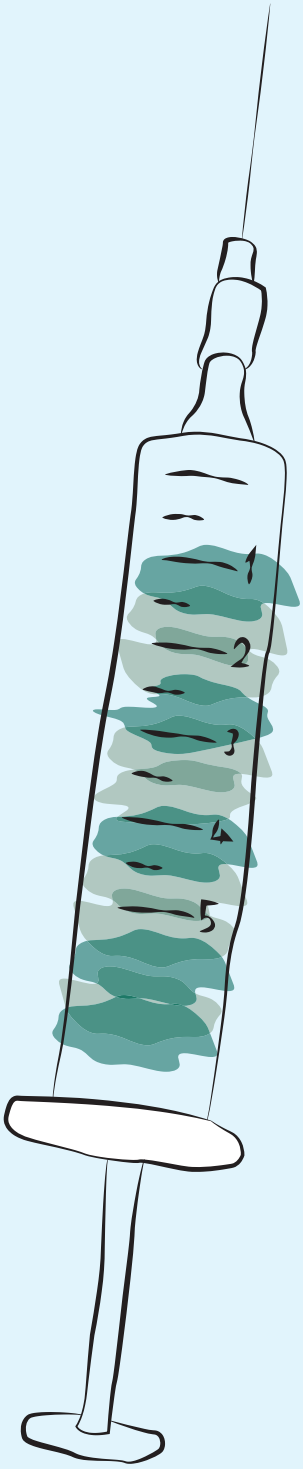
67. myPKFiT user guide. Westlake Village, CA: Baxter Healthcare Corporation; 2014.

68. van Steekelenburg E, Kersten I, Huber M. 'Positieve gezondheid' in Nederland - Wie, wat, waarom en hoe? Een inventarisatie. Amersfoort: Institute for Positive Health (IPH); 2016.

69. Porter ME. A strategy for health care reform--toward a value-based system. N Engl J Med 2009;361:109-12.

70. Jameson JL, Longo DL. Precision medicine--personalized, problematic, and promising. N Engl J Med 2015;372:2229-34.

71. Lock J, de Bekker-Grob EW, Urhan G, et al. Facilitating the implementation of pharmacokinetic-guided dosing of prophylaxis in haemophilia care by discrete choice experiment. Haemophilia 2016;22:e1-e10.



12

Summary / Samenvatting

SUMMARY

Hemophilia A is characterized by a (partial) deficiency of coagulation factor VIII (FVIII), caused by a mutation in the *F8* gene. As FVIII is crucial to maintain adequate secondary hemostasis. A deficiency in FVIII leads to (spontaneous) bleeding in joints and muscles or (prolonged) bleeding after trauma and/or surgery. Mainstay of treatment in hemophilia, is the replacement of the deficient coagulation factor with intravenously administered factor concentrate, also called factor replacement therapy. However, dosing of FVIII concentrate is challenging. Standard practice is to dose based on bodyweight and crude estimations of *in vivo* recovery and FVIII clearance. Several studies have shown that large interindividual differences exist in FVIII concentrate pharmacokinetics (PK), resulting in underdosing with a higher risk of bleeding, or overdosing with concomitant unnecessary high costs. Dosing based on a patient's individual PK takes interindividual differences of FVIII PK into account and therefore optimizes treatment. In this thesis, we focus on the conditions needed, strengths, limitations and potential applications of PK-guided dosing of FVIII concentrate in hemophilia A patients. In the first part, we focus on *current practice with regard to diagnostics and treatment monitoring*. In the second part, *implementation of PK-guided dosing* is addressed.

Firstly**,** the background for the studies presented in this thesis by description of clinical symptoms and complications of hemophilia A are provided in **chapter 1**. We also highlight current knowledge gaps in hemophilia care which we will address.

Part I - Current diagnostics and treatment monitoring

As FVIII measurements are essential to optimize diagnosis and to safeguard quality of treatment monitoring, accuracy of measurements is of great importance. Implementation of PK-guided dosing is only feasible if data on factor levels on which population PK models are constructed, are accurate and reproducible. Therefore, knowledge and expertise on coagulation factor laboratory assays are indispensable when providing PK-guidance of factor concentrate dosing.

Measurements of FVIII coagulation activity (FVIII:C) vary considerably and may result in misclassification of hemophilia A with delay in initiation of prophylactic treatment if not recognized as is reported in **chapter 2**. We reported on two young brothers who were diagnosed as moderate hemophilia A patients and therefore not prophylactically treated with factor VIII concentrate despite frequent bleeding events. At ages of 6 and 5 years, both brothers were referred to our hemophilia treatment center. Laboratory analyses in our center revealed FVIII:C of <0.01 IU/mL, indicating severe hemophilia, which was confirmed by DNA analysis showing an inversion of intron 22. These findings

emphasized the relevance of (i) multiple FVIII:C measurements by certified laboratories; (ii) centralized treatment for rare diseases in centers with expertise; (iii) importantly, critical adjustment of treatment when test results do not correspond with clinical symptoms, (iii) relevance of additional DNA mutation analysis in patients with hemophilia.

A study on the analytical variation in FVIII:C measurements, both in the one-stage assay (OSA) and chromogenic substrate assay (CSA) is reported in **chapter 3**. Both assays can be used to measure FVIII activity, however factors explaining analytical variation in FVIII activity levels are still to be completely elucidated. We collaborated with the External Quality Assessment Program for Thrombosis and Hemostasis (ECAT) Foundation and studied factors potentially determining analytical variation in lyophilized plasma samples with varying FVIII:C levels. To investigate causes of OSA variation, we exchanged deficient plasma between three manufacturing company set-ups. On average, 206 (range: 164-230) laboratories worldwide used OSA to measure FVIII activity and 30 (range: 12-51) used CSA. The coefficient of variation of OSA and CSA increased with lower FVIII levels (FVIII <0.05 IU/mL). This resulted in misclassification of a severe hemophilia A sample into a moderate or mild hemophilia A sample in 4/30 (13.3%) of CSA measurements, while this was 37/139 (26.6%) for OSA. The variation in FVIII activity levels was partly explained by specificity of manufacturer. Exchange of deficient plasma into a set-up with equipment of another manufacturing company however did not change FVIII level results. We concluded that standardization of FVIII measurements and further research into the etiology of analytical variation is required.

Part II - Implementation of pharmacokinetic-guided dosing of factor VIII concentrate in hemophilia A

To implement pharmacokinetic (PK)-guided dosing of replacement therapy with factor concentrates in hemophilia, it is essential to investigate the strengths and limitations of this innovative intervention in order to analyze its potential.

We reviewed the current insights on PK-guided dosing in hemophilia care and discussed its advantages and limitations in a broader sense **in chapter 4**. Dosing of factor concentrates is now primarily based on bodyweight and on crude estimates of *in vivo* recovery (IVR) and clearance, often resulting in underdosing with a higher risk of bleeding or overdosing resulting in unnecessary high costs. Benefits of PK-guided dosing include individualization of treatment with more optimal FVIII and FIX targeting, more flexible blood sampling, potential lowering of overall treatment costs and increased insight into association of coagulation factor levels and bleeding. The latter ultimately leading to PK-pharmacodynamic (PD) modelling. Limitations include a slight patient burden, consisting of outpatient clinic visits and blood sampling in a population accustomed to

frequent intravenous administration of medication. Conditional is availability of a close collaboration with population PK modeling, Bayesian analysis experienced clinical pharmacologists and hospital pharmacists.

In **chapter 5**, we presented the design of a unique randomized controlled trial (RCT) which compares PK-guided perioperative dosing of FVIII concentrate with standard dosing based on bodyweight in severe and moderate hemophilia A patients. The OPTI-CLOT trial is an open-label, prospective, multicenter RCT, aiming to detect a 25% difference in perioperative FVIII concentrate consumption. Minimally sixty hemophilia A patients ≥ 12 years of age, with FVIII plasma levels ≤ 0.05 $IUmL^{-1}$ had to be included. Stratification was performed for mode of FVIII concentrate administration (bolus administration versus continuous infusion) and severity of elective surgery (low versus medium risk surgery).

The results of the OPTI-CLOT RCT trial are presented in **chapter 6**. The main question in this RCT was to test the impact of PK-guided dosing on factor concentrate consumption perioperatively. We found that PK-guided dosing in the perioperative period resulted in similar consumption of FVIII concentrate (mean: 365±202 IU/kg) compared to standard treatment arm (mean: 379±202 IU/kg) (P=0.90). However, achievement of FVIII target levels was more effective in the PK-guided arm than in the standard dosing arm. PK-guided treatment resulted in 69% of FVIII measurements within prescribed target range while this was only 37% for standard treatment (P<0.001). Hospitalization period and the amount of perioperative bleeding events were similar between treatment arms. Therefore, PK-guided dosing leads to more optimal targeting of pre-specified FVIII levels and more precise perioperative dosing.

In **chapter 7,** we investigated the dynamics of von Willebrand factor (VWF) in perioperative severe and moderate hemophilia A patients. VWF is hypothetically crucial when determining FVIII concentrate dosage as VWF protects FVIII from premature clearance from the circulation. To date, it is unknown how VWF behaves and what its impact is on FVIII clearance in the perioperative setting. Therefore, VWF antigen (VWF:Ag), VWF activity (measured as VWF:GPIbM) and VWF propeptide (VWFpp) were determined in perioperative blood samples collected in the OPTI-CLOT RCT trial. Linear mixed effects modeling was applied to analyze VWF dynamics. It showed that VWF:Ag and VWF:GPIbM increased significantly postoperatively. Blood type non-O and medium risk surgery were associated with higher VWF:Ag and VWF:GPIbM levels compared to blood type O and low risk surgery. Unexpectedly, VWF:Ag had only a minimal effect on perioperative FVIII clearance. VWF levels were not associated with perioperative bleeding, although sample size was small. Future research is needed to investigate PK/PD of VWF and FVIII and

bleeding. Furthermore, a future population PK model should be created incorporating VWF levels to investigate VWF effects on FVIII PK more extensively.

As the prevalence of overweight and obese individuals in the general population is increasing, hemophilia A patients are also increasingly affected. Under-and especially overdosing of factor replacement therapy in hemophilia A patients may be prevented by applying other morphometric variables than bodyweight to dose FVIII concentrates. **Chapter 8** reported which morphometric variable best describes interindividual variability (IIV) of FVIII concentrate PK parameters in overweight and obese hemophilia A patients. We concluded that ideal body weight best explained observed variability between patients, as IIV for clearance and volume of distribution (V1) was reduced by incorporating ideal body weight from 45.1% to 37.6% and 26.8% to 14.1%, respectively. Strikingly, clearance, V1, and half-life were shown to remain similar when normal weight (BMI <25 kgm^{-2}), overweight (BMI 25-30 kgm^{-2}) and obese patients (BMI >30 kgm^{-2}) were compared. Simulated FVIII trough and peak levels using real world data were similar when FVIII concentrate dosing was based on ideal body weight, also in cases of dosing for life-threatening bleed. We therefore concluded, that ideal body weight most accurately predicts necessary FVIII concentrate doses in overweight and obese hemophilia A patients. Moreover, it seems that ideal body weight can be safely applied in all situations including life threatening bleeding. Although, we do remain careful and therefore still advise regular monitoring when dosing is critical.

We reported an extremely obese severe hemophilia A patient who underwent a laparoscopic sleeve gastrectomy to lose weight in **chapter 9**. We investigated the influence of extreme weight loss on the patient's individual FVIII PK parameters. Individual PK parameters were estimated with a prophylactic population PK model based on ideal body weight as most important morphometric variable for allometric scaling. One year postoperatively, our patient lost 27.1 kg and weighed 106.4 kg with a BMI of 34.0 kgm^{-2}. IVR decreased significantly with decreasing bodyweight. Strikingly, FVIII clearance and volume of distribution remained similar over time, resulting in a similar half-life over time. Time to 0.01 $IUmL^{-1}$ was calculated after a hypothetical prophylactic FVIII concentrate dose of 3500 IU. Simulations using the patient's individual PK parameters also showed that time to 0.01 IU/mL was not subject to change and remained a calculated period of 75.0 hours both before and after gastric bypass surgery. In conclusion, extreme weight loss does not influence individual FVIII parameters significantly and therefore does not lead to dose changes of prophylaxis. However, as hemostatic balances change, as is the case with extreme weight loss or obesity, monitoring of pharmacodynamics e.g. bleeding may become more relevant than pharmacokinetics.

In **chapter 10**, three PK tools e.g. myPKFiT®, Web-Accessible Population Pharmacokinetic Service-Hemophilia or WAPPS, and a prophylactic population PK model according to Bjorkman applied by a dedicated clinical pharmacologist (OPTI-CLOT group) using NONMEM, were compared. Patients underwent individual PK profiling. Subsequently, FVIII dose, FVIII levels and patient characteristics were entered into the three separate PK tools. Obtained PK parameters and dosing advises were then compared. MyPKFiT® provided PK parameters for 24 of 30 patients receiving Advate®, whereas WAPPS and NONMEM provided estimates for all patients. Half-life was different among the three methods: medians were 12.6 hours (n = 24), 11.2 hours (n = 30) and 13.0 hours (n = 30) for myPKFiT®, WAPPS and NONMEM, respectively. To maintain a FVIII trough level of 0.01 $IUmL^{-1}$ after 48 hours, doses for myPKFiT® and NONMEM were 15.1 and 11.0 $IUkg^{-1}$ and for WAPPS and NONMEM were 9.0 and 8.0 $IUkg^{-1}$. In nine patients receiving Kogenate®, WAPPS and NONMEM produced different PK-parameter estimates; half-life was 15.0 and 12.3 hours and time to 0.05 $IUmL^{-1}$ was 69.2 and 60.8 hours. However, recommended doses to obtain these levels were not different. In conclusion, the three evaluated PK tools produced different PK parameters and doses for recombinant FVIII concentrate. Hematologists should take this into account when obtaining dosing advices from the currently available PK tools.

The last chapter of this thesis**, chapter 11**, compromises an overview of the most important findings and discusses their clinical implications. Furthermore, we provide recommendations for future research.

SAMENVATTING

Hemofilie A wordt gekenmerkt door een tekort aan stollingsfactor VIII (FVIII) dat veroorzaakt wordt door een mutatie in het *F8* gen. Aangezien FVIII van belang is voor een adequate secundaire hemostase, leidt een tekort aan FVIII tot (spontane) bloedingen in spieren en gewrichten en forse bloedingen na minimaal trauma en/of een operatie als geen adequate behandeling wordt toegepast. De belangrijkste behandeling bij hemofilie is suppletie van de ontbrekende stollingsfactor met factor concentraat dat intraveneus wordt toegediend. Het doseren van factor concentraat is echter lastig. Het is gebruikelijk om te doseren op basis van lichaamsgewicht en door middel van ruwe schattingen van de opbrengst en klaring van het factor concentraat. Meerdere studies hebben laten zien dat er grote interindividuele verschillen bestaan in de farmacokinetiek (PK) oftewel wat het lichaam doet met het concentraat van FVIII concentraat waardoor beoogde factor spiegels in het bloed per individu niet goed kunnen worden voorspeld. Dit leidt in de praktijk tot onder doseren met een verhoogd risico op bloedingen of over doseren met bijkomende excessieve kosten aangezien factor concentraat behoort tot de categorie dure geneesmiddelen. Om deze reden is het doseren van factor concentraat op basis van een patiënts eigen PK een interessant concept aangezien op deze wijze de interindividuele verschillen van het FVIII concentraat PK in acht worden genomen en de behandeling wordt geoptimaliseerd.

In dit proefschrift beschrijven wij de voorwaarden die nodig zijn, als ook de voor-en nadelen en potentiele toepassingen van PK-gestuurd doseren van FVIII concentraat in hemofilie A patiënten. In het eerste deel focussen we op de huidige klinische praktijk met betrekking tot diagnostiek en het monitoren van een behandeling met FVIII concentraat. In het tweede deel wordt nader ingegaan op de implementatie van PK-gestuurd doseren in de praktijk.

In **hoofdstuk 1** wordt de achtergrond van de ziekte met de klinische symptomen en complicaties beschreven Daarnaast komen mogelijke verbeterpunten in de huidige van hemofilie behandeling aan bod.

Deel I – Huidige diagnostiek en monitoring van behandeling

Aangezien FVIII metingen essentieel zijn om de diagnose hemofilie A te stellen en om de kwaliteit van de behandeling te monitoren, is het van belang dat deze metingen nauwkeurig en betrouwbaar zijn. Implementatie van PK-gestuurd doseren is dan ook alleen mogelijk als de FVIII metingen waarop PK modellen zijn gebouwd, accuraat en reproduceerbaar zijn. Kennis en expertise met betrekking tot stollingsfactor testen zijn daarom onmisbaar wanneer PK-gestuurd doseren van stollingsfactor concentraat wordt toegepast.

FVIII activiteit metingen (FVIII:C) variëren en kunnen resulteren in misclassificatie van de ernst van hemofilie A met een mogelijke vertraging in het starten van een profylactische behandeling zoals beschreven in **hoofdstuk 2**. We beschrijven hier twee jonge broers die in eerste gediagnosticeerd zijn als matige-ernstige hemofilie A patiënten op basis van de uitgevoerde stollingstesten. Hierdoor werden ze niet tijdig profylactisch behandeld met stollingsfactor concentraat ondanks frequente bloedingen. Op de leeftijd van vijf en zes jaar oud werden de broers verwezen naar ons hemofiliebehandelcentrum. Laboratorium testen lieten FVIII:C waardes zien van <0.01 IU/mL, dat past bij een diagnose van ernstige hemofilie A. Dit werd bevestigd door DNA analyse waarbij een inversie van intron 22 werd aangetoond. Deze bevindingen benadrukken de noodzaak van (i) meerdere FVIII:C metingen rondom diagnose door gecertificeerde laboratoria; (ii) belang van concentratie van zorg voor zeldzame ziektes in centra met expertise; (iii) een kritische instelling en aanpassing van de behandeling als de laboratorium resultaten niet corresponderen met klinische symptomen; (iv) en als laatste de relevantie van additionele DNA mutatie analyses in patiënten met hemofilie.

Een studie over de variatie in FVIII:C metingen, zowel in de one-stage assay (OSA) als in de chromogene substraat assay (CSA), is opgenomen in **hoofdstuk 3**. Beide testen worden gebruikt om FVIII activiteit te meten, maar niet alle factoren die de analytische variatie verklaren zijn bekend. Samen met de External Quality Assessment Program voor Trombose en Hemostase (ECAT) Foundation hebben we de factoren onderzocht die de analytische variatie zouden kunnen beïnvloeden en verklaren in bloed plasma monsters met verschillende FVIII:C levels. Om de variatie in de OSA te onderzoeken, werd er FVIII deficiënt plasma uitgewisseld tussen drie verschillende instrumentarium opstellingen van drie verschillende leveranciers. Gemiddeld werd de OSA gebruikt door 206 (range: 164-230) en de CSA door 30 (range: 12-51) laboratoria. De variatie coëfficiënt nam toe wanneer de FVIII levels resultaten lager werden (FVIII<0.05 IU/mL). Dit resulteerde in misclassificatie van een ernstige hemofilie A patiënten monster in een matig of milde hemofilie A in 4 van de 30 (13.3%) CSA metingen, terwijl dit in 37 van de 139 (26.6%) plaats vond wanneer de OSA gebruikt werd. De variatie in FVIII activiteit metingen werd voor een gedeelte verklaard door specifieke leverancier. Het testen van het FVIII deficient plasma met de opstelling van een andere fabrikant veranderde de FVIII activiteits metingen echter niet. We concludeerden daarom dat verdere standaardisatie van FVIII metingen en minimaliseren van de analytische variatie van belang is.

Deel II – Implementatie van farmacokinetisch-gestuurd doseren van factor VIII concentraat in hemofilie A.

Om farmacokinetisch(PK)-gestuurd doseren van factor concentraat te implementeren in de huidige behandeling van hemofilie is het essentieel om de voor-en nadelen te onderzoeken van deze innovatieve techniek.

We bespreken de laatste ontwikkelingen ten aanzien van het PK-gestuurd doseren van factor concentraat binnen de hemofilie zorg en bediscussiëren de voordelen en beperkingen van deze behandeling in **hoofdstuk 4**. Voordelen van PK-gestuurd doseren zijn het individualiseren van de behandeling met meer als gevolg het beter bereiken van beoogde FVIII en FIX spiegels in het bloed, grotere flexibiliteit ten aanzien van bloedafnames, een mogelijke verlaging van kosten gerelateerd aan een behandeling met duur factor concentraat, en beter inzicht in de associatie tussen stollingsfactor spiegels in het bloed en (het risico op) bloedingen. Wanneer meer data beschikbaar is die stollingsfactor spiegels relateert aan het optreden van bloedingen dan is het mogelijk niet alleen populatie PK modellen te construeren, maar ook populatie PK-farmacodynamiek (PD) modellen. Beperkingen van een PK-gestuurde behandeling met factor concentraat zijn enige belasting voor de patiënt wegens alsnog frequentere bloedafnames en polikliniekvisites. Een belangrijke voorwaarde voor het toepassen van het PK-gestuurd doseren is een goede samenwerking met de afdeling Klinische Farmacologie of de ziekenhuisapotheek en ervaring met de complexe toepassingen van populatie PK modellen.

In **hoofdstuk 5** presenteren we de studie opzet van een unieke gerandomiseerd gecontroleerde trial (RCT) die perioperatief PK-gestuurd doseren van factor concentraat vergelijkt met het standaard doseren gebaseerd op gewicht in ernstige en matig-ernstige hemofilie A patiënten. De OPTI-CLOT trial is een open-label, prospectieve, multicenter RCT, met als doel om een 25% verschil in het perioperatief verbruik van FVIII concentraat aan te tonen. In de studie is gestratificeerd voor toedieningswijze (bolus infusies versus continu infuus) en complexiteit van de operatie (laag risico versus medium risico).

De resultaten van de OPTI-CLOT RCT trial worden gepresenteerd in **hoofdstuk 6**. Het primaire eindpunt van deze RCT was of het verbruik van FVIII concentraat met behulp van PK-gestuurd doseren verlaagd kon worden in een perioperatieve setting. Belangrijkste uitkomsten waren dat het verbruik van FVIII concentraat tussen de twee behandelarmen niet verschilde (PK gestuurde behandelarm FVIII concentraat gemiddelde: 365±202 IU/kg en standaard doseren (379±202 IU/kg) (P=0.90)). Echter, PK-gestuurd doseren leidde tot het feit dat 69% van de FVIII spiegels binnen de streefwaarden werden gemeten en dat bij standaard doseren dit slechts in 37% het geval was (P<0.001). De opnameduur en het voorkomen van bloedingen waren vergelijkbaar in beide behandelarmen. Conclude-

rend, PK-gestuurd doseren leidt een meer optimale behandeling doordat de streefwaarden in klinische richtlijnen en van tevoren bepaald door de behandelaar beter worden behaald doordat er nauwkeuriger wordt gedoseerd.

In **hoofdstuk 7** is binnen de OPTI-CLOT RCT trial gekeken naar hoe von Willebrand factor (VWF) zich gedraagt in perioperatieve ernstige en matig-ernstige hemofilie A patiënten. VWF beschermt FVIII voor vroegtijdige klaring uit de lichaamscirculatie. Daarom was onze hypothese dat de hoeveelheid VWF van belang is voor de PK van het FVIII concentraat. Er is tot op heden weinig bekend over hoe VWF zich gedraagt en wat de impact is van de hoeveelheid VWF op FVIII spiegels in de perioperatieve periode. Daarom is VWF antigeen (VWF:Ag), VWF activiteit (gemeten als VWF:GPIbM) en VWF propeptide (VWFpp) bepaald in perioperatieve bloed monsters die verzameld zijn binnen de OPTI-CLOT RCT trial. Linear mixed effects modelering werd toegepast om de VWF dynamiek te analyseren. Dit toonde aan dat VWF:Ag en VWF:GPIbM toenamen direct na de operatie. Patiënten waarbij sprake was van bloedgroep non-O en een operatie met een middelmatig risico vertoonden hogere VWF:Ag and VWF:GPIbM waarden dan patiënten met bloedgroep O of een operatie met een laag risico. Opvallende bevinding was dat de VWF waardes een kleiner effect hadden op de perioperatieve klaring van het FVIII concentraat dan verwacht. De hoogte van de VWF waardes was ook niet geassocieerd met bloedingen die plaats vonden rondom de operatie. Van belang is dat de hoeveelheid patiënten binnen deze studie uiteraard klein was. Meer onderzoek is daarom nodig om de belangrijke relatie tussen de PK en farmacodynamiek van VWF en FVIII en bloedingen te onderzoeken. Daarnaast zou er gestreefd moeten worden naar een populatie PK model waarbij de interactie van FVIII en VWF wordt geïncorporeerd zodat de effecten van VWF op FVIII beter kunnen worden onderzocht.

Aangezien de prevalentie van overgewicht en obesitas in de algemene bevolking toeneemt, zijn er ook steeds meer hemofilie A patiënten die zich hiermee presenteren. Onder-en vooral overdoseren van FVIII concentraat in hemofilie A patiënten kan waarschijnlijk worden voorkomen door andere morfometrische variabelen in plaats van lichaamsgewicht te gebruiken om het FVIII concentraat te doseren. In **hoofdstuk 8** beschrijven wij welke morfometrische variabele het beste de interindividuele variabiliteit (IIV) van FVIII concentraat beschrijft in hemofilie A patiënten met overgewicht en obesitas. We observeerden dat “ideaal lichaamsgewicht” (IBW) het beste deze variabiliteit verklaart aangezien de IIV voor klaring en distributievolume werd gereduceerd van 45.1% naar 37.6% en 26.8% naar 14.1%, respectievelijk. Klaring, V1 en de halfwaardetijd waren gelijk wanneer normaal gewicht (BMI <25 kg/m^2), overgewicht (BMI 25-30 kg/m^2) en obese patiënten (BMI >30 kg/m^2) werden vergeleken. Daarnaast waren de gesimuleerde FVIII dal- en topspiegels gelijk wanneer data van patiënten werd gebruik om

FVIII te doseren op basis van IBW, vooral belangrijk in het geval van een levensbedreigende bloeding. We concludeerden dat IBW moet worden gebruikt om meer accuraat FVIII doseringen te berekenen in hemofilie A patiënten met overgewicht en obesitas. Wel adviseren we ook bij het gebruik van IBMW nog steeds FVIII waardes regelmatig te monitoren wanneer doseringen en bereikte FVIII waardes van kritisch belang zijn.

We beschrijven een ernstige hemofilie A patiënt met extreme obesitas, die een laparoscopische gastrectomie heeft ondergaan om gewicht te verliezen in **hoofdstuk 9**. We onderzochten de invloed van dit extreme gewichtsverlies op de individuele FVIII PK parameters van deze patient. De individuele PK parameters werden geschat met een profylactisch populatie PK model waarbij IBW werd gebruikt voor de allometrische schaling. Een jaar na operatie, was de patiënt 27.1 kilogram afgevallen en woog nog 106.4 kg met een Body Mass Index (BMI) van 34.0 kg/m^2. De *in vivo* recovery daalde significant met afname van het lichaamsgewicht. FVIII klaring en het distributievolume bleef echter gelijk over de tijd, waardoor er uiteindelijk sprake was van een gelijke FVIII halfwaardetijd. De tijd tot 0.01 IU/mL werd berekend na een hypothetische profylactische FVIII dosering van 3500 IU. Simulaties waarbij de individuele PK parameters van de patiënt werden gebruikt, lieten zien dat de tijd tot een FVIII van 0.01 IU/mL niet veranderde na zijn gewichtsverlies en dat deze rond de 75 uur was zowel voor als na de operatieve maagverkleining. We concludeerden dat extreem gewichtsverlies de individuele FVIII PK parameters niet significant beïnvloedt en dus dat dit niet leidt tot veranderingen in de dosering van FVIII profylaxe. Aangezien, er nog weinig kennis is met betrekking tot verschuivingen binnen hemostatische balans na extreem gewichtsverlies, is monitoring van de farmacodynamiek, oftewel registratie van bloedingen, waarschijnlijk meer relevant dan de FVIII PK in deze omstandigheden.

In **hoofdstuk 10** zijn drie PK tools (myPKFiT®, Web-Accessible Population Pharmacokinetic Service-Hemophilia oftewel WAPPS en een profylactisch populatie PK model beschreven door Björkman en toegepast door een klinisch farmacoloog met behulp van NONMEM) met elkaar vergeleken. Na een individueel PK profiel werden de FVIII dosering, FVIII waardes en patiënt karakteristieken ingevoerd in deze drie PK tools, waarna de verkregen PK parameters en doseeradviezen werden vergeleken. MyPKFiT® gaf de PK parameters voor maar 24 van de 30 patienten die FVIII-Advate® kregen, waarbij WAPPS en NONMEM de PK parameters gaven van alle patiënten. De FVIII halfwaardetijden verschilden tussen de drie tools, waarbij de medianen 12.6 uur (n = 24), 11.2 uur (n = 30) en 13.0 uur (n = 30) waren voor respectievelijk myPKFiT®, WAPPS en NONMEM. Om boven een FVIII dal spiegel van 0.01 IU/mL te blijven na 48 uur, waren de doseringen van myPKFiT® en NONMEM 15.1 en 11.0 IU/kg, en voor WAPPS en NONEM 9.0 en 8.0 IU/kg. In de negen patiënten die Kogenate® kregen toegediend , produceerden WAPPS en

NONMEM verschillende PK parameters; de FVIII halfwaardetijd was hierbij 15.0 en 12.3 uur en de tijd tot 0.05 IU/mL was 69.2 en 60.8 uur. Desondanks waren de geadviseerde FVIII doseringen niet verschillend. We concludeerden dat de drie PK tools verschillende PK parameters en doseringen voor recombinante FVIII concentraten berekenen en dat kinderarts-hematologen en internist-hematologen hiervan bewust moeten zijn bij de keuze van een PK tool.

In het laatste hoofdstuk van dit proefschrift, **hoofdstuk 11**, geven we een overzicht van de meest belangrijke bevindingen van de verrichtte studies en bediscussiëren wij de klinische implicaties en aanbevelingen voor toekomstig onderzoek.

A

LIST OF PUBLICATIONS

Hazendonk HCAM, **van Moort I**, Fijnvandraat K, Kruip MJHA, Laros-van Gorkom BAP, van der Meer FJM, Meijer K, Peters M, Schutgens REG, Zwaan CM, Driessens MH, Polinder S, Leebeek FWG, Mathôt RAA, Cnossen MH. The OPTI-CLOT trial. Study design of a randomized controlled trial on perioperative pharmacokinetic-guided dosing of clotting factor concentrate in hemophilia A.
Thromb Haemost. 2015 Aug 31;114(3):639-44.

van Moort I, Joosten M, de Maat MPM, Leebeek FWG, Cnossen MH. Pitfalls in the diagnosis of hemophilia: What to do?
Pediatr Blood Cancer. 2017 Apr;64(4).

van Moort I*, Preijers* T, Fijnvandraat K, Leebeek FWG, Cnossen MH, Mathôt RAA. Cross-evaluation of Pharmacokinetic-Guided Dosing Tools for Factor VIII.
Thromb Haemost. 2018 Mar;118(3):514-525.

Hazendonk HCAM, Heijdra JM, de Jager NCB, Veerman HC, Boender J, **van Moort I**, Mathôt RAA, Meijer K, Laros-van Gorkom BAP, Eikenboom J, Fijnvandraat K, Leebeek FWG, Cnossen MH for the OPTI-CLOT and WIN study group. Analysis of current perioperative management with Haemate® P/Humate P® in von Willebrand disease: Identifying the need for personalized treatment. *Haemophilia. 2018 May;24(3):460-470.*

Hazendonk HCAM, **van Moort I**, Mathôt RAA, Fijnvandraat K, Leebeek FWG, Collins PW, Cnossen MH for the OPTI-CLOT study group. Setting the stage for individualized therapy in hemophilia: What role can pharmacokinetics play?
Blood Rev. 2018 Jul;32(4):265-271.

van Moort I, Meijer P, Priem-Visser D, van Gammeren AJ, Péquériaux NCV, Leebeek FWG, Cnossen MH, de Maat MPM. Analytical variation in factor VIII one-stage and chromogenic assays: Experiences from the ECAT external quality assessment programme.
Haemophilia. 2019 Jan;25(1):162-169.

van Moort I, Bukkems LH, Heijdra JM, Schutgens REG, Laros-van Gorkom BAP, Nieuwenhuizen L, van der Meer FJM, Fijnvandraat K, Ypma P, de Maat MPM, Leebeek FWG, Meijer K, Eikenboom J, Mathôt RAA, Cnossen MH, for the OPTI-CLOT study group. Von Willebrand factor and factor VIII clearance in perioperative hemophilia A patients (OPTI-CLOT trial).
Thromb Haemost. 2020 Jul;120(7):1056-1065.

Schütte LM, Hodes LS, **van Moort I**, Stoof SCM, Leebeek FWG, Cnossen MH, de Maat MPM, Kruip MJHA. The one-stage assay or chromogenic assay to monitor baseline factor VIII levels and desmopressin effect in non-severe haemophilia A: Superiority or non-inferiority?
Haemophilia. 2020 Jul 26; doi:10.1111/hae.14106

van Moort I[*], Preijers T*, Hazendonk HCAM, Schutgens REG, Laros-van Gorkom BAP, Nieuwenhuizen L, van der Meer FJM, Fijnvandraat K, Leebeek FWG, Meijer K, Mathôt RAA[**], Cnossen MH[**], for the "OPTI-CLOT" study group. Dosing of factor VIII concentrate by ideal body weight is more accurate and seems safe in overweight and obese hemophilia A patients.
Submitted

van Moort I, Bukkems LH, Nieuwenhuizen L, Leebeek FWG, Mathôt RAA & Cnossen MH for the OPTI-CLOT study group. Impact of extreme weight loss on factor VIII concentrate pharmacokinetics in hemophilia.
Submitted

van Moort I, Preijers T, Bukkems LH, Hazendonk HCAM, van der Bom JG, Laros-van Gorkom BAP, Beckers EAM, Nieuwenhuizen L, van der Meer FJM, Ypma P, Coppens M, Fijnvandraat K, Fischer K, Schutgens REG, van Leeuwen N, Meijer K, Leebeek FWG, Mathôt RAA, Cnossen MH, for the OPTI-CLOT study group. A randomized controlled trial comparing perioperative dosing of factor VIII concentrate in hemophilia A based on pharmacokinetics with standard treatment (OPTI-CLOT trial).
Submitted

LIST OF ALL OPTI-CLOT MEMBERS

The investigators and institutions participating in the OPTI-CLOT research programme in the Netherlands are as follows. Steering committee – M.H. Cnossen (principal investigator and chair), Erasmus University Medical Center – Sophia Children's Hospital, Rotterdam; F.W.G. Leebeek, Erasmus University Medical Center, Rotterdam; R.A.A. Mathôt, K. Fijnvandraat, M. Coppens, Amsterdam University Medical Centers; K. Meijer, University Medical Center Groningen.

Principal Investigators, local Collaborators in the Netherlands – M.H.J.A. Kruip, S. Polinder, E.W de Bekker-Grob, Erasmus University Medical Center, Rotterdam; R.A.A. Mathôt, K. Fijnvandraat, M. Coppens, Amsterdam University Medical Centers; K. Meijer, R.Y.J. Tamminga, University Medical Center Groningen; P. Brons, B.A.P. Laros-van Gorkom, Radboud University Medical Center, Nijmegen; F.J.M. van der Meer, H.C.J. Eikenboom, Leiden University Medical Center; R.E.G. Schutgens, K. Fischer, University Medical Center Utrecht; L. Nieuwenhuizen, Maxima Medical Center, Veldhoven; E.A.M. Beckers, Maastricht University Medical Center, Maastricht; P. Ypma, Haga Hospital, the Hague; M.H.E. Driessens, J. de Meris, Netherlands Hemophilia Patient Society; Clinical research coordination – C.M. Zwaan, I. van Vliet, Erasmus University Medical Center, Rotterdam.

PhD graduates on OPTI-CLOT projects
J. Lock (PhD defense 26th October 2016) , H.C.A.M. Hazendonk (PhD defense 30th May 2017), T. Preijers (PhD defense 8th April 2020),
I. van Moort, J.M. Heijdra, M.C.H.J. Goedhart,
N. de Jager, L.H. Bukkems,
L.G.R. Romano ("DAVID" studies), W. Al Arashi, M.E. Cloesmeijer, A. Janssen.

Principal investigators and local collaborators in the United Kingdom: P.W. Collins, Arthur Bloom Haemophilia Centre, Institute of Infection and Immunity, School of Medicine, Cardiff University, Cardiff; R. Liesner, Great Ormond Street Haemophilia Centre, Great Ormond Street Hospital for Children NHS Trust, London; P. Chowdary, Katharine Dormandy Haemophilia Centre and Thrombosis Unit, Royal Free London NHS Foundation Trust, London; C.M. Millar, Hammersmith Hospital- Imperial College Healthcare NHS Trust, London; D. Hart, The Royal London Hospital Barts Health NHS Trust, London; D. Keeling, Oxford Haemophilia and Thrombosis Centre, Oxford University Hospitals, Churchill Hospital, Oxford.

DANKWOORD

Een promotietraject doorloop je niet alleen, daarom zou ik graag de volgende mensen willen bedanken voor hun hulp in de afgelopen jaren.

Zonder patiënten geen onderzoek! Daarom wil ik als eerst alle **patiënten en ouders** bedanken voor hun deelname en vertrouwen in de OPTI-CLOT studies. Ieder verhaal van elke patiënt was weer anders en inspirerend, wat ertoe leidde dat ik nog meer passie voor dit onderzoek kreeg en doorzettingsvermogen om studies voort of op te zetten. Daarbij een speciaal bedankje voor de Nederlandse Vereniging voor Hemofilie Patiënten (NVHP), bedankt voor jullie hulp en toewijding!

Lieve Marjon, oftewel **dr. Cnossen**. Vijf jaar geleden hadden we gelijk een 'klik' bij het sollicitatiegesprek. Ondanks dat ik niet kon bloedprikken, had jij het vertrouwen dat dit 'wel goed moest komen' en dat 'goedkomen' is zeker gelukt! Dank je voor al je hulp, cappuccinootjes als het onderzoek even niet liep zoals het zou moeten lopen, je inspiratie en vooral de mogelijkheden die jij mij gaf tijdens mijn promotie. Een meer 'personalized promotietraject' bestaat er denk ik niet. Als kers op de taart hebben we samen met het SYMPHONY consortium de NWO-NWA toegewezen gekregen. Daarom nemen we gelukkig niet helemaal afscheid en blijven we nog een klein beetje samenwerken. Ik kijk er naar uit!

Beste Frank, beste **prof. dr. Leebeek**. Toen ik startte, was ik een van de weinige 'niet-artsen' tussen alle dokters, maar daardoor hebben jullie mij veel geleerd over het vak van de benigne hematologie. Dank je voor al je hulp in de kliniek, constructieve feedback en ook vooral de humor! Ik vergeet niet snel meer dat we samen met de Rotterdamse hemofiliepatiënten gingen ijskarten, en wie was er nou ook alweer sneller, jij of ik?

Beste Ron, beste **prof. dr. Mathôt**. Een OPTI-CLOT-patiënt in het weekend? Jij stond klaar om doseeradviezen te controleren op zondagochtend. Daarnaast is je PK kennis niet up-to-date als je niet bij de maandelijkse NONMEM meeting in het AMC bent. Door jou en Reinier was er iedere maand een leerzame meeting, waar alle ins en outs van het PK modelleren werden besproken. Ik wil je bedanken voor alle goede feedback en tijd in de afgelopen jaren.

Een studie opzetten in alle **hemofiliebehandelcentra's** in Nederland gaat ook niet zonder slag of stoot. Daarom wil ik ieder hemofiliebehandelcentrum en vooral de trialbureaus en/of verpleegkundigen/verpleegkundig specialisten bedanken. Jullie hebben ervoor gezorgd dat in alle centra de OPTI-CLOT-studie ging lopen, van contracten tot

site files, van bloedafnames tot databases aanvullen. Zonder jullie was dit niet gelukt, bedankt! Vooral **Ineke** wil ik in het bijzonder bedanken, zonder jouw kennis over alle regels van METC, CCMO en andere instanties kon de OPTI-CLOT niet bestaan. Daarnaast wil ik ook alle hematologen bedanken voor hun inzet 24/7 rondom de operaties, we konden altijd bij jullie terecht, dank!

Beste **prof. dr. Voorberg, prof. dr. de Wildt en prof. dr. de Maat,** dank dat jullie plaats willen nemen in de kleine promotiecommissie. Daarnaast wil ik **prof. dr. Meijer, prof. dr. van der Ploeg en prof. dr. Rings** bedanken voor hun deelname aan de grote promotiecommissie.

Beste Sander en Maartje, beste **prof. dr. Meijer en dr. van den Biggelaar**. Nadat de OPTI-CLOT-studie opgestart was in ieder centrum, miste ik het laboratorium. Bedankt voor de mogelijkheid om in jullie lab de wereld van proteomics te ontdekken. Samen gaan we nog een mooi project afronden.

Directe collega's maken of kraken het werkplezier, nou bij de stolling in het Erasmus MC en Sophia Kinderziekenhuis is het een gezellige boel! Zonder jullie was deze promotie zeker niet zo leuk geweest! **Carolien**, nadat jij al het voorwerk hebt uitgevoerd voor de OPTI-CLOT, nam ik het stokje van je over. Dank je voor je adviezen, goede gesprekken op congressen (als we "op tijd" gingen slapen en het dan toch weer 2 uur 's nachts was) en vooral de gezelligheid. Jij bent een super kinderarts! Johan, Jossi en Caroline, samen waren we toch een lekker koffieclubje (en oké Lisette hoorde er ook bij met haar theetje). **Johan**, wat mis ik jouw humor en wat hebben we gelachen op 'ons eiland' samen met Jossi. Gelukkig komen we elkaar nog tegen op congressen. **Jossi**, naast dat we samen heel goed Jochem Meijer na konden doen op de vroege ochtend, konden we daarna ook heel hard werken. Hopelijk komt er (binnenkort?) een moment dat je je passie voor stolling en statistiek weer oppakt? Lijkt mij heel leuk! **Lisette**, samen begonnen we aan het avontuur 'promotie', ik zie ons nog braaf zitten met zijn tweetjes in het kantoor in ons eerste jaar tussen kerst en oud en nieuw. Dankjewel voor alle goede gesprekken en leuke discussies! **Caroline**, of zal ik dr. Veen zeggen? Samen hebben we heel wat meegemaakt, van een lach tot een traan, baby-en puppyverhalen en van logistieke zaken in het onderzoek tot statistische uitdagingen, altijd konden (en kunnen!) we bij elkaar terecht. Ik vind het dan ook echt heel fijn dat je bij het laatste deel van mijn promotietraject naast me wil staan als mijn paranimf!

Uiteraard zijn er ook een heleboel "nieuwe" PhD studenten begonnen tijdens mijn promotie, die ik ook graag wil bedanken voor alle serieuze en minder serieuze momenten! **Jessica**, een rots in de branding. Jouw kalmte, gezelligheid en kennis over VWD en het is

daarnaast ook heerlijk om lekker even bij te kletsen over van alles en nog wat. Daarnaast was de OPTI-CLOT zonder jou niet doorgegaan tijdens mijn vakantie; daarvoor ook heel erg bedankt! **Tine**, ook al kennen we elkaar nog niet heel lang, het voelt toch heel vertrouwd. Ik heb er dan ook het volledige vertrouwen in dat die PhD en kinderartsencarrière bij jou goed gaat komen. En als je nog eens een lift nodig hebt in Brabant, weet je me te vinden. **Ferdows**, mijn statistiekmaatje! Na de statistiekcursussen samen, neem ik aan dat we nog vaak met elkaar gaan sparren over de juiste statistiek en waarom een statistiekprogramma niet doet wat het zou moeten doen. Daarnaast zal ik de Indische waterlelies uit Madrid ook niet snel vergeten. **Judith**, ik hoop dat we, naast dat we elkaar nog regelmatig tegen gaan komen op het lab, ook nog regelmatig naar een hardloopwedstrijdje gaan. Is een van de beste manieren om te ontspannen, toch? Ook wil ik **Maite, Samantha, Lorenzo en Joppe** bedanken voor de gezellige momenten gewoon op het kantoor of op congressen!

Laura en Tim, zonder jullie had de OPTI-CLOT niet draaiende gehouden kunnen worden. **Tim**, met onze gezamenlijke OPTI-hobby in het weekend hebben we toch stiekem best lol gehad, wat heeft geleid tot mooie artikelen. Succes met je opleiding tot ziekenhuisapotheker! **Laura**, jij hebt je vliegensvlug ingewerkt in alle studies in de hemofilie. Petje af! Ook jij bedankt voor je hulp en gezelligheid bij de OPTI-CLOT.

Daarnaast is de OPTI-CLOT in het Erasmus MC ook vlekkeloos verlopen door de hulp van de geweldige hemofilieverpleegkundigen/verpleegkundig specialisten: **Marjo, Floor, Greta, Carolien en Sasja**. Dames ontzettend bedankt voor al jullie hulp, het meedenken over de logistiek van de studie, helpen met het bijvullen van de prikkoffer (“even shoppen” he Marjo) en uiteraard het leren prikken (Floor dank je!). Greta, dank je wel voor je oplettendheid tijdens iedere poli, de gezellige momenten en goede adviezen. Zonder jullie had Rotterdam niet de “beste includeerder” kunnen zijn.

Ook het **hemostaselab** in het Erasmus MC moet natuurlijk even genoemd worden. De ‘cadeautjes’ die ik langs kwam brengen met citraatbuisjes werden altijd keurig door jullie verwerkt en de FVIII bepalingen werden vliegensvlug uitgevoerd als een patiënt opgenomen lag. Daarnaast is het gewoonweg ook heel gezellig bij jullie op het lab, dat heb ik wel gemerkt tijdens het uitvoeren van de VWF bepalingen. Dank je voor jullie assistentie! Ook de andere hemostaselaboratoria in Nederland wil ik bedanken voor hun bijdrage!

Many thanks for all my colleagues from Sanquin! **Nadia, Iris, Gosia, Bart, Eelke, Esmee, Arjan, Carmen, Floris and Mariette**, many thanks for all your help and assistance in the laboratory. You taught me how to prepare mass spectrometry (MS) samples, create

beautiful figures in R and the basics of the MS. Thanks for answering all my questions, and for the introduction into this very interesting and promising field. Most of all, thank you for all the enjoyable moments, good jokes and good conversations.

Beste **dr. Kruip en prof. dr. de Maat**, beste Marieke en Moniek. Marieke, dank voor al je hulp met de OPTI-CLOT en ook de goede gesprekken en adviezen. Moniek, jij ook bedankt dat ik altijd even binnen kon lopen met een lab of statistiekvraag. Daarnaast vind ik het een eer dat je wilt plaatsnemen in mijn promotiecommissie!

Beste **dr. Bierings,** beste Ruben, dank je dat ik tijdens het begin van mijn postdoc soms nog 'even' aan mijn proefschrift kon werken. Hierdoor is de overgang van hemofilie naar BOECs vloeiend verlopen. Ik kijk er naar uit om verder te werken aan het SYMPHONY project binnen de gezellige "Bierings" groep samen met **Petra**, **Maurice en Bas**!

Naast collega's zijn er ook genoeg andere mensen die indirect een bijdrage hebben geleverd aan dit proefschrift. Mijn wetenschappelijke carrière begon op de VU samen met jullie dames: **Valerie, Maralinde, Willemijn en Iris**. Het klinkt gewoon gek om jullie namen voluit te schrijven, dus Val, Mara, Wil en Ier, gewoon even lekker zo. Zo begint een vriendschap met een naamsverwisseling in de collegezaal (wie verzint het ook, Iris van Moort en Iris van Noord), en zo zijn we opeens volwassen vrouwen met een huis en baan. Nu we allemaal weer in Europa wonen, komt dat weekendje London (of Oxford!) eraan! Val, na ons geweldige avontuur in Londen, hebben we je heel lang moeten missen door je verhuizing naar Melbourne. Hoe toevallig was het ook dat het stollingscongres (ISTH) in het laatste jaar van mijn PhD in Melbourne werd gehouden! Heerlijk was het om eindelijk weer in 'real life' met elkaar te kletsen bij jouw favoriete tentje in Brunswick. Ik bewonder je doorzettingsvermogen ("even" emigreren naar Australië!) en relativeringsvermogen. Daarnaast is het ook gewoonweg heerlijk om keihard met jou te lachen over de serieuze en minder serieuze zaken van het leven. Mara, jij als mede-PhD'er, of nou, nu dr. Mara, wat is het heerlijk om samen de hoogte-en dieptepunten over de wetenschap te bespreken onder het genot van een wijntje. Je staat op je strepen bij de zaken die er toe doen, en je hebt altijd een oplossing klaar staan. Ik vind het dan ook super fijn dat je mijn paranimf wilt zijn! Je bent een kanjer!

Ook mijn oude huisgenootjes, **Linda en Karlijn**, wil ik graag bedanken. Zoals Lin zo mooi zei, van diner in badjas naar dames met een 'echte' baan. Lin en Karlijn, door jullie heerlijke baksels, goede gesprekken en diners hadden we altijd een heerlijk 'thuis' tussen de colleges, feestjes en sportlesjes door. Ondanks dat we nu niet meer samenwonen, hoop ik dat onze etentjes nog heel vaak plaats gaan vinden!

Je kan het meisje uit Zeeland halen, maar Zeeland niet uit het meisje. **Jessica en Heleen**, dank jullie voor de Zeeuwse nuchterheid, die ons denk ik verder brengt dan dat we zelf beseffen! Binnenkort maar weer eens in de mooiste provincie van Nederland een wijntje drinken?

Maartje en Maartje, dames ook jullie bedankt voor de gezelligheid en het aanhoren van mijn PhD verhalen. Maartje Heitzman, van een drankje in de tuin tot serieuze gesprekken op de bank, ik ben blij dat we daarvoor terecht kunnen bij elkaar. Maartje Thompson, na alle avonturen die we samen hebben gedeeld, van carnaval tot aan het kerstdiner bij de schoonfamilie, hebben we ondertussen een nieuwe hotspot, onze etentjes bij Mangiare zijn ondertussen een begrip. Er zijn weinig mensen die ik ken die zo goed op de hoogte zijn en zo weinig vergeten over iemands leven. De wereld zou een beetje meer Maartjes moeten hebben.

Het liefst had ik vijf wetenschappelijke stellingen over sporten aan mijn proefschrift toegevoegd, en dat is niet zonder reden. De bootcamp in Bergen op Zoom en Halsteren is gewoonweg een van de gezelligste sportclubs van Nederland en die zorgt voor de perfecte ontspanning! **Mitchell, Madeleine, Matthijs, Joyce, Tessa, Marchel, Ascania, Marije en Eveline**, dank jullie voor de slappe lach tijdens de meest vervelende oefeningen, de heerlijke competitie tijdens een sprintje, de gezelligheid tijdens een obstakelrun of tijdens een Ardennenweekend rondom het kampvuur. **Madeleine**, ik ken weinig mensen die blij zijn met een verjaardagscadeautje van 14 km door de duinen rennen en daarom vind ik onze vriendschap ook zo fijn. Dank je voor je luisterend oor tijdens, maar vooral naast het sporten!

Lieve **Rien, Loes en Gijs**, na al die jaren voelen jullie als een ‘tweede paar ouders en broer’ en is het ook in het Eindstraatje een gevoel van thuiskomen. Dankjewel voor alle fijne goede gesprekken en natuurlijk voor de oppas op onze kleine Joep! Volgens mij heb ik Rien nog bijna nooit zo blij gezien nadat we hadden verteld dat we voor een puppy gingen kijken.

Lieve **Papa, Mama, Joris en Roosmarijn.** Wie kent je beter dan je eigen familie? Pap en Mam, dank je voor de vrijheid die jullie me hebben gegeven om zelf een studie te kiezen die ik leuk vond (en geen zeevaartschool…). Jullie staan altijd voor me klaar en voelden het haarfijn aan als iets toch anders gaat dan ik in mijn hoofd had gepland. Ik denk dat de uitspraak: “A ship in a harbor is safe, but that is not what ships are built for”, zowel letterlijk als figuurlijk op jullie van toepassing is. Ik ben trots op hoe jullie alles regelen op de schepen en ons (Joris, Roos en ik) de vrijheid geven om zelf met ons figuurlijke bootje vanuit het veilige Veerse Meer richting de wilde zee gaan. Joris, jouw passie voor

muziek is eindeloos. Muziek maken, luisteren, recensies schrijven en publiceren, en dat allemaal in je vrije tijd. Ik ben trots op je en ik wacht op de dag dat jij je eigen column of wekelijkse reportage in de krant krijgt! Roosmarijn, hoe jij je binnen een paar jaar hebt ontwikkeld van een verpleegkundige die net begon tot een verpleegkundige die praktisch een heel complex regelt en bijspringt als er (weer) te weinig personeel is, vind ik bewonderingswaardig. Ik ben trots op je dame! En je weet het, de hemofiliezorg zou een verpleegkundige als jij heel goed kunnen gebruiken.

Dan als laatst, had ik dit boekje niet kunnen afmaken zonder **Bart**. Ik denk dat jij ondertussen ook een PhD kan behalen over PK-gestuurd doseren van stollingsfactor VIII na al mijn verhalen. Zoals Rosalind Franklin zei: “Science and everyday life cannot and should not be separated.” Lieverd, jij houdt me met beide benen op de grond, stimuleert me om vol voor het onderzoek te gaan, maar remt me af waar nodig en zorgt ervoor dat ook de kleine successen worden gevierd. Ik wil je bedanken voor je warmte, liefde en vooral betrokkenheid. Voordat het echt heel sentimenteel gaat worden, hoop ik dat we deze dag vooral goed gaan vieren en samen nog heel veel heerlijke reizen gaan maken, mooie avonturen gaan beleven met Joep en nog vele kilometers door de polders gaan rennen; samen onze mooie toekomst tegemoet.

ABOUT THE AUTHOR

Iris van Moort was born on the 7th of April 1991 in Wolphaartsdijk, the Netherlands. After graduating from secondary school (Ostrea Lyceum, Goes), she moved to Amsterdam to study Health and Life Sciences at the VU University, followed by a Research Master in Cardiovascular Research, in which she participated in the Top Master program. This program offered her the opportunity to participate in extracurricular courses and to perform an internship in a renowned research center abroad. She left for King's College London for seven months to investigate phosphorylation of a new potential pharmacological target for patients with heart failure. For this research project, she received the Dekker student grant and graduated cum laude in 2014.

As she was interested in both pediatric and cardiovascular pharmacological research, she started her PhD project entitled: "Personalizing Factor Replacement Therapy in Hemophilia" at the Erasmus University Medical Center- Sophia Children's Hospital in October 2014. In her PhD project under supervision of Dr. Marjon H. Cnossen, Prof.dr. Frank W.G. Leebeek and Prof.dr. Ron A.A. Mathôt she investigated the introduction of pharmacokinetic-guided dosing of factor concentrates into hemophilia care. Besides her clinical research, she performed laboratory studies to discriminate endogenous and exogenous plasma factor VIII by mass spectrometry under supervision of Prof.dr. Sander Meijer at Sanquin Research Laboratories. During her PhD program, she received several awards, e.g. young investigator award of the International Society of Thrombosis and Hemostasis, the Ulla Hedner haemostasis award, and a research grant for translational research in hemophilia. She also followed the PhD curriculum of Training Upcoming Leaders in Pediatric Science (TULIPS) and the Female Talent Class of the Erasmus MC. Finally, she assisted her co-promotor Dr. Marjon H. Cnossen in setting up a Dutch National Science Agenda Grant (NWO-NWA) which was granted to the SYMPHONY consortium, a national collaboration of talented multidisciplinary researchers which aim to orchestrate personalized treatment for patients with bleeding disorders. This grant will allow her to start her postdoctoral research project at the Erasmus University Medical Center in the research group of Dr. Ruben Bierings.

PHD PORTFOLIO

Name PhD student: Iris van Moort	PhD period: October 2014 – October 2019
Erasmus MC Department: Pediatric Hematology	Promotors: Prof.dr. F.W.G. Leebeek, Prof.dr. R.A.A. Mathôt
Research School: COEUR	Co-promotor: Dr. M.H. Cnossen

1. PhD training	Year	Workload (ECTS)
General academic skills		
Biomedical English Writing and Communication	2017	3
Research integrity	2017	0.3
Good Clinical Practice (BROK)	2015	1
Open Clinica course	2015	0.3
CPO course (Center for Patient Oriented Research)	2015	0.3
Systematic Literature Retrieval	2014	0.5
Specific courses		
Repeated Measurements (NIHES)	2019	1.7
3x NVTH annual PhD course on hemostasis and thrombosis	2015-2018	3.0
NONMEM journal clubs	2014-2018	3.0
Biostatistical Methods II: classical regression models (NIHES)	2017	4.3
Biostatistical Methods I: Basic Principles (NIHES)	2015	5.7
Vascular Medicine (COEUR)	2015	1.5
Clinical cardiovascular epidemiology (COEUR)	2014	1.5
(Inter)national scientific presentations and conferences		
Oral presentations		
Dutch Association of Thrombosis and Hemostasis (NVTH)	2019	1.0
ECAT symposium (invited)	2018	1.0
Dutch Society of Pediatrics	2017	1.0
Hemophilia Nursing Conference (invited)	2017	1.0
International Society on Thrombosis and Hemostasis Congress, Berlin (2x oral presentation)	2017	1.0
Pharmacology meeting Erasmus MC	2017	1.0
COEUR PhD day (2x)	2016, 2017	1.0
Netherlands association for Hemophilia Patients (NVHP), patient day (invited)	2016	1.0
COEUR symposium	2016	1.0
ESDK day – Day for young hemophilia patients (invited)	2016	1.0
Sophia Research Day	2016	1.0

Poster presentations		
International Society on Thrombosis and Hemostasis Congress, Melbourne, Australia	2019	0.3
European Congress on Thrombosis and Hemostasis, Den Haag	2016	0.3
International Society on Thrombosis and Hemostasis Congress, Toronto, Canada	2015	0.3
International conferences		
3x International Society on Thrombosis and Hemostasis Congress	2015, 2017, 2019	4.4
2x European Association of Hemophilia And Allied Disorders Congress	2015, 2018	1.8
European Congress on Thrombosis and Hemostasis	2016	1.0
Bari international conference, Bari, Italy	2014	0.9
National conferences		
NVTH Symposium	2015-2019	1.5
Annual meeting of the Dutch Society of Pediatrics	2016, 2017	0.6
AMSTOL Symposium	2016	0.3
Von Creveld Symposium	2014	0.3
Seminars and workshops		
Masterclass with Prof.dr. David Lillicrap	2019	0.3
Sophia Research Day	2015-2019	1.5
COEUR PhD Day	2015-2018	1.2
Nijmegen Symposium on Hemophilia and Rare Bleeding disorders	2015, 2017	0.6
TULIPS Young Investigator Day	2015-2017	0.9
COEUR symposium personalized medicine	2015	0.1
Masterclass with Prof.dr. Evan Sadler	2015	0.3
2. Teaching activities		
Lecturing		
Teacher 'Biochemistry of coagulation' & 'PK and Hemophilia' - second year medical students	2015-2019	0.3
Lecturing at massive blood loss symposium for anesthesiology department	2018	0.1
Supervision of master's thesis		
Sam Reerds, medical student	2016	1.0
Supervision of scientific internship		
Colin Spence, medical student	2018, 2019	0.3

Hematology PhD training		
Work discussions and journal clubs Erasmus MC	2014-2019	8.0
Work discussions at Sanquin	2017-2019	0.5
3. Other		
Participation in Female Talent Class–Erasmus MC	2019	0.6
Participation in TULIPS PhD program	2016-2018	3.0
TULIPS - Organizing Young Investigator Day	2016, 2017	1.0
Pediatric Pharmacology meetings	2014, 2015	0.6
4. Awards and grants		
Top scoring abstract, EAHAD	2020	
Dutch National Research Agenda, SYMPHONY consortium (€4.100.000)	2019	-
Sobi Dutch research grant for translational research in hemophilia (€25.000)	2018	-
Ulla Hedner Haemostasis Award (€15.000)	2017	-
Young Investigator Award International Society on Thrombosis and Haemostasis (€750)	2017	-
Winner Slam session Bayer Hemophilia nurse conference	2017	-
Poster Award International Society on Thrombosis and Haemostasis	2015	-
Total		**69.1**

ECTS = European Credit Transfer and Accumulation System (1 ECTS represents 28 hours).