Individualizing Treatment in Von Willebrand Disease

One size does not fit all

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Het individualiseren van de behandeling van Von Willebrandziekte

Proefschrift

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Chapter 1

General introduction and outline of the thesis

INTRODUCTION

Von Willebrand factor

Von Willebrand factor (VWF) is a multifunctional coagulation protein, which is important for both primary hemostasis and secondary hemostasis as it mediates platelet adhesion and aggregation, as well as protects coagulation factor VIII (FVIII) from degradation and clearance from the blood circulation (1). In the body, VWF is found in blood plasma and is stored in the vascular endothelial cells and in platelets. When stress or injury occurs, VWF is mobilized from its storage sites, leading to a concomittant increase in FVIII.

Von Willebrand disease

Von Willebrand disease (VWD) is caused by a partial or complete absence of VWF or by functionally impaired VWF. The variation in VWD types, the underlying mutations and their possible functional effects on VWF and FVIII, as well as other intrinsic and extrinsic modifiers, lead to a wide variation in clinical phenotype of VWD (1). The clinical phenotype is characterized by mucocutaneous bleeding, such as bruising, menorrhagia, epistaxis, postpartum bleeding and bleeding after trauma or surgery. More severe bleedings, such as joint bleeding and gastrointestinal bleeding may occur in certain patients. The prevalence of VWD in the general population is approximately 1%, however only a minority of 0.01% has clinically relevant bleeding symptoms (2).

VWD is classified into three types: type 1, 2 and 3. Individuals with type 1 VWD have a partial deficiency of VWF, individuals with type 2 VWD have impaired function of VWF, and individuals with type 3 VWD have a complete absence of VWF. Type 2 VWD is further subdivided into: 1) type 2A, in which binding of VWF to platelets is impaired and the hemostatically active high molecular-weight VWF multimers (HMWM) are reduced; 2) type 2B, in which VWF-platelet binding is increased, leading to thrombocytopenia; 3) type 2M, which leads to decreased platelet binding or collagen binding activity, accompanied by normal HMWM; 4) type 2N, in which the binding of VWF to FVIII is impaired, leading to severely decreased FVIII levels (3). Due to the absence of VWF and the concomitantly decreased FVIII levels, type 3 VWD patients generally have severe (spontaneous) bleeding symptoms such as gastro-intestinal bleeds and joint bleeds, and may therefore require prophylactic treatment (4).

Many known - and probably also unknown - intrinsic and extrinsic factors modify the VWD phenotype. Intrinsic factors include a wide range of pathogenic mutations in different VWF gene domains causing VWD. Other patient characteristics, such as sex, age and blood group are of influence as well (5-8). Extrinsic factors, such as stress,

hormonal cycle, physical exercise and comorbidities are also known to temporarily increase VWF, as well as other factors illustrated in Figure 1 (9). These modifying factors lead to different VWF levels in patients with a similar genotype, as well as varying VWF levels within the individual patient over time (10).

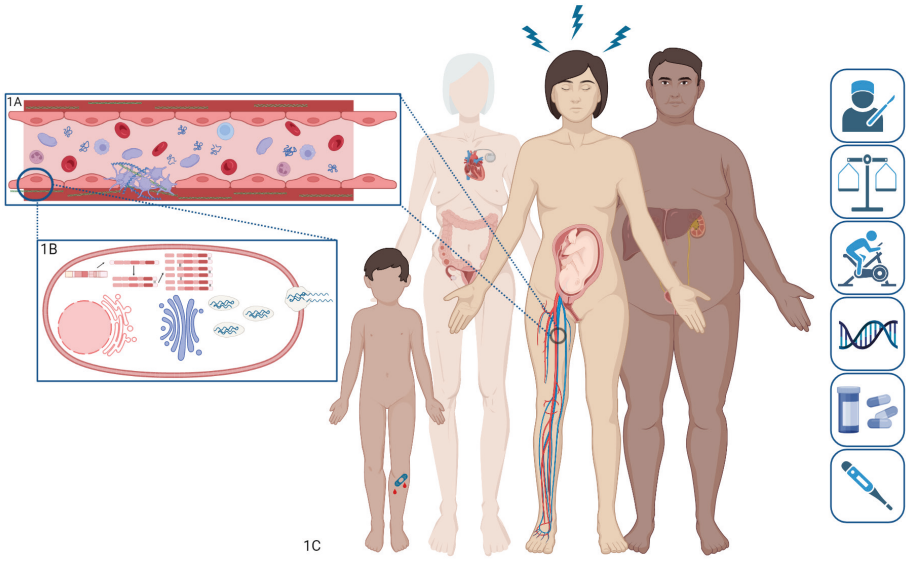

Figure 1. Function and formation of VWF, and intrinsic and extrinsic modifying factors of VWF and FVIII levels which are known to modify bleeding phenotype.

1A: Blood vessel with circulating erythrocytes, leukocytes, platelets and von Willebrand factor (VWF). The vessel wall consists of endothelial cells. When the endothelium is damaged, VWF and subendothelial collagen bind to platelets, forming a clot.

1B: Inside the endothelial cell, VWF is synthesized. In the endoplasmic reticulum, VWF is synthesized as a propolypeptide, consisting of several structural domains. After the signal peptide is cleaved, the VWF subunits dimerize in the endoplasmic reticulum. Thereafter, VWF multimers are formed in the Golgi. The propeptide is cleaved, but remains noncovalently bound to the forming VWF multimer. The forming VWF multimer organizes into a helix, folding itself for storage in Weibel-Palade bodies. When the Weibel-Palade bodies fuse with the endothelial membrane, the VWF multimers unwind into long VWF strings.

1C: Intrinsic factors known to modify VWF and FVIII levels and bleeding phenotype include genotype -and thereby the balance between synthesis, secretion and clearance of VWF and FVIII-, hormonal changes during the menstrual cycle and pregnancy, aging, and comorbidities including cardiovascular disease and cancer. Extrinsic factors include bleeding due to trauma or surgery, stress, physical exercise, and medication. It is likely there are other still unknown intrinsic and extrinsic factors that influence VWF and FVIII levels and bleeding type.

VWD is often compared to the more widely known and often more severe congenital X-linked bleeding disorder hemophilia A, in which FVIII is absent or decreased (11). However, VWD is in many ways very different from hemophilia A and actually more complex.

This is due to VWF's multiple roles within hemostasis, as well as its role in angiogenesis and its presence in the vascular endothelium and in platelets (12). Furthermore, consistent genotype-phenotype associations in VWD types and subtypes are still lacking in most cases (4). In addition, due to the important carrier function of VWF for FVIII, protecting FVIII from degradation in the circulation, patients with VWD may also have reduced FVIII levels. This is especially observed in type 2 and 3 VWD. Moreover, while significant progress has been made over the last 30 years in the development of new therapies for hemophilia A, treatment innovations for VWD have been sparse (11). Therefore, mainstay of treatment for VWD still are desmopressin and VWF-containing coagulation factor concentrates. Only recently, recombinant VWF has become available, providing an alternative for plasma-derived VWF-containing concentrates.

Due to the heterogeneity of VWD, the development of new therapies is more complicated, and therefore investing in research may be less appealing. In addition, most patients with VWD are treated on demand, leading to less replacement therapy consumption and therefore less incentive to develop alternative therapies. Illustrative is the development of recombinant factor concentrates for hemophilia A as early as in the 1990s, while the first recombinant factor concentrate for VWD was only introduced in 2015 (13). Other novel therapies, such as extended half-life factor concentrates, monoclonal antibodies and gene therapy, have been introduced and implemented for hemophilia. None of these approaches are being tested in clinical trials for VWD patients yet (11). However recently, some promising initiatives have been undertaken to develop innovative therapeutic modalities for VWD as well (14, 15). For a complete overview of the similarities and differences between VWD and hemophilia A, see Table 1.

In our opinion, lack of research initiatives for personalization of treatment in VWD is an unmet need. We have therefore taken first steps to develop a more individualized approach to the treatment of individuals with VWD. In recent years, population pharmacokinetic (PK)-guided dosing in hemophilia has been widely studied by the OPTI-CLOT study group together with other research groups. Using this methodology, optimal dosage, frequency and timing of factor VIII or IX concentrate dosing can be calculated for the individual patient. This has hardly been investigated in individuals with VWD. In this thesis, we specifically present population PK-guided treatment strategies in VWD, as one of the first research groups worldwide.

Basic pharmacokinetic concepts

In order to understand PK-guided dosing based on population PK modelling, it is important to describe the basic PK concepts (16), which we will summarize briefly. When a drug, e.g. a clotting factor concentrate, is administered intravenously, it

is transported through the blood stream and distributed to various body tissues. *Distribution* can be affected by many factors, e.g. body weight and height, blood flow, drug lipophilicity and the bodies' water/fat ratio, molecular size of the drug, and how the drug interacts with other blood components, such as proteins. After distribution, the body starts to break down the drug. This is called *metabolism* and is mainly done by liver enzymes. Factors that influence metabolism are for instance genetics, decreased liver function, and drug-drug interactions. In the *excretion* phase, the drug is removed from the body, mostly by the kidneys into the urine or by the liver into the stool. Many factors can influence excretion. A few examples are: kidney or liver dysfunction, and diseases causing decreased blood flow through the kidneys.

Importantly, in physiologically based PK, there are two fundamental parameters: *clearance* and *volume of distribution*. Clearance (*CL*) describes how efficiently a drug is eliminated from the body, and volume of distribution (*V*) describes the relationship between the drug concentration in blood and the drug concentration in the body tissue at the site of action. The *half-life* or *elimination rate constant* (*Kel*) is determined by both clearance and volume of distribution of a drug.

Age is a significant factor in all of the above mentioned pharmacokinetic stages, as growth and developmental changes profoundly affect body composition and the body's response to medication.

Individualized PK-guided dosing

Knowledge of these basic drug PK parameters is essential to understand how a drug is handled by the body and how it should be dosed. The classical approach for determining these PK parameters requires large numbers of blood samples -often ten or more- from healthy volunteers or patients over a relatively short time period. In these samples, drug concentrations are measured, after which a concentration-versus-time curve can be constructed and *CL*, *V* and half-life can be calculated for the individual (17).

In the population PK approach, data from the total population is used, as well as PK data from the individual. As population PK studies are performed in relatively large groups of patients, intensive sampling in each individual is not necessary. Instead, sparse data, only including a few (for example one to four) samples from each individual, are needed to construct a concentration-versus-time curve for the total population (18). If needed, time points can be randomized or allocated between patients or patient groups. It also allows for less strict timing of sampling, as long as the exact sampling time is registered meticulously. Using specific software, drug concentration-versus-time data is subsequently analyzed by performing nonlinear

mixed-effects modelling (NONMEM) (19). This type of modelling describes data in terms of fixed effects and random effects. Fixed effects are 1) population average values of the PK parameters (e.g. *CL* and *V*) found in that population, and 2) parameters that possibly cause variation in the PK parameters (e.g. age, weight, sex or liver function). The random effects include 1) residual inter-individual variability due to factors that have not been measured or are unknown, 2) residual intra-individual variability, including random variation of parameters in an individual over time, measurement errors and other unknown errors.

The basic population PK model is fitted to the population data by non-linear regression. The other fixed effects parameters are then added using a forward stepwise approach to evaluate if they improve the fit of the model to the data significantly. A backward stepwise approach is then used to remove fixed effect parameters that do not contribute to the final model using more strict criteria for significance. The population PK model can be used for dose adjustments based on a patients' individual PK profile and characteristics, from which the individual PK parameters can be estimated (Bayesian analysis) (Figure 2).

Individualized PK-guided dosing is already widely applied in hemophilia care, facilitated by easy-to-use online dosing tools and web portal support (20). Application of population PK-guided dosing in hemophilia A leads to better targeting of adequate FVIII levels (21). In VWD, only two studies have previously examined PK-guided dosing prior to or during surgery (22, 23). However, these studies did not take the information of the population and possible covariates into account, and therefore lack clinical effectiveness and generalizability. There are several factors that make modelling of VWD more difficult than modelling hemophilia A, as both VWF and FVIII rather than only FVIII is affected, and target levels for VWF and FVIII during treatment may differ. Furthermore, pathophysiology varies between the different types of VWD, and production, secretion and function of endogenous VWF differs greatly. Another complicating factor is that different clotting factor concentrates for VWD contain different VWF/FVIII ratios and VWF activity (VWF:Act)/VWF antigen (VWF:Ag) ratios as well as varying multimer content and composition.

We hypothesize that modelling VWD treatment will not only make dosing of the currently available treatment more efficient and effective by tailoring the treatment to the individual patient, but will also help clinicians and researchers to better understand the complexity of VWD and the intricate interactions between endogenous and exogenous VWF and FVIII. Implementing these new treatment strategies will ultimately help us to better serve VWD patients and their specific needs.

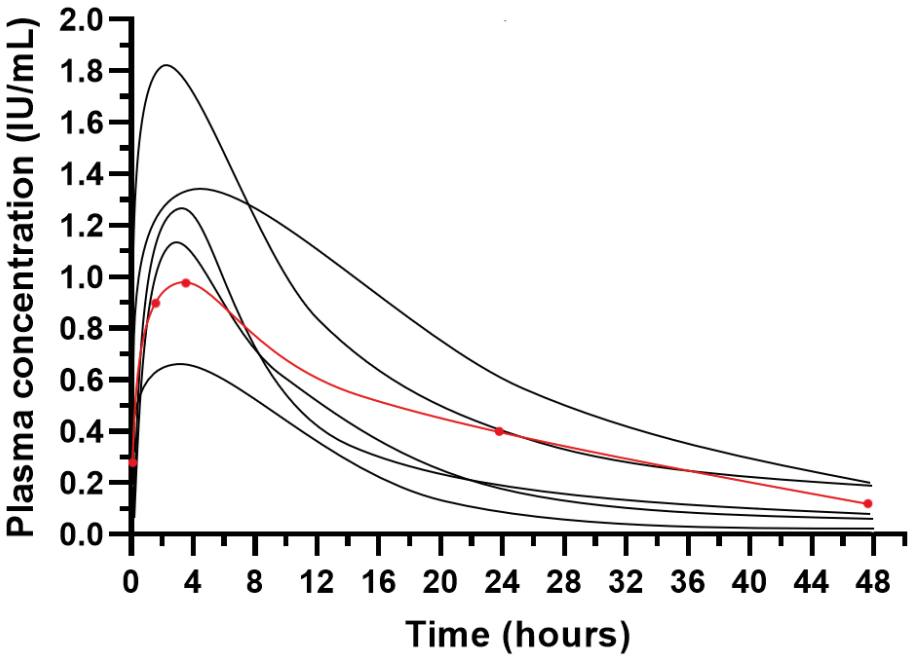

Figure 2. Estimating individual PK parameters using Bayesian analysis. The black lines represent all the information from the individuals present in the population PK model. The red dots are the measured coagulation factor levels in an individual patient. Using all information available, individual PK parameters (red line) can be estimated. Based on the calculated individual PK parameters (*V*, *CL* and *Kel*), a personalized dosing advice for targeting coagulation factor ranges is created.

Table 1. Characteristics of Von Willebrand disease (VWD) versus hemophilia A

Characteristics	
	**Sex**
	Prevalence
	**Inheritance**
	Deficient coagulation factor
	Disease classification
	**Effect on hemostasis**
	Intra-individual variability
	**Symptoms**

	Von Willebrand disease	**Hemophilia A**
	Males and females are equally affected More often diagnosed in females due to female-specific bleeding (menstruation, postpartum hemorrhage)	Almost solely males, women are carriers but may be symptomatic
	~1% (~0.01% symptomatic)	~0.02% of males
	Autosomal dominant or autosomal recessive	X-linked recessive
	Deficiency or qualitative defect of VWF FVIII may also be decreased	Deficiency of FVIII
	Type 1 (partial VWF deficiency) *Type 1 Vicenza (partial VWF deficiency due to strongly increased clearance)* Type 2 (qualitative VWF defect) *Type 2A (decreased platelet binding, decreased HMWM)* *Type 2B (increased platelet binding, decreased HMWM)* *Type 2M (decreased platelet binding, normal HMWM)* *Type 2N (decreased FVIII binding)* Type 3 (complete VWF deficiency)	Severe (FVIII <1%) Moderate (FVIII 1-5%) Mild (FVIII >5-40%)
	Mainly affects primary hemostasis (decreased platelet adhesion) Also affects secondary hemostasis (decreased FVIII)	Affects secondary hemostasis
	Large intra-individual variability in VWF Large inter-individual differences in clinical phenotype, unexplained by residual VWF and FVIII levels Multiple known and unknown intrinsic and extrinsic factors which influence VWF and FVIII levels	Smaller but present intra-individual variability in FVIII Inter-individual differences in clinical phenotype, not completely explained by residual FVIII levels Limited intrinsic and extrinsic factors which influence FVIII levels in non-severe hemophilia A
	Mainly mucocutaneous bleeding as well as bleeding due to trauma or surgery In severe cases also recurring gastro-intestinal bleeding and (spontaneous) joint and muscle bleeding	Typically (spontaneous) joint and muscle bleeding and bleeding due to (minor) trauma or surgery

Table 1. Continued

Characteristics
Assays for analysis
Currently available therapies
Intensity of treatment
Phase 3 clinical trials

VWD: von Willebrand disease; VWF: von Willebrand factor; FVIII: factor VIII; HMWM: high molecular-weight multimers; VWF:Ag: VWF antigen; VWF:Act: VWF activity; VWF:RCo: VWF ristocetin cofactor activity; VWF:Ab: VWF antibody; GP1b: VWF glycoprotein 1b; VWF:CB: VWF collagen binding; RIPA: ristocetin-induced platelet aggregation; VWFpp: von Willebrand factor propeptide; TFPI: tissue factor pathway inhibitor; siRNA: small interfering ribonucleic acid; AAV: adeno-associated virus.

Von Willebrand disease	Hemophilia A
VWF:Ag VWF:Act (VWF:RCo, VWF:Ab, VWF:GP1bM; GP1bR) One-stage FVIII VWF:CB (for subtyping type 2 VWD) Multimer assay RIPA (for distinguishing between type 2A and 2B VWD) VWF:FVIIIB assay (for type 2N diagnosis) VWFpp (for distinguishing between severe type 1 and type 3 VWD; not part of clinical routine)	One-stage FVIII assay Chromogenic FVIII assay
Oral Tranexamic acid/aminocaproic acid ***Subcutaneous*** Desmopressin ***Intravenous*** Desmopressin Plasma-derived VWF-containing concentrates Recombinant VWF-containing concentrate	***Oral*** Tranexamic acid/aminocaproic acid ***Subcutaneous*** Desmopressin Bispecific monoclonal antibody (emicizumab) ***Intravenous*** Desmopressin Plasma-derived FVIII concentrates Recombinant standard half-life FVIII concentrates Recombinant extended half-life concentrates (PEGylated, FC-fusion or albumin fusion) Bypassing agents (activated recombinant FVII, activated prothrombin complex concentrates, recombinant porcine FVIII) FVIII/VWF-D'D3-fusion variant (efanesotocog alpha)
Usually on demand, seldom long-term prophylaxis	On demand in all patients, and severe and moderate patients often receive long-term prophylaxis
None	Monoclonal antibodies against TFPI (concizumab, marstacimab) Anti-thrombin siRNA (fitusiran) AAV-based gene therapy

OUTLINE OF THE THESIS

In this thesis, we aim to provide tools for improving treatment in individuals with VWD by individualization of therapy by applying population PK modelling. First, current treatment strategies are evaluated and unmet needs are described. Subsequently, alternative dosing strategies of currently available medication using population PK modelling will be investigated. Lastly, future research perspectives and opportunities for innovation are discussed.

The thesis consists of three parts:

In **Part I**, current treatment in VWD is evaluated and unmet needs are discussed, which may be improved by individualization of therapy.

In **Chapter 2**, current management of VWD is reviewed. In **Chapter 3**, the aim is to predict desmopressin responsiveness based on VWF and FVIII response measurements during desmopressin testing. The goal is to reduce the amount of desmopressin tests required, lowering the testing burden for patients and health care providers, and thereby reducing costs. In **Chapter 4** current perioperative treatment with the most used VWF-containing concentrate in the Netherlands (Haemate® P) is analyzed, to provide information on the effectiveness of the current body weight-dependent dosing regimen and to gather data for the construction of population PK models.

In **Part II**, the construction of several population PK models for VWF-containing concentrates and desmopressin, based on retrospective, real-life patient data is described.

In the pharmacodynamic study in **Chapter 5**, the aim is to investigate and assess the relationship between desmopressin concentration and VWF in type 1 VWD patients, and to model the feasibility of capped dosing, i.e., giving all patients within the same weight range the same desmopressin dose, and to analyze if this will lead to an equal response rate compared to weight-based dosing. The aim in **Chapter 6** is to construct and describe a population PK model of VWF:Act in VWD after administration of desmopressin. The development of two different PK models for a specific FVIII/VWF concentrate (Haemate P) in VWF patients undergoing a medical procedure are reported in **Chapter 7 and Chapter 8**. In the first model, FVIII PK is analyzed, whereas in the second model, we include the complex interaction between VWF and FVIII. Aim of these studies is to facilitate better targeting of VWF and FVIII levels, and to gain further insight into the pathophysiological mechanisms underlying VWD.

In **Part III**, future studies and perspectives are presented. In **Chapter 9**, a protocol is presented for studying efficacy and feasibility of PK-guided dosing of desmopressin and VWF-containing concentrates in VWD and its implementation in clinical practice. Novel laboratory tests and methods may help to improve diagnosis and treatment of VWD in the future. In **Chapter 10**, the release of VWF propeptide (VWFpp) - a measure of VWF synthesis - in patients with either VWD or hemophilia A after desmopressin is studied. Analyzing VWFpp may help to distinguish between VWD patients with impaired synthesis and increased clearance of VWD. Furthermore, as VWD is a heterogeneous disease due to many possible genetic variants and mutations in the VWF gene, we aim to explain differences in desmopressin response by the presence and type of genetic variants in **Chapter 11**.

Finally, the results of the studies in this thesis are discussed in **Chapter 12**.

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Part I

Current treatment in VWD


2

Chapter 2

Current and emerging options for the management of von Willebrand disease

Jessica M. Heijdra, Marjon H. Cnossen, Frank W.G. Leebeek

Drugs. 2017 Sep;77(14):1531-1547

ABSTRACT

Von Willebrand disease (VWD) is the most common inherited bleeding disorder with an estimated prevalence of ~1% and clinically relevant bleeding symptoms in approximately 1:10.000 individuals. VWD is caused by a deficiency and/or defect of von Willebrand factor (VWF). The most common symptoms are mucocutaneous bleeding, hematomas and bleeding after trauma or surgery. For decades, treatment to prevent or treat bleeding has consisted of desmopressin in milder cases and of replacement therapy with plasma derived concentrates containing VWF and Factor VIII (FVIII) in more severe cases. Both are usually combined with supportive therapy, e.g. antifibrinolytic agents, and maximal hemostatic measures.

Several developments such as the first recombinant VWF concentrate, which has been recently licensed for VWD, will make a more "personalized" approach to VWD management possible. As research on new treatment strategies for established therapies, such as population pharmacokinetic-guided dosing of clotting factor concentrates, and novel treatment modalities such as aptamers and gene therapy are ongoing, it is likely that the horizon to tailor therapy to the individual patients' needs will be extended. Thus, further improving the already high standard of care in VWD in most high-resource countries.

INTRODUCTION

Von Willebrand disease (VWD) is the most common inherited bleeding disorder with an estimated prevalence of ~1% (1). Clinically relevant bleeding symptoms are present in approximately 1:10.000 individual (2). Von Willebrand disease is caused by a quantitative and/or qualitative defect of von Willebrand factor (VWF).

Function of VWF

VWF plays an important role in primary hemostasis. It circulates in the plasma in a globular, inactive form. When vascular damage occurs, VWF binds to the exposed vascular subendothelial collagen and uncoils. Once VWF is uncoiled, the binding site for platelet glycoprotein Ibα on the VWF A1 domain becomes exposed, allowing platelets to bind (3). Concomitantly, platelets also bind to vascular collagen. After activation by thrombin and other agonists, platelets undergo shape changes and platelet integrin αIIbβ3 (the GPIIb-IIIa complex) becomes able to bind VWF with high affinity, but also fibrinogen and fibronectin, leading to subsequent platelet aggregation (4).

Pathophysiological mechanisms in VWD

The function of VWF and pathophysiology of VWD is better understood if the different phases of VWF- synthesis, -secretion and -clearance are regarded.

Synthesis of VWF

VWF is synthesized in endothelial cells and megakaryocytes. The protein pre-pro-VWF is produced after primary translation and glycosylation of mRNA by ribosomes in the endoplasmic reticulum of endothelial cells and megakaryocytes. This protein includes a signal peptide, a large propeptide and the mature VWF subunit, which is composed of several structural domains, named A to D. After cleavage of the signal peptide, the VWF subunits dimerize and are transported into the Golgi apparatus, where disulfide bridges are formed between the D3 domains. This leads to formation of VWF multimers. The propeptide is subsequently cleaved but remains noncovalently bound to the forming VWF multimer, facilitating the disulfide bond formation. These ultra large VWF multimers are the most hemostatically potent multimers (5).

Secretion of VWF

After synthesis, up to 95% of VWF is secreted constitutively into the circulation, whereas the remainder is stored in Weibel-Palade bodies in the endothelium, and in platelet α-granules (6). Adrenergic stress, thrombin generation or treatment with desmopressin (DDAVP) stimulates the release of stored VWF (7). After secretion, the ultra large multimers are proteolyzed by ADAMTS13 -a disintegrin and

metalloproteinase with a thrombospondin type 1 motif, member 13-, into smaller multimers that circulate in plasma (8).

Clearance of VWF

After secretion of VWF into the circulation, the survival of the VWF multimers depends on their size, interaction with platelets and other cells, susceptibility to proteolysis, and the rate of clearance from the circulation (9). These mechanisms of VWF clearance are not yet fully understood. Abnormal clearance of VWF may also contribute to the pathogenesis of VWD, as several gene mutations have been identified that are specifically associated with increased clearance of endogenous VWF (10).

Epidemiology and diagnosis

Patients are diagnosed based on a personal- or family history of bleeding and laboratory abnormalities in VWF, Factor VIII (FVIII), or both. VWD is classified into three types. Type 1, which accounts for 70-80% of cases, is a partial quantitative deficiency of von Willebrand factor due to either reduced production and/or secretion, or increased clearance of VWF. Type 2, which accounts for approximately 20% of cases, includes several qualitative defects of VWF defined as subtypes 2A, 2B, 2M and 2N. Type 3 (accounting for <5% of cases) is defined as a virtually complete absence of VWF, making this the most severe type of von Willebrand disease (Table 1) (9).

Table 1. Von Willebrand Disease classification according to the International Society on Thrombosis and Hemostasis (25)

Type of VWD	Description	% of total VWD population
1	Partial quantitative deficiency of VWF	70-80%
2	Qualitative VWF defects	~20%
2A	Decreased VWF-dependent platelet adhesion and a deficiency of high-molecular-weight VWF multimers	
2B	Increased affinity for platelet glycoprotein Ib (GpIb) and a deficiency of high-molecular-weight VWF multimers	
2M	Decreased VWF-dependent platelet adhesion without a deficiency of high-molecular weight VWF multimers	
2N	Markedly decreased binding affinity for FVIII	
3	Virtually complete deficiency of VWF	<5%

To systematically quantify the bleeding symptoms in an individual, bleeding scores may be a helpful diagnostic tool (11). Different bleeding questionnaires have been developed over the years, but since 2010, the International Society for Thrombosis and Hemostasis Bleeding Assessment Tool (ISTH-BAT), intended for use in both adults and children, is recommended (12). This questionnaire scores 14 different bleeding symptoms on a scale of 0-4. The values for an abnormal bleeding score are ≥3 in children, ≥4 for adult males and ≥6 for adult females (13). A limitation of the score is that it is a cumulative score, which means that the score is age dependent, can be saturated and that bleedings in the past may reveal a high bleeding score, which may not reflect the current bleeding phenotype.

Key measurements in the evaluation of VWD include VWF Ristocetin Cofactor (VWF:RCo), which measures the ability of VWF to interact with platelets; VWF antigen (VWF:Ag), as a measure of the total amount of VWF; and FVIII, which reflects the ability of VWF to chaperone FVIII through the circulation. According to most guidelines, in order to establish a definite diagnosis of type 1 VWD, a patient requires VWF:RCo levels <0.30 IU/ml and a ratio of VWF:RCo to VWF antigen (VWF:Ag) >0.6. Patients with a bleeding tendency and VWF:RCo levels of 0.30-0.50 IU/ml are regarded to as individuals with 'low VWF levels'; which are considered a risk factor for bleeding (5). Patients with type 2 VWD have VWF:RCo levels <0.30 IU/ml and a VWF:RCo to VWF:Ag ratio or a FVIII to VWF:Ag ratio of ≤0.60. Type 2N, which is characterized by reduced binding of FVIII to VWF, is characterized by low FVIII levels and a reduced FVIII to VWF:Ag ratio. A patient is diagnosed with type 3 VWD when VWF:Ag is <0.05 IU/ml.

Clinical presentation and complications

The most common symptoms in von Willebrand disease patients are mucocutaneous bleedings, such as epistaxis (~50%), oral cavity bleeding (~60%), hematomas and bleeding from minor wounds (~80%). In women, menorrhagia is often present, eventually leading to iron-deficiency anemia necessitating iron administration or blood transfusions in some cases (14). The risk of severe post-partum hemorrhage is increased, especially in women with low factor levels in the third trimester. This risk remains higher than in healthy women, despite specialized treatment (15). One of the most difficult complications to manage is gastrointestinal bleeding, which occurs mainly in elderly type 2A and type 3 VWD patients. Most commonly, gastrointestinal bleeding is caused by angiodysplasia, although it is difficult to establish this diagnosis. Joint- and muscle bleeding are rare, although these may be underestimated complications, as in a recent study 23% of moderate and severe VWD patients reported joint bleeding (16). Bleeds in joints and muscles are explained by the fact that VWF functions as chaperone protein of FVIII, protecting FVIII from proteolysis in the circulation. Therefore, severe deficiency of VWF causes a concomitant deficiency of

FVIII. Patients with both a severe VWF- and FVIII deficiency may present with joint bleeds, which are more typical for hemophilia and may cause long-term impairment.

Bleeding symptoms leading to a diagnosis of VWD often present peri- or postoperatively or after dental procedures in index patients. When this occurs, a family history should be taken subsequently and a hemostatic work-up should be performed evaluating both the primary and secondary hemostasis, and including laboratory evaluation of VWF- and FVIII levels in order to identify hemostatic abnormalities (12).

Inheritance and molecular genetics

There is a large variation in mutations described in VWD. Quantitative deficiencies of VWF as observed in severe type 1 and type 3 are mostly caused by null alleles (large gene deletions, stop codons, frameshift mutations, or splice-site mutations), but may also be caused by mutations in the promotor regions of the VWF gene (17, 18). Type 3 patients are usually homozygous or compound heterozygous for these defects (19). Type 1 VWD is mostly caused by heterozygous missense mutations (17, 20, 21). However, in approximately 30% of type 1 VWD patients, no mutations in the VWF gene are identified (20, 21). Type 2 VWD is characterized by missense mutations, which are located in the affected functional domain. The inheritance of subtypes 2A, 2B and 2M is autosomal dominant. Type 2N VWD has a recessive inheritance pattern and is caused by homozygosity for two type 2N mutations, or compound heterozygosity, with a type 1 defect and a type 2N defect (22).

Variation in VWF levels

It is well known that even in individuals with similar gene mutations, plasma VWF levels show a large intra- and interindividual variability. A major determinant of interindividual variation in von Willebrand factor levels is ABO blood group, as VWF plasma levels are approximately 25% lower in individuals with blood group O, when compared to non-O individuals (23). In these individuals, an increased clearance is described, possibly regulated by the ABO blood group antigens on N-linked oligosaccharides of VWF (24).

Genome wide association studies (GWAS) have also identified several other genetic loci that are associated with VWF levels in healthy individuals. Mutations or polymorphisms in these loci may explain variability in VWF levels between individuals with VWD but also the varying bleeding phenotype in patients without variations or mutations in the gene coding for VWF. C-type lectin domain family 4 member M (CLEC4M) and Lipoprotein Receptor 1 (LRP1) have been associated with VWF clearance (25, 26), and Syntaxin Binding Protein 5 (STXBP5) seems to affect VWF exocytosis (27).

New candidate genes for VWF levels found in GWAS include SCARA5, STAB2, STX2, TC2N and UFM1 (27, 28). Recently, a linkage analysis identified a highly significant quantitative trait locus (QTL) on chromosome 2, that was not detected earlier by large GWAS. The effect size on VWF variation of this locus was comparable to the effect of the ABO locus (19.2% vs. 24.5%) (29). The effect mechanism of this genetic variant has not yet been elucidated.

Besides these endogenous factors (blood group, gene mutations and modifying genetic loci), many exogenous factors have been identified that clearly influence VWF levels; such as physical exercise, stress, inflammation, hypertension, diabetes, hormones and pregnancy (30, 33). Moreover, VWF levels also increase with age, possibly explained by increasing arterial rigidity over time (34, 35). All these different factors lead to challenges in establishing normal and abnormal VWF levels in individuals with and without a clinically significant bleeding phenotype.

Current treatment options

In this review article, we aim to give an overview of current treatment in congenital VWD. Firstly, we will discuss treatment for acute bleeding events and how to prevent bleeding during surgical- and dental procedures ("on demand" treatment). Secondly, we will discuss "prophylactic" treatment which aims to prevent spontaneous bleeding in VWD patients who experience frequent and severe bleeding. Furthermore, we will elaborate on novel developments and future perspectives with regard to treatment of this frequently diagnosed bleeding disorder.

Goal of treatment in VWD patients is to stop or prevent bleeding by increasing plasma VWF- and FVIII levels to adequate hemostatic levels by stimulation of the release of endogenous VWF by administration of DDAVP, or by infusing VWF-containing factor concentrates. Choice of treatment is dependent on the type of disease and the severity of the bleeding. A multidisciplinary approach involving a (pediatric) hematologist and other specialists, such as (orthopedic) surgeon, gynecologist, anesthesiologist or clinical geneticist of course dependent on the specific hemostatic challenge and situation, is of great importance to provide optimal care for the individual VWD patient.

Desmopressin (DDAVP)

Desmopressin (1-deamino-8-d-arginine vasopressin, DDAVP) is a synthetic vasopressin analogue. The drug increases VWF and FVIII plasma levels by releasing

VWF from Weibel-Palade bodies in the endothelium (7). Due to this effect, it is the most widely used drug in the treatment of VWD.

DDAVP can be administered intravenously or subcutaneously at a standardized dose of 0.3 µg/kg every 12-24 hours. Although some groups have suggested the use of a capped dose of 15 or 20 µg, further research is warranted to prove the effectiveness of this concept (36, 37). DDAVP is also available as an intranasal spray, often used for home treatment in case of bleeding. Intranasal dosing is 150 µg (1 puff) in patients <50 kg or 300 µg (2 puffs) in patients ≥50 kg. Due to variable adsorption in case of intranasal administration, increase in VWF and FVIII may be lower than after intravenous or subcutaneous administration (38).

Interindividual response to DDAVP differs greatly. Most type 1 VWD patients respond well to DDAVP. In type 2 VWD, responsiveness to DDAVP varies significantly and is difficult to predict. Understandably, type 3 VWD patients are unresponsive as they have little to no endogenous VWF to mobilize. Individual characteristics such as VWF gene mutation and baseline VWF:Ag and VWF:RCo levels have been reported to influence the increase of VWF and FVIII plasma levels and duration of response (39). In general, response in the individual patient has been proven to be reproducible and consistent over time (40). However, it is important to realize that DDAVP response decreases when DDAVP is administered sequentially at short intervals (tachyphylaxis), due to depletion of VWF storage in the endothelium (41).

Due to the great interpatient variability in response, a DDAVP test is required to establish DDAVP response in each individual patient. Different protocols dictate different blood sampling regimens, but there is general agreement that plasma levels of VWF and FVIII should be measured prior to, and at least 1 (peak level) and 4 hours after DDAVP infusion. According to most investigators, a patient is defined as responsive to DDAVP when VWF and FVIII levels increase at least two- to threefold and VWF and FVIII levels are >0.30 IU/ml 30-90 minutes after DDAVP administration (42, 43). In most patients with rapid clearance of VWF, the initial response to DDAVP is substantial. However, VWF and FVIII levels may decrease to inadequate levels within several hours when half-life of VWF and FVIII is short (44). Therefore, whether DDAVP is an adequate treatment option is dependent on both type and severity of the bleeding or surgical procedure as well as on the initial response and duration of response.In individuals with type 2B von Willebrand disease, DDAVP treatment is contra-indicated because of aggravation of the tendency towards thrombocytopenia (45).

DDAVP is considered safe but may have mild side effects, such as flushing, transient headache or hypotension (46). To prevent occurrence of more severe side effects such as hyponatremia and cardiovascular events, fluid intake should be restricted to 1500 ml during the first 24 hours after administration of the drug. This applies for pediatric patients eligible for DDAVP with a body weight >20 kilograms and adult patients without adjusting for body weight. Due to the risk of side effects, in very young (<4 years) and older (>70 years) patients desmopressin should be used with caution (47). The use of DDAVP in pregnant women also remains controversial due to the lack of evidence of safety and efficacy in this group. Several cases have been reported, describing complications such as hyponatremia, pre-term delivery and uterine contractions in this patient group (48).

Plasma derived factor concentrates

Until the 1980's, patients unresponsive to DDAVP were usually treated with cryoprecipitate. The emergence of virally-inactivated FVIII concentrates containing VWF for the treatment of hemophilia A proved a more optimal therapeutic option for patients with VWD.

Eligible for treatment with plasma derived factor concentrates are type 3 VWD patients who do not produce any endogenous VWF, and type 2B VWD patients in whom DDAVP can cause thrombocytopenia. Furthermore, factor concentrates are used in patients with type 1 and type 2 VWD who are insufficiently responsive to DDAVP, or patients with contraindications for DDAVP therapy. VWF/FVIII concentrates can be administered in case of bleeding or surgery, but also as prophylaxis in severe VWD patients with recurrent spontaneous bleeding, including joint bleeds, gastro-intestinal bleeds in the elderly and severe epistaxis in children.

Nowadays, several plasma derived, virally inactivated factor concentrates containing VWF and FVIII are licensed for treatment of VWD. However, the availability of replacement therapy for bleeding disorders in general is strongly dependent on the economic situation and health care organization in countries. Because in most severe cases, VWF- as well as FVIII levels are decreased, both factors often require substitution. The different available products contain different ratios of VWF and FVIII, with differences in specific activity (Table 2) (49-51). Therefore, before treating a patient with a VWF/FVIII concentrate, the specific activity and the VWF:RCo/VWF:Ag and VWF:RCo/FVIII ratios should be considered.

For on demand treatment, calculation of the required dose of VWF or FVIII is based on the empirical finding that 1 IU VWF:RCo per kilogram body weight raises VWF with ~1.5% and 1 IU FVIII:C per kilogram raises FVIII plasma level by ~2%. Dosing is

based on both VWF:RCo and FVIII:C levels. The aim is to increase or normalize both factor levels in order to ensure adequate hemostasis. The more recent applicability of quickly available results of VWF:RCo or VWF:GPIbM assays has greatly improved and facilitated VWF/FVIII concentrate dosing (52).

In case of treatment with bolus infusions, the required dose is determined using the following formula: Required IU of VWF concentrate (based on VWF:RCo content) = body weight (kg) x desired VWF:RCo rise (%) (IU/dl) / 1.5. In case of continuous infusion, the initial infusion rate is calculated as follows: Infusion rate (IU/kg/h) = clearance (ml/kg/h) x desired steady state level (IU/ml). Continuous infusion is feasible as in a study by Lubetsky et al., it was described that reconstituted Humate P was stable for 14 days at room temperature (53).

Table 2. Von Willebrand factor-containing concentrates for the treatment of von Willebrand disease tested in prospective clinical studies

Product	Manufacturer	Preparation	Purification	Viral inactivation	Ratio VWF:RCo/ VWF:Ag[a]	Ratio VWF:RCo/ FVIII[a]
Alphanate	Grifols	PD	Heparin ligand chromatography	S/D + dry heat	0.47 ± 0.1	0.91 ± 0.2
Factor 8Y	Bioproducts Laboratory	PD	Heparin/glycine precipitation	S/D + dry heat	0.29	0.81
Fanhdi	Grifols	PD	Heparin ligand chromatography	S/D + dry heat	0.47 ± 0.1	1.04 ± 0.1
Humate-P (US) Haemate P (EU)	CSL Behring	PD	Multiple precipitation	Pasteurization	0.59 ± 0.1	2.45 ± 0.3
Immunate	Shire	PD	Ion-exchange chromatography	S/D + vapor heat	0.47	1.1
Koate-DVI	Kedrion Biopharma	PD	Multiple precipitation + size exclusion chromatography	S/D + dry heat	0.48	1.1
Voncento	CSL Behring	PD	Heparin/glycine precipitation + gel filtration chromatography	S/D+ dry heat	0.87 - 0.95	2.0
Vonvendi	Shire	Rec	-	-	>1	>10
Wilate	Octapharma	PD	Ion-exchange + size exclusion chromatography	S/D + dry heat	-	0.9
Wilfactin	LFB	PD	Ion-exchange + affinity chromatography	S/D + nanofiltration + dry heat	0.7	60

PD: plasma derived; Rec: recombinant; S/D: solvent detergent. [a]*Data derived from (49-51)*

In principle, the endogenous FVIII synthesis in VWD patients is normal. The low FVIII plasma concentrations are the result of low von Willebrand factor and/or decreased binding affinity of VWF for FVIII. When exogenous VWF is infused, it binds and stabilizes FVIII; thereby increasing the FVIII plasma level. Furthermore, clearance of FVIII is known to be lower than that of VWF (54). Subsequently, VWF/FVIII concentrate infusions in a short time period may lead to very high FVIII:C plasma concentrations (>270 IU/dl), and thus form a possible risk factor for thromboembolic complications (55-57). Therefore, daily measurements of plasma FVIII levels after surgery are important in patients receiving repeated doses of VWF/FVIII concentrate not only to assess the risk of bleeding but also to monitor the risk of thrombosis. Terminal half-life of VWF:Ag and VWF:RCo differs greatly between patients (58). Monitoring of VWF:RCo levels intraoperatively and during the first postoperative days is important to determine timing and dosing of the follow-up bolus infusions to ensure hemostatically adequate levels of VWF in the first phases of wound healing (Table 3) (1, 14, 52, 59). For a treatment algorithm for bleeding and dental- and surgical procedures with VWF/FVIII concentrates according to the National Heart, Lung and Blood Institute (NHLBI), see Fig. 1.

In theory, a product with a VWF/FVIII ratio of approximately 1:1 is the easiest to dose, because the rise of VWF and FVIII plasma levels after infusion can be easily predicted (60, 61). However, although no randomized trials have been performed, all different products with different ratios show good to excellent hemostatic properties in observational clinical studies (62, 63). Moreover, there is broad clinical experience in treatment of VWD patients with Humate-P® -the first virus-inactivated VWF/FVIII concentrate- , which has been on the market for more than 30 years (64).

The rationale for treatment with highly purified concentrates containing nearly no FVIII or treatment with recombinant VWF, is that patients with von Willebrand disease all have normal production of FVIII, but lack adequate VWF levels to protect FVIII from degradation. When VWF levels are normalized by infusion of exogenous VWF, a subsequent rise of endogenous FVIII is expected. However, the rise of FVIII after infusion is slow and a peak is achieved only after 6 to 8 hours (65). Therefore, patients with low circulating FVIII levels require a priming dose of FVIII in addition to the VWF concentrate when hemostasis needs to be corrected promptly. In case of elective surgery, a VWF concentrate infusion should be administered at least 6-8 hours before the operation, to allow FVIII to rise to adequate levels in time for the procedure when no additional FVIII is administered.

Table 3. Recommendations for FVIII and VWF target levels in minor and major surgical – and dental procedures according to a selection of guidelines

	Minor procedures			Major procedures		
Guideline	FVIII target levels (IU/ml)	VWF:RCo taret levels (IU/ml)	Duration (days)	FVIII target levels (IU/ml)	VWF:RCo target levels (IU/l)	Duration (days)
NHLBI	nd	>1.00	perioperative	nd	>1.00	perioperative
(US) (19)	>0.50	>0.50	3-5	>0.50	>0.50	7-14
AICE (Italy) (70)	>0.30	nd	2-4	>0.50	nd	5-10
NVHB (the	>0.80	>0.80	perioperative	>0.80	>0.80	perioperative
Netherlands)	>0.50	nd	3	>0.50	nd	7-10
(30)	>0.30	nd	4-7			
UKHCDO	>0.50	>0.50	nd	≥1.00	nd	perioperative
(UK)(63)				>0.50	>0.50	6-10

NHLBI: National Heart, Lung and Blood Institute; AICE: The Italian Association of Hemophilia Treatment Centers; NVHB: Dutch Society for Hemophilia Treaters; UKHCDO: United Kingdom Haemophilia Centre Doctors' Organisation. Nd: not defined in guidelines.

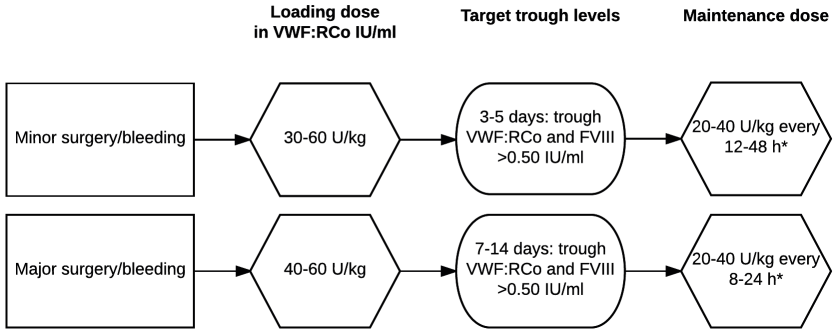

Figure 1. Treatment of bleeding and dental- and surgical procedures with VWF/FVIII concentrate according to National Heart, Lung and Blood Institute (NHLBI) (1)

Treatment in patients with alloantibodies

Alloantibodies against VWF are a rare complication in VWD, with an estimated prevalence of 6-10% in type 3 VWD patients (66, 67). Almost all cases occur in type 3 VWD patients with partial or complete VWF gene deletions (68, 69), although a case of alloantibodies in a type 2B VWD patient has recently been described (70).

Patients with antibodies against VWF generally present with impaired response to infused VWF-containing concentrates . When re-exposed to VWF, some patients –especially those with high-titer alloantibodies- may develop severe anaphylactic reactions (71, 72). Recombinant FVIII has been used successfully for hemostatic therapy in patients with anti-VWF antibodies. Due to the lack of stabilization by VWF, the half-life of FVIII is

decreased. This problem can be overcome by continuous infusion of higher doses of FVIII concentrate (67). Another option is treatment with recombinant Factor VIIa (rFVIIa) or activated prothrombin complex, which function as a FVIII and Factor IX (FIX) bypassing agent (73-75). These are regularly used to treat hemophilia patients with inhibiting antibodies. To extrapolate this experience to VWD patients with inhibiting antibodies seems reasonable, but sparse evidence for effectiveness and safety of rFVIIa treatment in patients with anti-VWF antibodies requires caution with regard to these products.

Immune tolerance induction (ITI) therapy, using high doses of factor concentrates and immunosuppressive therapy, is widely applied in hemophilia A. A case report of a 20-year old male with alloantibodies to VWF, treated with ITI was published in 2012. After 3 years of ITI treatment, inhibiting antibodies could still not be detected anymore, but half-life of VWF containing concentrates did not normalize (76). Therefore, on the basis of this sporadic evidence, more research is required to assess safety and efficacy of ITI in VWD patients, especially as anaphylactic reactions may occur in this setting.

Recombinant VWF concentrate

For patients with hemophilia, recombinant coagulation factor concentrates have been available for nearly two decades. These products reduce the transfer risk of viral infections and potentially other infectious agents. Another advantage is the independence of donor availability for the supply of plasma-derived concentrates. For VWD, Turecek and coworkers have recently developed a recombinant VWF (Vonicog alfa, rVWF), which is produced in genetically altered CHO cells expressing both VWF and FVIII (77). As VWF is synthesized in the absence of the VWF protease ADAMTS13, this rVWF contains intact high molecular weight- and ultra large multimers, resulting in a higher specific activity (ratio VWF:RCo:VWF:Ag >1.0) than in plasma-derived VWF concentrate. In 2013 Mannucci et al. reported a phase 1 trial to study the pharmacokinetic parameters of this product. The terminal half-life of of rVWF was comparable to that of plasma derived VWF (78). In a recent phase 3 clinical study on the treatment of bleeding episodes in patients with severe type1, 2 or 3 VWD, rVWF showed a high efficacy in cessation of bleeding (79). The first dose of rVWF was administered together with rFVIII and subsequently without rFVIII. The outcome of treatment was rated as excellent in over 96% of bleeding episodes. Additional pharmacokinetic studies showed that FVIII normalized after sole infusion of rVWF within 6 hours. Treatment was considered safe, as no thrombosis, allergic reactions or development of inhibitors to rVWF were demonstrated. Currently, studies are ongoing on the efficacy of rVWF in surgery, as well as studies on the use of long-term prophylaxis with rVWF concentrate in patients suffering from recurrent bleeding. Recently rVWF has been registered and approved for clinical use in the USA for the treatment of bleedings in adults with VWD.

Supportive treatments

Antifibrinolytic agents

Antifibrinolytic agents, such as tranexamic acid and aminocaproic acid, inhibit the interaction of plasminogen with fibrin, thus preventing the degradation of the fibrin clot. These agents are especially effective in the mucosa due to the high fibrinolytic activity present in these tissues (80). Therefore, in case of mucocutaneous bleeding, supportive treatment with antifibrinolytics is strongly recommended in the light of the low cost and few side effects. The hemostatic effectiveness of tranexamic acid has also been demonstrated in large placebo-controlled randomized trials in patients undergoing high-risk cardiac- or orthopedic surgery (81-83). In patients with bleeding disorders undergoing surgical- or dental procedures, antifibrinolytics are widely used to prevent perioperative blood loss. Although evidence from randomized controls is lacking for efficacy in VWD, this is generally accepted to be likely (84).

Antifibrinolytic agents can be administered systemically as an oral or intravenous formulation, or topically, as a mouthwash (for available formulations, concentrations and dose see Table 4). Importantly, hematuria of unknown origin or caused by renal or ureteral bleeding is a contraindication for antifibrinolytic treatment as treatment of blood loss in the urinary tract may lead to clotting in the ureters and subsequent painful colic episodes with risk of ureter obstruction(85, 86).

Table 4. Antifibrinolytic agents for the treatment of von Willebrand disease

Formulation	Available concentration	Dose[a]
Tranexamic acid intravenous	10 mg/ml	0.5-1 g, 2-3x daily (1 ml/min) Children ≥ 1 year: 20 mg/kg/day in 2-3 doses a day
Tranexamic acid oral	650 mg (US) / 500 mg (EU)	0.5-1 g in 2-4 doses a day Children ≥ 1 year: 20 mg/kg/day, in 2-3 doses a day
Tranexamic acid mouth rinse	50 mg/ml	0.5-1.5 g (15-25 mg/kg), in 2-3 doses a day "swish and swallow or spit" Children ≥ 1 year: 20 mg/kg/day, in 2-3 doses a day
Aminocaproic acid intravenous	250 mg/ml	Starting dose: 4-5 g slowly during the first hour, followed by continuous infusion of 1 g/hr Children: 100 mg/kg or 3 g/m^2 slowly (> 1 hr), followed by continuous infusion of 33.3 mg/kg/hr or 1 g/m2/hr
Aminocaproic acid oral	500 mg and 1000 mg	Starting dose: 4-5 g, followed by 1-1.25 g/hr or 4-6 g every 4-6 hours, with a max. dose of 24 g/day Children: starting 100 mg/kg, followed by 3 g/m^2 during the first hour, followed by 33.3 mg/kg or 1 g/m^2 every hour. Max. dose: 18 g/m^2/day or 600 mg/kg/day
Aminocaproic acid mouth rinse	250 mg/ml	Starting dose: 4-5 g, followed by 1-1.25 g/hr, with a max. dose of 24 g/day "swish and swallow or spit" Children: starting 100 mg/kg, followed by 3 g/m^2 during the first hour, followed by 33.3 mg/kg or 1 g/m^2 every hour. Max. dose: 18 g/m^2/day or 600 mg/kg/day

[a] *Data derived from (84)*

Hormonal treatment

Menorrhagia is a very common symptom in women with VWD, with a prevalence of 62-81% (87-89). In women with VWD presenting with menorrhagia, it is important to first rule out anatomic and hormonal causes. Thereafter, hormonal treatment with oral contraceptives containing both progestin and estrogen can be initiated if there is no wish for pregnancy. Oral contraceptive treatment leading to non-ovulatory bleeding will significantly reduce uterine blood loss during the oral contraception-free week. When administered continuously (≥28 days), total bleeding days can be reduced drastically (90).

Another hormonal treatment option is the levonorgestrel intrauterine device (IUD). This device suppresses endometrium- and spiral arteriole growth and increases capillary thrombosis. It also has no effect on endometrial FVIII activity, while copper-containing intrauterine devices have been described to decrease FVIII activity (91). In a study in 16 women with bleeding disorders receiving a levonorgestrel IUD, nine women became amenorrhoeic, and the remaining seven reported a significant decrease in menstrual blood loss (92). Bleeding complications did not occur at the time of insertion of the device, in the presence of adequate hemostatic- or replacement therapy.

In women with menorrhagia, often a combination of antifibrinolytic and hormonal therapy is used. Despite the fact that the combination of tranexamic acid and oral contraceptives may be pro-thrombotic, no reports of thrombo-embolic events in women with VWD using this combination of medication have been reported. Therefore, it is assumed that a combination of antifibrinolytic and hormonal therapy is safe (93).For all treatment options in women with menorrhagia, see the treatment algorithm in Fig. 2 (94).

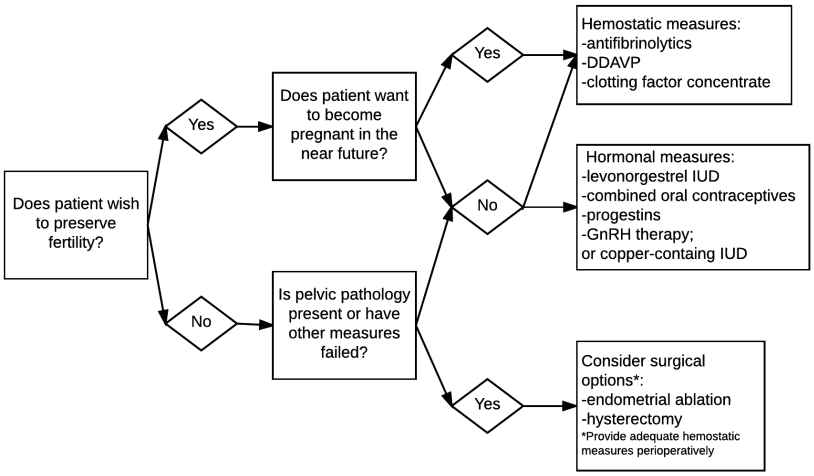

Figure 2. Treatment of menorrhagia (94)

Additional measures

In case of epistaxis, xylometazoline nose drops can be applied intranasally to induce vasoconstriction. When the active bleeding focus can be identified, chemical or electrical cauterization performed by an emergency- or ear-, nose- and throat physician is the preferred method of treatment (95). In addition, to prevent bleeding after surgical- or dental procedures, secure suturing is important to achieve local hemostasis. Additional measures for wound sealing and promotion of wound healing include application of (autologous) fibrin glue or platelet-rich clots (96, 97).

Management of pregnancy and delivery

In case of pregnancy in a VWD patient, a hematologist should be consulted in the first trimester in order to coordinate treatment for pregnancy and delivery if necessary. In VWD type 1 and 2, VWF:Ag, VWF:RCo and FVIII:C should be monitored at 12 weeks and 30-34 weeks. If VWF and FVIII levels are inadequate (<0.50 U/ml) at 30-34 weeks, a multidisciplinary team consisting of a hematologist, pediatric hematologist, gynecologist and anesthesiologist with expertise in bleeding disorders should establish a treatment plan which includes timing and dosing of factor concentrate and/or antifibrinolytic agent administration during childbed and hospitalization as well as mode of (regional) anesthesia and indication for atraumatic delivery. In case of post partum hemorrhage, factor concentrate should be administered, taking possible other obstetric causes of bleeding into account (98). When good responsiveness to DDAVP has been demonstrated prior to the event, DDAVP can be administered after clamping of the umbilical cord. Because of the decrease of VWF and FVIII to pre-existent levels after delivery (99), it is recommended to supply tranexamic acid 4 times a day 500-1000 mg or aminocaproic acid 4-6 g every 4-6 hours orally during the first seven days post partum.

Generally, in type 1 VWD, VWF- and FVIII levels generally rise to relatively normal values in the third trimester and maternal problems are not expected during delivery. Historically, guidelines consistently advised to aim for target levels of VWF and FVIII >0.50 U/ml before delivery (1). However, Szecsi et al. reported that in normal pregnancies FVIII:C at 38-42 weeks was 130-430% (n=73) (100). This fact, combined with the observation that the risk of post-partum hemorrhage despite specialized care is greater in women with VWD, it is likely that women may be 'undertreated' currently at time of delivery (15, 98).

To determine the indication for an atraumatic delivery, invasive prenatal diagnostic procedures can be performed in week 33-34 of the pregnancy if the causative VWF gene mutation is known. If maternal FVIII and/or VWF is <0.50 IU/ml, treatment with factor concentrate is indicated during such procedures. When a child with (potentially) type 3

VWD or a clinically severe type 1 or 2 VWD may be born, an atraumatic delivery should be pursued. Vaginal delivery is usually preferred. Only in cases of severe emergency, a forceps should be performed (no vacuum extraction) and no vaginal breech delivery and no expulsion for >1 hour are to be allowed. A caesarean section should be performed without hesitation under adequate replacement therapy when complications are expected. Fetal scalp blood testing and placement of a fetal scalp electrode should be avoided. VWF and FVIII cord blood analysis to diagnose the newborn is only indicated in severe type 1 and 2 and in type 3 VWD. Due to relatively high VWF- and FVIII levels directly after birth due to activation in cord blood, measurement of VWF and FVIII in milder cases should be repeated a few weeks after birth if results are dubious (101). Intramuscular injections in the neonate should be avoided or replaced by subcutaneous injection as long as VWF- and FVIII levels are unknown. In case of a (possible) severe VWD (VWF <0.05 U/ml and/or FVIII <0.05 U/ml), observation of the neonate during the first 24 hours is indicated. Routine ultrasound screening is not recommended in neonates with type 3 VWD, but should be performed consequently and rapidly when additional bleeding risks are present or clinical symptoms suspect for intracranial bleeding are observed. When ultrasound is not acutely available, replacement therapy should be given prior to imaging when symptoms are most suspect.

Treatment of angiodysplasia-related gastrointestinal bleeding

Gastrointestinal bleeding is a common and sometimes life-threatening problem in VWD patients with a prevalence of 11-27%, depending on disease type (14). In patients lacking high-molecular weight (HMW) VWF multimers -as in VWD type 2A-, angiodysplastic lesions are often found to be causative (102). In vitro and in vivo studies have identified VWF as a regulator of angiogenesis through different pathways, although the exact mechanisms remain unclear (103). Identification of angiodysplasia is often difficult. The diagnostic approach starts with endoscopy, however in a substantial part of cases results are negative, especially when the lesions are small and not abundant. Additional methods include video capsule endoscopy, helical computed tomography and angiography. Often repetitive investigations are necessary to ultimately diagnose angiodysplasia (104). Therefore, it is important to repeat diagnostic procedures in VWD patients presenting with unexplained iron-deficient anemia or clinical symptoms of gastrointestinal bleeding, especially in those lacking HMW VWF multimers.

In most patients with congenital VWD, replacement therapy with factor concentrates is sufficient, but often seems to be less effective than in other types of bleeding (102, 105). Several other pharmacological therapies have been proposed for the often complex management of recurrent gastrointestinal bleeding due to angiodysplasia. A problem with these therapies is that effectiveness has only been described in small case

series and case reports, with variable results. It is also likely that there is a significant publication bias, as reports of successful pharmacologic treatment are more likely to be published than unsuccessful ones. As a consequence, these drugs have not yet been approved for treatment of angiodysplasia and are currently only prescribed off-label. Therefore, further studies on potentially effective pharmacological agents are required.

Octreotide

Treatment with octreotide, a somatostatin analogue, has shown a high efficacy and safety in studies involving non-VWD patients with chronic bleeding due to angiodysplasia. Regretfully, these studies included relatively small numbers of patients and differed strongly in drug dosage, route and duration of administration of the drug. Also, follow-up time was relatively short (106). In literature, three cases of VWD patients treated with octreotide are described. A case series of two VWD patients with massive and prolonged gastrointestinal bleeding resistant to conventional treatment was described by Bowers (107). A rise in baseline VWF:Act and hemoglobin was observed after initial intravenous administration and continued subcutaneous administration of octreotide and no hospital admissions were required during follow-up. In 2005, Krikis et al. reported on a case of a VWD patient with recurrent and life threatening gastrointestinal bleeding. This patient was treated with octreotide long-acting release (LAR) 20 mg by intramuscular injection once a month and propranolol 20 mg three times a day. During follow-up, the patient experienced no bleeding. Laboratory evaluations however showed no rise in VWF levels (108).

Thalidomide

Thalidomide inhibits angiogenesis by suppression of vascular endothelial growth factor (VEGF) (109), making it an interesting drug candidate for the treatment of angiodysplasia. In a literature review, Engelen et al. described 19 relevant publications on thalidomide use in angiodysplasia-related gastrointestinal bleeding (110). These articles included one randomized controlled trial, two prospective cohort studies, seven case series and 14 case reports. A total of 115 patients receiving thalidomide were described. Dosing ranged from 50 mg/day to 400 mg/day, with an average dose of 100 mg/day. In all studies, a beneficial effect of thalidomide was shown as in only 2 of the cases, thalidomide treatment had no effect on gastrointestinal bleeding. Four out of the total reported patients had congenital VWD. In all VWD patients, treatment with thalidomide was successful and bleeding episodes stopped. In one patient this effect was temporary and the dose needed to be increased. In 17 out of the total of 115 patients, thalidomide was withdrawn due to side effects. Although the results of thalidomide therapy are promising, severe side effects such as neurotoxicity and concerns on possible oncogenetic properties limit the use of thalidomide as long-term therapy.

Lenalidomide

Lenalidomide is a thalidomide analog, also with anti-angiogenic effect, but with a somewhat more favorable adverse effects pattern. A retrospective chart review of five VWD patients with angiodysplasia receiving lenalidomide was performed in 2013 by Kohli et al. (111). Patients received a starting dose of 5 mg daily. In one patient, it was necessary to increase dosage up to 15 mg daily due to recurrent gastrointestinal bleeding. Mean bleed-free duration was one year and the number of endoscopies was significantly lower after treatment. Fatigue was the most commonly reported side effect and one patient even discontinued treatment due to excessive fatigue.

Statins

Statins in high doses have been reported to inhibit angiogenesis (112). In 2008, Sohal and Laffan reported a severe type 1 VWD patient with refractory bleeding due to angiodysplasia in the gastrointestinal tract (113). Atorvastatin was administered at 10 mg daily, with dose escalation to 40 mg/day over the following three months. During six months of follow-up, bleeding gradually subsided and no side effects were reported. Following this report, Alikhan and Keeling reported on a type 2A VWD patient in whom 10 mg atorvastatin was commenced daily, increasing the dose to 40 mg daily over 4 months (114). A reduction in blood transfusions was observed. After a dose increase to 80 mg, the patient had not needed any blood transfusions or hospitalization over a follow-up period of 9 months. No side effects were reported.

Hormonal- and antihormonal therapy

Estrogen and progesterone have been investigated in a number of studies in non-VWD angiodysplasia patients. This mode of therapy has shown no beneficial effect and is no longer recommended in gastrointestinal angiodysplasia (115). In the recent years, tamoxifen has been identified as an effective therapy for the management of patients with hereditary hemorrhagic telangiectasia having recurrent bleeds (116). The counterintuitive benefits of an antiestrogen for treating telangiectasia were noted by coincidence. It is hypothesized that when estrogen binds to its receptors, it induces proliferation of the blood vessels, and thus telangiectatic lesions (117). A case report by Thachil on 2 VWD patients with angiodysplasia was published in 2013. In one patient, an immediate reduction of bleeding episodes was observed, and bleeding stopped completely after 3 months of tamoxifen treatment and persisted during the 14 month follow-up period. The other patient discontinued treatment after 4 months due to vaginal discharge. Six months after cessation of treatment in this patient, no further bleeds had occurred and no angiodysplastic lesions were observed during endoscopy.

Danazol

Danazol has been shown to increase FVIII levels and reduce bleeding frequency in hemophilia A patients (118). Other studies, however, could not reproduce these findings (119). Therefore, the effect is thought to likely be more at the endothelial level, rather than the result of increased coagulation factor levels. The only study of danazol in angiodysplasia has been performed by Botero et al. (120). Three VWD patients with refractory gastrointestinal bleeding were reported, receiving danazol 100-500 mg daily. One patient experienced two transfusion-free periods of six months almost directly after starting danazol. The other patients needed six months to three years to achieve transfusion independence. In all patients, concomitant endoscopic management was still required. In one patient, danazol had to be discontinued due to drug-induced liver toxicity.

Prophylactic prevention of bleeding

Severely affected VWD patients who suffer from recurrent bleeding episodes may be treated with VWF concentrates two or three times a week in order to prevent bleeding (prophylaxis). Several retrospective case series of VWD patients on prophylaxis reported beneficial results (121, 122). So far only one prospective dose-escalating study has been performed to evaluate the use of prophylaxis in type 1, 2 and 3 VWD patients with a severe bleeding phenotype. A major reduction of the number of bleedings, such as recurrent gastrointestinal bleeding and joint bleeding or severe epistaxis was shown. Nearly all patients required 50 VWF:RCo IU/kg two or three times a week. This study shows that bleeding may be reduced in patients by regular VWF concentrate administration, although the study was limited by slow and limited inclusion of patients (total number included 12) (123). In a related comment to the article of Abshire et al, Federici proposed that patients with severe VWD, irrespective of type of VWD, who suffer from recurrent bleedings may benefit from prophylaxis and this option must be discussed with and offered to patients with a severe bleeding phenotype (124).

Emerging therapies in VWD

Treatment for von Willebrand disease has not evolved much over the last decades. For years, the only option for VWD patients unresponsive to desmopressin was treatment with plasma derived factor concentrates. These products are effective in the prevention and treatment of bleeding von Willebrand disease, but adverse events such as allergic reactions and thrombosis have been reported (57, 125). In addition, possible transmission of viral or prion diseases remains a concern in products derived from donor plasma, although this has not occurred for a long time due to deployment of viral inactivation

technologies. Meanwhile in hemophilia, treatment with recombinant factor concentrates is already an established therapy for many years, and treatment with extended half-life products are a promising solution for current prophylaxis limitations. Here we will discuss possible future options for better and more personalized treatment in VWD.

Individualized management based on population pharmacokinetic modeling

Currently, VWF/FVIII concentrate is dosed according to body weight, type and location of bleeding while aiming for certain VWF- and FVIII target levels. Postoperatively, dosing is based on these parameters, but also on a crude approximation of clearance of VWF/FVIII concentrate in case of continuous dosing or crude half-life estimations in case of bolus infusions.

DDAVP is administered in a standardized dose of 0.3 µg/kg intravenously every 12-24 hours. When administered sequentially in short intervals, tachyphylaxis occurs due to depletion of the VWF storage in the endothelium.

In both of the above mentioned treatment strategies, other individual patient characteristics, such as age, lean body mass, liver- and kidney function, and baseline VWF and FVIII plasma levels are not taken into account. Furthermore, scarce data is available on the pharmacokinetics (PK) of VWF/FVIII concentrate during surgery and no population PK-models have been constructed (126, 127). Moreover, in patients with a partial deficiency of VWF and FVIII, the rise of these clotting factors during stress due to interactions with the vascular endothelium remains to be elucidated. A population PK model based for both DDAVP and factor concentrate administration may prove valuable in bleeding disorders such as VWD, as it has been for dosing regimens in hemophilia A (128). By taking individual clearance differences into account as well as modelling the interaction with the vascular endothelium in the different VWD types, treatment can be more tailored to the individual requirements of the patient.

Interleukin-11

Early studies in wild type mice and VWD mouse models showed that interleukin-11 (IL-11) significantly increases plasma VWF. Mice treated with subcutaneous IL-11 for 7 consecutive days had a 2-fold increase of FVIII and VWF. In 2008 Ragni et al. reported a phase II prospective trial in nine patients with mild VWD using different dosages of rIL-11 given subcutaneously for 7 days. This resulted in a 1.5 to 3-fold increase over baseline. Because platelet mRNA expression increased, they suggested that the mechanism of effect of rIL-11 was the upregulation of VWF mRNA (129). In additional clinical studies the same group showed that menstrual bleeding severity could be

reduced by rIL-11 in patients with mild VWD and refractory menorrhagia (130). More recently it was also shown that in patients with mild or moderate VWD, who were unresponsive to DDAVP, rIL-11 increased FVIII and VWF nearly 2-fold (131).

Aptamers

Aptamers are a new class of oligonucleotide-based drugs that are able to block various proteins. ARC1779 is an aptamer that binds to the A1 domain of VWF , thereby blocking the interaction with platelet GpIb. Animal studies have shown that this aptamer blocks thrombus formation. In humans this aptamer was studied in patients with type 2B VWD. This type of VWD is characterized by increased binding of the A1 domain to the GpIb receptor on platelets. In patients treated with DDAVP, the rise of VWF in plasma is accompanied by thrombocytopenia, due to platelet aggregation. The aptamer ARC1779 was able to reduce the platelet drop after DDAVP treatment, and increased VWF:Ag and VWF:RCo (132, 133). Therefore it is suggested that the aptamer can be used as an anti-bleeding drug in VWD patients (134). Another potential application can be the use of the aptamer in VWD type 2B patients with hepatitis C and thrombocytopenia. The aptamer may be able to raise platelet counts, making these patients eligible for interferon therapy (133). Blockade of VWF by a longer acting aptamer with subcutaneous bioavailability such as ARC15105, could potentially be useful (133). However, no clinical trials have been performed to determine efficacy.

Gene therapy

In recent years several advances have been reported using gene therapy in congenital bleeding disorders, especially in hemophilia B. Severely affected hemophilia B patients (FIX<1%) treated with adeno-associated virus 8 (AAV8)-mediated gene transfer with a codon-optimized wild type FIX gene showed FIX levels up to 5-8 % of normal and reported a strong reduction in bleeding and exogenous FIX concentrate use (135, 136).

Gene therapy in VWD is challenging due to the large size of the VWF gene, leading to difficulties in inserting VWF cDNA in most viral gene transfer vectors. For VWD, preliminary gene therapy mice studies have been reported. De Meyer et al. used a mouse model to study liver-specific gene transfer of murine VWF expressing vector by hydrodynamic injection. They showed a temporary expression of VWF by the liver, resulting in VWF levels and consequent restoration of in vivo platelet adhesion and aggregation (137). Furthermore, Wang et al. showed in a mouse model that lentiviral vectors could transfer intact murine VWF cDNA in vivo directly to the neonatal liver of VWF knockout mice. This resulted in production of VWF multimers and a partial correction of VWF levels in 33% of the treated mice (138). Although these results seem promising, further improvements in efficiency are needed before clinical application is within reach.

Conclusion

Over the last decades, treatment of VWD has mainly been based on DDAVP and plasma derived factor concentrates. With the FDA approval of a the first recombinant VWF concentrate for treatment of bleeding in VWD patients in 2015, treatment options for von Willebrand disease are now finally being expanded. As research on pathophysiology of VWD and on new treatment modalities is ongoing, it is likely that in the upcoming years, the options to tailor treatment to the individual patients' needs will improve.

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3

Chapter 3

Desmopressin testing in von Willebrand disease: lowering the burden

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Background

Individuals with von Willebrand disease (VWD) require desmopressin testing, due to inter-individual response differences. However, testing is burdensome, while not all patients may need extensive testing.

Objectives

To provide von Willebrand factor (VWF) cut-offs that predict desmopressin non-response and thereby identify individuals who do not need extensive testing in a retrospective cohort. Second, we validated these cut-offs in a prospective cohort.

Patients and methods

We included 376 patients (type 1 VWD with VWF activity (VWF:Act) <0.30 IU/mL: n = 112; with VWF:Act 0.30-0.50 IU/mL: n = 206; type 2 VWD: n = 58; age 5-76) from January 2000 - July 2020. We collected VWF:Act and factor VIII activity (FVIII:C) at baseline and several time points after desmopressin (T1-T6). We defined response as VWF:Act and FVIII:C ≥0.50 IU/mL at T1 and T4. We compared VWF:Act and FVIII:C distribution (historically lowest level, baseline and T1) between responders and non-responders, and determined cut-offs discriminating between these groups. Results were validated in a group of 30 individuals.

Results

All individuals with type 1 VWD and type 2VWD respectively with baseline VWF:Act ≥0.34 IU/mL or ≥0.28 IU/mL were responders. In individuals with T1 VWF:Act ≥0.89 IU/mL (type 1 VWD) or T1 VWF:Act ≥1.10 IU/mL (type 2 VWD), response remained at T4.

Conclusion

Desmopressin testing is not needed when lowest historical VWF:Act is ≥0.30 IU/mL. In type 1 VWD patients who require testing, measurements after T1 are often not needed. In type 2 VWD patients who require testing, we advise performing T1 and T4 measurements.

Introduction

Von Willebrand disease (VWD) is a bleeding disorder caused by a deficiency or qualitative defect of von Willebrand factor (VWF). VWF is essential for both primary and secondary hemostasis. It facilitates platelet plug formation at sites of vascular damage and functions as a chaperone protein for factor VIII (FVIII), which it protects from proteolytic degradation in the circulation. VWD is categorized into three types (1). Type 1 is defined as a partial VWF deficiency (VWF <0.50 IU/mL) in individuals with a family history of VWD and/or abnormal bleeding, and type 3 as a complete deficiency of VWF. Type 2 comprises several qualitative VWF defects, classified as types 2A, 2B, 2M and 2N.

Bleeding in individuals with VWD can be prevented or treated with either desmopressin (1-deamino-8D-arginine vasopressin, DDAVP) or VWF-containing concentrates. Desmopressin stimulates the release of VWF from vascular endothelial cells into the circulation, resulting in increased levels of FVIII (2). After desmopressin administration, the maximum VWF and FVIII response and the duration of response differ significantly between patients, whereas the response in a single individual is reproducible and consistent over time (3). It is therefore common practice for individuals who are potentially eligible for desmopressin treatment to first undergo desmopressin testing to determine their individual response. Individuals with type 2B and type 3 are not eligible for treatment with desmopressin, respectively due to the risk of thrombocytopenia and due to the severely impaired synthesis of VWF (4).

In most situations, desmopressin testing involves administering an intravenous dose of 0.3 µg/kg desmopressin diluted in 50 mL NaCl 0.9% over 30 minutes, and measuring VWF and FVIII at several time points (usually at baseline, 1 hour and 4 hours after desmopressin administration) (5). Various experts and studies have proposed different definitions of clinical response. Most commonly, complete responders are defined by VWF ristocetin cofactor activity (VWF:RCo) and FVIII levels of 0.50 IU/mL or higher after desmopressin (6-9). The most recent international guidelines on the management of von Willebrand state that a patient is considered responsive to desmopressin if their VWF level increases at least two times over baseline level, and if both VWF and FVIII levels of >0.50 IU/mL are achieved after administration of desmopressin (5). In these guidelines it is recommended that VWF:Act levels should be increased to ≥0.50 IU/mL before performing a minor invasive procedure, and a desmopressin test should be performed before starting treatment with desmopressin in patients with a VWF baseline level <0.30 IU/mL. This level is however mainly based on expert opinion.

In this study, we retrospectively collected desmopressin test data from a large group of individuals with different types of VWD, and analyzed plasma VWF and FVIII levels at various time points after desmopressin administration. Our primary aim was to provide relevant cut-off levels for prediction of an individuals' response to desmopressin and to identify individuals who do not require a complete desmopressin test or no desmopressin test at all. Our second aim was to validate these cut-off levels by applying them to a cohort of prospectively included patients whom underwent desmopressin testing. We hypothesize that many patients, especially those with type 1 VWD, will not need testing if certain cut-off levels are applied.

By limiting desmopressin testing in general and by decreasing the number of blood samples needed to be taken during testing, health care professionals will save time, and patient burden as well as health care costs will be reduced.

Patients and methods

Patient selection – initial cohort

The initial cohort was derived from a retrospective, single-center cohort study. We included all individuals with VWD (defined as having a positive family history of VWD and/or abnormal bleeding and historically lowest VWF antigen (VWF:Ag), VWF activity (VWF:Act) and/or VWF collagen binding (VWF:CB) <0.50 IU/mL -or FVIII <0.40 IU/mL in case of type 2N VWD-), in whom a desmopressin test was performed between January 1st 2000 and June 1st 2020 at the Erasmus University Medical Center Rotterdam, the Netherlands.

Patient selection - validation cohort

To validate the results from the initial cohort, we analyzed data of patients who were prospectively included in the OPTI-CLOT: To WiN study (Netherlands Trial Register trial registration number: NL7212, www.trialregister.nl) between June 2019 and July 2020 from the Erasmus University Medical Center Rotterdam and University Medical Center Groningen, using the same inclusion criteria as for the retrospective cohort. All individuals included in this cohort provided signed informed consent.

Ethics review

The study protocol for the retrospective study (number: MEC-2020-0683), as well as the study protocol for the prospective OPTI-CLOT: To WiN study was reviewed and approved by the Medical Ethics Committee of the Erasmus University Medical Center Rotterdam.

Desmopressin testing

In all patients, a single intravenous desmopressin test dose of 0.3 µg/kg was administered in 30 minutes. Venous blood samples were routinely obtained immediately before desmopressin administration (baseline) and at 1, 3 and 6 hours after desmopressin administration (T1, T3, T6) in adults, and at baseline, T1, T2, T4 and T6 in children, according to local protocol.

Laboratory measurements

VWF:Ag, VWF:Act, VWF:CB and FVIII activity (FVIII:C) were measured for routine diagnostics in the hemostasis laboratory of the Erasmus University Medical Center. VWF:Act was measured using different assays over the years: a VWF ristocetin cofactor (VWF:RCo) assay from 2000 to 2005, a monoclonal antibody (VWF:Ab) assay from 2005 to 2012, and a VWF glycoprotein 1b binding (VWF:GP1bM) assay from 2012 onwards. These specific laboratory measurements have been described in detail in an earlier publication (10).

Clinical Response Definition

Primarily, we defined responders as individuals with both VWF:Act and FVIII:C ≥0.50 IU/mL at T1 and T4, as the most recent international guidelines recommend that levels of VWF:Act and FVIII:C before performing a minor invasive procedure should be ≥0.50 IU/mL (5). Non-responders were defined as individuals with VWF:Act and/or FVIII:C <0.50 IU/mL at T1 and/or T4. Secondarily, we investigated the fold-increase in VWF:Act over baseline as an additional measure of efficacy.

Statistical analysis

Descriptive data are presented as numbers with percentages for categorical variables and as means with standard deviations or medians with interquartile ranges (IQR) for continuous data, depending on the distribution of the data.

In case the VWF or FVIII level measured was below the lower limit of quantification (LLOQ), we calculated and imputed the outcome. As timing of measurements differed between children and adults, we calculated VWF:Act and FVIII:C at T4 for adults as follows:

$$T4\ level = T3\ level - \frac{1}{t1/2} * T3\ level.$$

We compared the distribution of VWF:Act and FVIII:C between responders and non-responders to establish sensitivity and specificity of the test for type 1 VWD and type 2 VWD separately. In addition, we performed receiver operator characteristics (ROC) analysis to determine specific cut-offs that discriminated best between responders

and non-responders. We performed logistic regression analysis to assess the influence of sex and age on desmopressin response.

We performed statistical analysis with IBM SPSS statistics for Windows, version 25.0 and GraphPad Prism, version 8.4.3.

Results

Patients

We included 376 individuals in the initial cohort: 112 with type 1 VWD and historically lowest VWF levels <0.30 IU/ml, 206 with type 1 VWD and historically lowest VWF levels between 0.30-0.50 IU/mL and 58 with type 2 VWD (2A: n = 41; 2M: n = 14 and 2N: n = 3). Sixty-nine percent were females. Mean age was 29 ± 15 years, mean body weight was 66 ± 20 kg, and 65% had blood group O. Median VWF:Act at baseline immediately before desmopressin administration was 0.31 IU/mL in type 1 VWD with historically lowest VWF <0.30 IU/mL, 0.55 IU/mL in type 1 VWD with historically lowest VWF <0.30-0.50 IU/mL and 0.18 IU/mL in type 2 VWD and. Median FVIII:C at this time point was: 0.62 IU/mL in type 1 VWD (VWF <0.30 IU/mL), 0.80 IU/mL in type 1 VWD (VWF 0.30-0.50 IU/mL) and 0.58 IU/mL in type 2 VWD. Patient characteristics of the initial cohort are shown in table 1.

We found 37 individuals eligible for inclusion in the prospective validation cohort. Four potential inclusions were missed, one patient was planned to have a short desmopressin test with only one measurement after administration of desmopressin, and two patients declined to participate. In total we included and analyzed 30 individuals in the validation cohort: 11 with type 1 VWD (VWF <0.30 IU/mL), 14 with type 1 VWD (VWF 0.30-0.50 IU/mL), 4 with type 2A VWD and 1 with type 2M VWD, whom all completed the desmopressin test. Seventy-three percent were females and mean age was 23 ± 16 years. Mean body weight was 60 ± 23 kg and 75% had blood group O. Median VWF:Act at baseline directly before desmopressin administration was 0.37 IU/mL in type 1 VWD (VWF <0.30 IU/mL), 0.48 IU/mL in type 1 VWD (VWF 0.30-0.50 IU/mL) and 0.13 IU/mL in type 2 VWD. Median FVIII:C at this time point was 0.78 IU/mL in type 1 VWD (VWF <0.30), 0.80 IU/mL in type 1 VWD (VWF 0.30-0.50 IU/mL) and 0.62 IU/mL in type 2 VWD. Patient characteristics of the validation cohort are shown in table 2.

Table 1: Patient characteristics of the initial cohort

Patient characteristics	Total cohort	Type 1 VWD (VWF <0.30 IU/mL)	Type 2 VWD		Type 1 VWD (VWF 0.30-0.50 IU/mL)
Number of patients	376 (100%)	112 (29.8%)	58 (15.4%)		206 (54.8%)
Disease type (type 2)	-	-	*Type 2A*	*41 (10.9%)*	-
	-	-	*Type 2M*	*14 (3.7%)*	-
	-	-	*Type 2N*	*3 (0.8%)*	-
Age (years)	29 ± 15	29 ± 16	32 ± 18		29 ± 14
Sex (females)	259 (69%)	70 (63%)	31 (53%)		158 (77%)
Body weight (kg)*	66 ± 20	67 ± 22	65 ± 22		66 ± 19
Blood group O*	244 (65%)	73 (65%)	25 (43%)		146 (71%)
Historically lowest levels plasma levels (IU/mL)					
VWF:Ag	0.42 [0.32-0.50]	0.30 [0.25-0.36]	0.34 [0.22-0.49]		0.48 [0.42-0.54]
VWF:Act	0.36 [0.23-0.47]	0.25 [0.19-0.29]	0.14 [0.07-0.23]		0.46 [0.39-0.51]
FVIII:C	0.62 [0.46-0.78]	0.50 [0.39-0.65]	0.42 [0.29-0.59]		0.69 [0.58-0.85]
Plasma levels immediately before desmopressin administration (T0) (IU/mL)					
VWF:Ag	0.50 [0.37-0.61]	0.36 [0.28-0.50]	0.39 [0.24-0.60]		0.56 [0.47-0.64]
VWF:Act	0.46 [0.29-0.59]	0.31 [0.24-0.46]	0.18 [0.08-0.28]		0.55 [0.47-0.63]
VWF:CB*	0.51 [0.32-0.69]	0.32 [0.23-0.50]	0.20 [0.11-0.36]		0.63 [0.51-0.75]
FVIII:C	0.73 [0.56-0.93]	0.62 [0.47-0.88]	0.56 [0.38-0.71]		0.80 [0.68-0.97]
Fold increase over baseline					
VWF:Ag	3.29 [2.57-3.89]	3.55 [2.64-4.47]	3.35 [2.57-4.58]		3.17 [[2.52-3.68]
VWF:Act	3.69 [2.99-4.80]	3.85 [3.05-5.41]	4.29 [3.31-6.63]		3.54 [2.91-4.20]
VWF:CB*	3.64 [2.83-4.84]	4.25 [3.04-6.79]	4.20 [3.14-6.47]		3.45 [2.73-4.35]
FVIII:C	3.65 [3.06-4.45]	3.73 [3.10-4.96]	4.37 [3.36-5.87]		3.53 [3.00-4.11]

VWD = von Willebrand disease; VWF:Ag = von Willebrand factor antigen; VWF:Act = von Willebrand factor activity; FVIII:C = factor VIII activity; VWF:CB: von Willebrand factor collagen binding.
Data are presented as mean ± SD, n (%) or median [interquartile range].
**Number of subjects (total cohort) with missing data: weight (19); blood group (46); VWF collagen binding at T0 (26). As VWF collagen binding was not routinely measured during the early 2000's, historically lowest VWF collagen binding levels are not stated.*

Desmopressin response rates in the initial cohort

Ninety percent of patients (n = 338/376) were responders (VWF:Act and FVIII:C ≥0.50 IU/mL at T1 and T4). We observed large differences between disease types: all type 1 VWD patients with historically lowest VWF levels between 0.30-0.50 IU/mL (n= 206/206); 88% of type 1 VWD patients (n = 99/112); and 57% of type 2 patients (n = 33/58) were responders (table 3). All patients with a VWF:Act response also showed a FVIII:C response. In figure 1, the individual VWF:Act levels measured in the different disease types at different time points during desmopressin testing are plotted and categorized into responders and non-responders.

Table 2: Patient characteristics of the validation cohort

Patient characteristics	Total cohort	Type 1 VWD (VWF <0.30 IU/mL)	Type 2 VWD		Type 1 VWD (VWF 0.30-0.50 IU/mL)
Number of patients	30 (100%)	11 (36.7%)	5 (16.6%)		14 (46.7%)
Disease type (type 2)	-	-	*Type 2A*	*4 (13.3%)*	-
	-	-	*Type 2M*	*1 (3.3%)*	-
	-	-	*Type 2N*	-	-
Age (years)	23 ± 16	31 ± 21	11 ± 5		20 ± 11
Sex (females)	22 (73%)	8 (73%)	3 (60%)		11 (79%)
Body weight (kg)	60 ± 23	65 ± 23	38 ± 12		64 ± 22
Blood group O*	15 (75%)	7 (78%)	0 (0%)		8 (100%)
Historically lowestplasma levels (IU/mL)					
VWF:Ag	0.39 [0.28-0.50]	0.28 [0.21-0.39]	0.35 [0.17-0.62]		0.47 [0.38-0.52]
VWF:Act	0.37 [0.22-0.45]	0.26 [0.22-0.31]	0.20 [0.08-0.32]		0.44 [0.41-0.50]
FVIII:C	0.65 [0.48-0.80]	0.53 [0.38-0.54]	0.48 [0.19-0.86]		0.79 [0.67-0.87]
Plasma levels immediately before desmopressin administration (baseline) (IU/mL)					
VWF:Ag	0.50 [0.32-0.56]	0.35 [0.24-0.59]	0.33 [0.16-0.47]		0.52 [0.50-0.56]
VWF:Act	0.40 [0.31-0.54]	0.37 [0.30-0.58]	0.13 [0.12-0.25]		0.48 [0.38-0.55]
VWF:CB*	0.47 [0.25-0.55]	0.41 [0.24-0.52]	0.07 [0.04-0.25]		0.53 [0.48-0.67]
FVIII:C	0.76 [0.62-0.97]	0.78 [0.47-1.09]	0.62 [0.36-0.62]		0.80 [0.67-0.89]
Fold increase over baseline					
VWF:Ag	3.57 [3.01-4.14]	3.14 [2.62-3.98]	4.55 [3.31-5.46]		3.60 [3.19-3.96]
VWF:Act	3.94 [3.32-4.79]	3.36 [2.93-4.34]	4.46 [3.35-6.02]		4.06 [3.71-4.91]
VWF:CB*	3.45 [2.77-4.79]	3.88 [2.59-4.77]	5.43 [3.38-6.83]		3.23 [2.85-3.58]
FVIII:C	4.01 [3.17-4.81]	3.21 [2.59-4.93]	4.69 [4.28-6.95]		4.01 [3.25-4.61]

VWD = von Willebrand disease; VWF:Ag = von Willebrand factor antigen; VWF:Act = von Willebrand factor activity; FVIII:C = factor VIII activity; VWF:CB: von Willebrand factor collagen binding.
Data are presented as mean ± SD, n (%) or median [interquartile range].
**Number of subjects (total cohort) with missing data: blood group (n=10), VWF:CB at baseline and fold increase over baseline (n=5).*

Table 3: Response to desmopressin in the initial cohort and the validation cohort, according to disease type

Initial cohort	Total cohort	Type 1 (VWF <0.30 IU/mL)	Type 2	*Type 2A*	*Type 2M*	*Type 2N*	Type 1 VWD (VWF 0.30-0.50 IU/mL)
Number of patients	376	112	58	*41*	*14*	*3*	206
Responder	338 (90%)	99 (88%)	33 (57%)	*22 (54%)*	*8 (57%)*	*3 (100%)*	206 (100%)
Non-responder	38 (10%)	13 (12%)	25 (43%)	*19 (46%)*	*6 (43%)*	-	-
Validation cohort							
Number of patients	30	11	5	*4*	*1*	-	14
Responder	26 (87%)	10 (91%)	2 (40%)	*1 (25%)*	*1 (100%)*	-	14 (100%)
Non-responder	4 (13%)	1 (9%)	3 (60%)	*3 (75%)*	-	-	-

VWF = von Willebrand factor

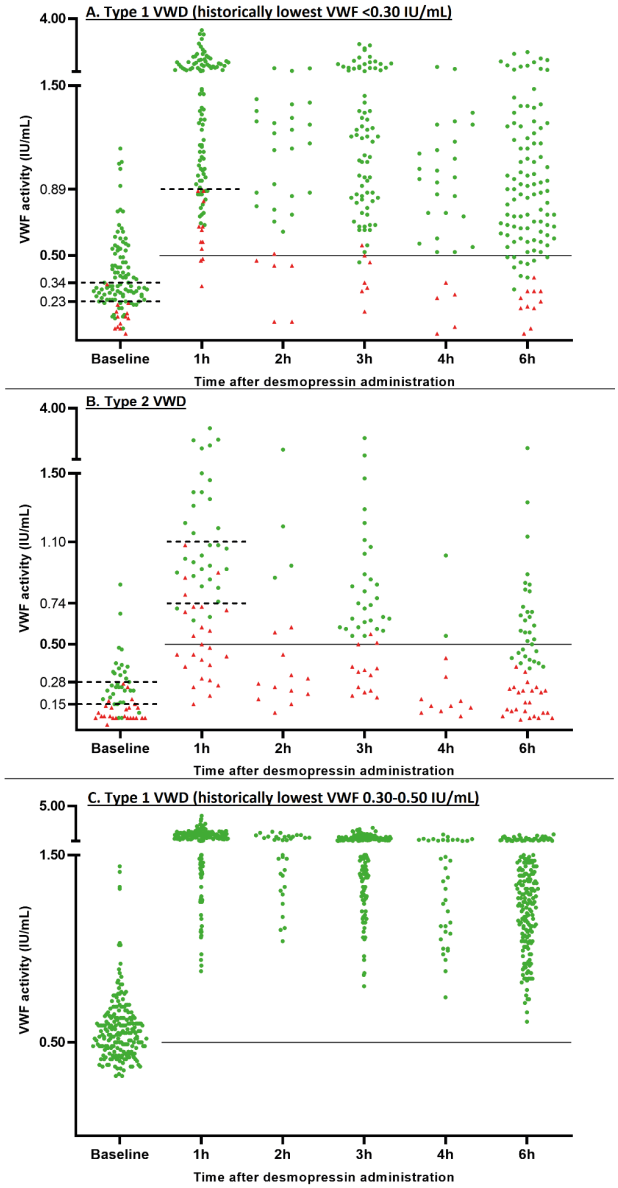

Figure 1: VWF activity (IU/mL) in responders and non-responders during desmopressin testing in type 1 VWD (VWF <0.30 IU/mL) (upper panel), type 2 VWD patients (middle panel) and type 1 VWD (VWF 0.30-0.50 IU/mL) (lower panel). Every green dot depicts a single VWF:Act measurement in one of the responders; every red triangle depicts a single VWF:Act measurement in one of the non-responders. Dashed lines in upper panel depict: optimal threshold at baseline (0.23 IU/mL), threshold with sensitivity 100% at baseline (0.34 IU/mL), and both optimal threshold and threshold with sensitivity 100% at T1 (0.89 IU/mL) in type 1 VWD (VWF <0.30 IU/mL). Dashed lines in middle panel depict: optimal threshold at baseline (0.15 IU/mL), threshold with sensitivity 100% at baseline (0.28 IU/mL), optimal threshold at T1 (0.74 IU/mL), and threshold with sensitivity 100% at T1 (1.10 IU/mL) in type 2 VWD. The uninterrupted line at 0.50 IU/mL in all panels depicts the threshold for response at T1 and T4.

In patients with type 1 VWD and historically lowest VWF <0.30 IU/mL, females were more likely to respond than males (OR 4.5; 95% CI: 1.3 - 16.1; p = 0.02). Mean historically lowest VWF:Act did not differ between females and males with type 1 VWD and historically lowest VWF:Act <0.30 IU/mL (0.24 IU/mL vs. 0.22 IU/mL, p=0.44), however males were more than twice as likely to have historically lowest VWF:Act <0.10 IU/mL. We did not find a difference in response between children (<16 years) and adults (≥16 years). In type 2, we did not find a significant difference in response between males and females, but children (<16 years) were less likely to respond than adults (≥16 years) (OR 0.08); 95% CI: 0.02 – 0.42; p 0.003).

All individuals who showed an increase in VWF:Act also showed an increase in FVIII:C and vice versa. We did not observe very large or unexpected discrepancies between fold increase in VWF:Act and FVIII:C in any of the subjects. In 10 out of the 376 patients (3%), VWF:Act increased less than two-fold over baseline at T1 (range: 1.30-1.97 fold). Three of these patients were non-responders: one type 1 VWD patient with historically lowest VWF <0.30 IU/mL, and two type 2A VWD patients. The seven responders with a less than two-fold increase were type 1 VWD patients with VWF:Act ≥0.50 IU/mL at baseline already, and included one individual with historically lowest VWF levels <0.30 IU/mL and six individuals with historically lowest VWF levels between 0.30-0.50 IU/mL.

Desmopressin response rates in the prospective validation cohort

Twenty-six out of the thirty patients were responders (87%). In type 1 VWD (VWF <0.30 IU/mL), 91% (n = 10/11) classified as responder and all patients with historically lowest VWF levels between 0.30-0.50 IU/mL (100%) were responders. Forty percent of the type 2 VWD patients (n = 2/5) were responders (table 3). All VWF:Act responders were also FVIII:C responders. None of the patients had a VWF:Act or FVIII:C increase less than two-fold over baseline.

Receiver Operating Characteristic (ROC) analysis

We used ROC curves to analyze the potential of VWF:Act and FVIII:C at different time points (baseline, T1 and historically lowest level) to predict desmopressin non-response. As only three type 2N patients were present in our cohort, we excluded these patients from the analysis. Comparison of the areas under the curve (AUCs) shows that VWF:Act measured at T1 has the highest accuracy to distinguish responders from non-responders with an AUC of 0.98 in type 1 VWD (VWF <0.30 IU/mL) and an AUC of 0.94 in type 2 VWD, followed by VWF:Act at baselinewith an AUC of 0.93 in type 1 VWD (VWF <0.30 IU/mL) and an AUC of 0.88 in type 2 VWD. Historically lowest VWF:Act was least predictive of desmopressin response.

The optimal predictive baseline cut-off –the VWF:Act level with the highest sensitivity and specificity- is 0.23 IU/mL in type 1 VWD (VWF <0.30 IU/mL) and 0.15 IU/mL in type 2 VWD. The most sensitive predictive baseline cut-off–the level with 100% sensitivity, at which no non-responders will be missed- is 0.34 IU/mL in type 1 VWD (VWF <0.30 IU/mL) and 0.28 in type 2 VWD. In figure 1, the different cut-offs at baseline, T1 and historically lowest level are visualized. The predictive potential of VWF:Act is shown in figure 2 and table 4.

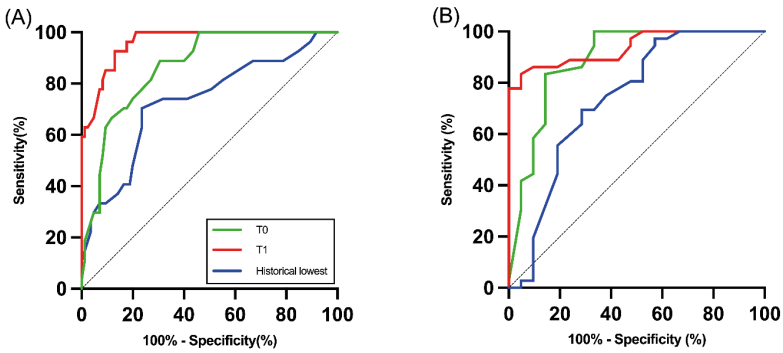

Figure 2: ROC curves comparing the potential of VWF:Act at different time points to discriminate between responders and non-responders.

A) VWF:Act in type 1 VWD (VWF <0.30 IU/mL) patients; B) VWF:Act in type 2 VWD patients (excluding type 2N patients). Figures show that VWF:Act at T1 predicts response to desmopressin best (AUC of 0.98 in type 1 VWD (VWF <0.30 IU/mL) and 0.94 in type 2 VWD), followed by measurements at baseline (AUC of 0.93 in type 1 VWD (VWF <0.30 IU/mL), 0.88 in type 2 VWD). Historical lowest VWF:Act is the least predictive of desmopressin response (AUC of 0.79 in type 1 VWD (VWF <0.30 IU/mL), and 0.79 in type 2 VWD). All individuals who show a VWF:Act response also show a FVIII response.

Validation of cut-offs in the prospective cohort

In the only non-responding type 1 VWD (VWF <0.30 IU/mL) patient, VWF:Act was 0.14 IU/mL at baseline and 0.47 IU/mL at T1. Historical lowest VWF:Act was 0.07 IU/mL. The three type 2A VWD non-responders had baseline VWF:Act of 0.10 - 0.13 IU/mL, T1 VWF:Act of 0.30 – 0.58 IU/mL and historically lowest VWF:Act of 0.05 – 0.22 IU/mL. All of these values are below the most sensitive predictive cut-off. In one type 2A VWD patient, the historically lowest level was above the optimal predictive cut-off of 0.15 IU/mL.

Table 4: Receiver Operating Characteristic (ROC) analysis of VWF:Act and FVIII at baseline (directly before desmopressin administration), one hour after desmopressin administration (T1) and at historically lowest level

	Type 1 VWD (VWF <0.30 IU/mL)	Type 2 VWD*
	VWF:Act at baseline	
Area under the ROC curve (95% CI)	0.93 (0.85-1.00)	0.88 (0.79-0.98)
Optimal cut-off (IU/mL)	0.23	0.15
Sensitivity % (95% CI)	92 (67-100)	80 (61-91)
Specificity % (95% CI)	87 (79-92)	90 (74-97)
Cut-off with sensitivity 100%	0.34	0.28
Sensitivity % (95% CI)	100 (77-100)	100 (87-100)
Specificity % (95% CI)	48 (39-58)	40 (25-58)
	VWF:Act at T1	
Area under the ROC curve (95% CI)	0.98 (0.95-1.00)	0.94 (0.87-1.00)
Optimal cut-off	0.89	0.74
Sensitivity % (95% CI)	100 (77-100)	84 (65-94)
Specificity % (95% CI)	86 (78-91)	90 (74-97)
Cut-off with sensitivity 100%	-	1.10
Sensitivity % (95% CI)	-	100 (87-100)
Specificity % (95% CI)	-	37 (22-54)
	Historically lowest VWF:Act level	
Area under the ROC curve (95% CI)	0.79 (0.62-0.95)	0.79 (0.64-0.93)
Optimal cut-off (IU/mL)	0.22	0.15
Sensitivity % (95% CI)	85 (58-97)	92 (75-99)
Specificity % (95% CI)	72 (62-80)	67 (45-83)
Cut-off with sensitivity 100%	0.33	0.29
Sensitivity % (95% CI)	100 (77-100)	100 (87-100)
Specificity % (95% CI)	7 (4-14)	19 (8-40)

VWD = von Willebrand disease; VWF:Act = von Willebrand factor activity; FVIII:C = factor VIII activity; CI = confidence interval

**Type 2N patients (n = 3) were excluded from this analysis.*

P-values for all area's under the ROC curve are <0.001

Discussion

The results of this study show that desmopressin testing is not needed in individuals with type 1 VWD with historically lowest VWF levels between 0.30-0.50 IU/mL as well as in a substantial number of individuals with type 1 VWD with historically lowest VWF levels <0.30 IU/mL, and those with type 2A and type 2M VWD.

In individuals with type 1 (VWF <0.30 IU/mL), type 2A and type 2M VWD, we suggest using the most recently measured VWF:Act during a regular outpatient clinic visit as a surrogate for the baseline measurement during a desmopressin test, as this is in essence a random time point. In our study, all type 1 VWD patients with historically lowest VWF levels <0.30 IU/mL with baseline VWF:Act ≥0.23 IU/mL were responders except for one patient who had a baseline VWF:Act of 0.33 IU/mL. All type 2 VWD patients with baseline VWF:Act ≥0.28 IU/ml also were responders. For practical

reasons, we therefore propose to only test those type 1 (VWF <0.30 IU/mL), type 2A and type 2M VWD patients in whom the most recent VWF:Act measured is below 0.30 IU/mL. This is in accordance with the 2021 guidelines on the management of VWD, which suggest performing a desmopressin test over not performing a test before starting treatment with desmopressin in patients with a VWF baseline level <0.30 IU/mL (5). Our data therefore confirm this guideline, that was mainly based on expert opinion.

If a desmopressin test is required, VWF:Act should be measured before and at least at one and four hours after desmopressin administration, in order to quantify the peak as well as the duration of the response. If it is logistically possible to acquire VWF:Act results from the laboratory rapidly after T1 blood withdrawal, the test may be terminated in type 1 VWD (VWF <0.30 IU/mL) patients if T1 VWF:Act is <0.50 IU/mL or ≥0.89 IU/mL, as the patient will surely be a non-responder or a responder respectively. In type 2A and type 2M VWD patients who qualify for desmopressin testing (baseline VWF:Act <0.30 IU/mL), we strongly advise to always perform measurements at T1 as well as T4 (figure 3).

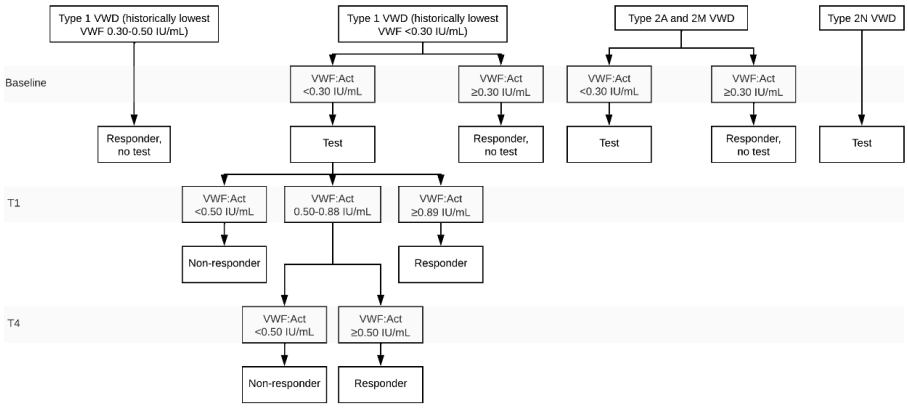

Figure 3: flowchart for desmopressin testing.
VWF = von Willebrand factor; VWD = von Willebrand disease; VWF:Act = von Willebrand factor activity.

Our results show that the use of historically lowest VWF:Act levels is not recommended when deciding if desmopressin testing should be performed, as these levels are least predictive of desmopressin response. This is in accordance with the most recent guidelines, which recommend to perform a desmopressin test shortly after diagnosis (11). Our results do not apply to type 2N patients, as the number of type 2N patients in our study was too small and was therefore excluded from the analysis.

If the approach as described above is adopted in clinical practice, the number of desmopressin tests performed can be reduced by 55% in type 1 VWD (VWF <0.30 IU/mL) patients and by 20% in type 2A and type 2M VWD patients. Of the individuals with type 1 VWD (VWF <0.30 IU/mL) who *will* need a desmopressin test, 64% will only require blood sampling at T1. Our data also demonstrate that FVIII does not necessarily have to be measured in type 1, type 2A and type 2M VWD patients during a desmopressin test, as in all individuals who showed a VWF:Act response, a FVIII response was observed as well.

In the 2021 guidelines on the management of VWD, responsiveness to desmopressin is defined as an increase of the baseline VWF level of at least two-fold, combined with the achievement of both VWF and FVIII levels of >0.50 IU/mL (5). However, when evaluating the criterion of a two-fold VWF:Act increase over baseline, we found that this does not add any value when VWF:Act and FVIII:C of 0.50 IU/mL or above at T1 and T4 are regarded as responsiveness, as the few patients who showed a less than two-fold increase over baseline already had baseline levels ≥0.50 IU/mL.

We found that in type 1 VWD, females are more likely to respond than males, and that the number of responders in type 2 VWD seems to increase with age. These results correlate with earlier findings that clearance of VWF is lower in females, and that bioavailability of VWF increases with age (10). The difference between females and males in type 1 can possibly be explained because females are more often diagnosed with VWD type 1 than men due to the hemostatic challenges they undergo, such as menstruation and childbirth. Overall, women diagnosed with type 1 VWD therefore tend to have milder laboratory abnormalities (12). As it is well known that coagulation factor levels do not always correlate with bleeding tendency, it is important that clinicians do not only establish desmopressin responsiveness based on coagulation factor levels when deciding which treatment modality to choose, but also take the bleeding tendency and type of VWD of the individual patient into account.

In the initial cohort, three out of the 112 type 1 VWD patients had a VWF:Act elimination half-life <2 hours. These patients had a VWFpp/VWF:Ag ratio >7 and a gene variant (R1205H or S2179R), associated with rapid clearance of VWF. In the validation cohort, none of the type 1 VWD patients had a VWF:Act half-life <2 hours. Data regarding genetic variants and their association with desmopressin response in type 1 VWD patients with historically lowest VWF levels <0.30 IU/mL and in type 2 patients have been described in an another article by our group (13). In type 1 VWD patients with a known VWFpp/VWF:Ag ratio >7 and/or a gene variant associated with rapid clearance, desmopressin testing is therefore unnecessary.

Our study has several strengths. Firstly, we included a large number of patients, likely representative for the VWD populations in hemophilia treatment centers worldwide, as a wide range of disease types and ages are included. We consider inclusion bias to be low as it is standard protocol at our center to perform a desmopressin test shortly after VWD diagnosis. Secondly, our study was conducted in a single center, using the same desmopressin test protocol over the studied time period. Thirdly, we were able to validate our results in a prospective cohort of VWD patients.

A limitation of this study is that in many centers, immediate laboratory measurement of VWF:Act is not possible. In those centers, a complete desmopressin test with measurements one hour as well as four hours after desmopressin will have to be conducted, when desmopressin testing is required. This may take away some of the benefits of implementing our advised testing protocol. Another limitation of our study is that ethnicity and socio-economic status of the participants was not registered. However, the Erasmus University Medical Center is situated in the city of Rotterdam, where more than half of the population is from non-Western descent. Furthermore, it is a tertiary referral hospital for the larger area, including suburban and rural areas. We are therefore convinced that the studied population is racially, culturally and socio-economically diverse.

In conclusion, our results show that individuals with type 1 VWD with historically lowest VWF levels between 0.30-0.50 IU/mL do not require desmopressin testing, as well as 55% of type 1 with historically lowest VWF levels <0.30 IU/mL, 20% of type 2A and 21% of type 2M VWD patients. Current guidelines are in accordance with our finding that type 1 VWD patients with VWF levels <0.30 IU/mL need testing (5). The results of the type 2 VWD cohort would however benefit from replication in a larger cohort, with especially larger numbers of type 2M and 2N VWD patients. Furthermore, in type 1, 2A and 2M VWD patients, it is not strictly necessary to measure FVIII, as all VWF:Act responders in our study were also FVIII responders. Application of this testing protocol in clinical practice will reduce both patient burden and time investments by health care professionals, as well as health care costs.

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4

Chapter 4

Analysis of current perioperative management with Haemate® P / Humate P® in von Willebrand disease: Indicating the need for personalized treatment

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ABSTRACT

Introduction Von Willebrand disease (VWD) patients are regularly treated with VWF-containing concentrates in case of acute bleeding, trauma and dental or surgical procedures.

Aim In this multicentre retrospective study, current perioperative management with a von Willebrand factor (VWF)/Factor VIII (FVIII) concentrate (Haemate® P) in VWD patients was evaluated.

Patients/Methods VWD patients undergoing minor or major surgery between 2000 and 2015, requiring treatment with a VWF/FVIII concentrate (Haemate® P) were included. Achieved VWF activity (VWF:Act) and FVIII during FVIII-based treatment regimens were compared to predefined target levels in national guidelines.

Results In total, 103 VWD patients (148 surgeries) were included: 54 type 1 (73 surgeries), 43 type 2 (67 surgeries) and 6 type 3 (8 surgeries). Overall, treatment resulted in high VWF:Act and FVIII levels, defined as ≥0.20 IU/mL above predefined levels. In type 1 VWD patients, respectively 65% and 91% of trough VWF:Act and FVIII levels were higher than target levels. In type 2 and type 3 VWD respectively, 53% and 57% of trough VWF:Act and 72% and 73% of trough FVIII levels were higher than target level. Furthermore, FVIII accumulation over time was observed, while VWF:Act showed a declining trend, leading to significantly higher levels of FVIII than VWF:Act.

Conclusion High VWF:Act and accumulation of FVIII was observed after perioperative FVIII-based replacement therapy in VWD patients, both underlining the necessity of personalization of dosing regimens in order to optimize perioperative treatment.

INTRODUCTION

Von Willebrand disease (VWD) is the most common inherited bleeding disorder with an estimated prevalence of approximately 1% with clinical relevant bleeding in 0.01% (1). It is caused by a quantitative or qualitative defect of von Willebrand factor (VWF) and is characterized by mucocutaneous bleeding and bleeding after trauma or surgery (2). In more severe VWD, there also may be a concomitant factor VIII (FVIII) deficiency, as VWF prevents FVIII from proteolysis (3). Generally, VWD patients are treated with desmopressin (DDAVP) or VWF-containing concentrates when acute bleeding or trauma occurs, or to prevent bleeding in the surgical setting. The aim of treatment is to correct the VWF deficiency, and also to correct a FVIII deficiency, if this is present. In patients who do not respond adequately to DDAVP or have contra-indications for its use, treatment usually consists of combined VWF/FVIII factor concentrates amongst which the ratios of VWF activity (VWF:Act) over FVIII may differ (4).

Although clinical symptoms are generally milder than in hemophilia, dosing of perioperative treatment in VWD is more challenging due to variation in VWD types and mutations (2, 5), interpatient variability of residual endothelial VWF production, VWF secretion and clearance, as well as heterogeneity in types of factor concentrates with different ratios of VWF:Act/FVIII and VWF:Act/VWF antigen (VWF:Ag) (6, 7). Previous studies have however reported that surgical procedures can be performed safely in VWD patients and that treatment with VWF-containing concentrates is efficacious (8-17).

In many countries, specific target levels are defined in national guidelines to safeguard hemostasis during surgery. These target values are based on expert opinion and limited observational research (Figure 1) (18). Currently, calculation of the required doses of VWF and/or FVIII is based on body weight. In the Netherlands, dosing is FVIII level-based, due to the fact that FVIII is considered crucial in preventing surgical bleeding by its role in thrombin generation and consolidation of the fibrin plug (17). However, momentarily VWF levels are increasingly monitored as rapid availability of VWF activity assay results is becoming mainstream. This may facilitate a more VWF-based dosing regimen in the near future (19). Furthermore, it is increasingly common to label factor concentrates according to both FVIII and VWF content.

VWF/FVIII concentrates can be classified into three different groups according to VWF:Act/FVIII and VWF:Act/VWF:Ag ratios (7). Firstly, products with a VWF:Act/FVIII ratio of approximately 1 (with low or high VWF:Act/VWF:Ag ratio). Secondly, with a VWF:Act/FVIII ratio of >1 (with high VWF:Act/VWF:Ag ratio) and lastly VWF

concentrates with a VWF:Act/FVIII ratio of >10 (with also high VWF:Act/VWF:Ag ratio). In case the last concentrates are used perioperatively, patients with low circulating FVIII levels should receive this concentrate intravenously 6-8 hours before surgery, in order to allow endogenous FVIII to rise to haemostatically adequate levels. Therefore, in emergency situations, a priming dose of FVIII in addition to VWF-concentrate is often required (20). Because FVIII production and secretion are normal in VWD patients, infusion of exogenous VWF, which stabilizes and increases endogenous FVIII levels, together with exogenous FVIII, may lead to very high levels of FVIII (>2.70 IU/mL) (21). This is of course a possible risk factor for thrombosis (22). It has been demonstrated that repetitive dosing of concentrates with a VWF:Act/FVIII ratio >1, will result in less accumulation of FVIII than concentrates with a ratio of approximately 1 (8). Worldwide, the most frequently used VWF/FVIII concentrate is Haemate® P, a plasma-derived virus-inactivated VWF/FVIII concentrate with a VWF:RCo/FVIII ratio of 2.45 (23).

Choice of perioperative treatment is dependent on type and severity of VWD, while dosing of replacement therapy is dependent on type and extent of the surgical procedure (18). In addition, treatment may differ due to interindividual differences in pharmacokinetic (PK) parameters such as clearance and half-life of both exogenous and endogenous VWF and FVIII. Studies report that perioperative VWF/FVIII concentrate consumption indeed varies substantially, from 27 to 146 VWF:Act $IUkg^{-1}day^{-1}$ (8, 17). As achieved VWF and FVIII levels have rarely been evaluated and reported in relation to efficacy (24), we aimed to evaluate current perioperative management with VWF/FVIII concentrate in VWD patients in relation to target levels as stated in national guidelines. This was done by assessing the extent to which predefined VWF:Act and FVIII target levels were actually achieved as well as by analysis of predictors of higher or lower VWF:Act and FVIII levels than targeted. Insight in these factors will help realize more efficacious and individualized treatment in VWD in the near future. In addition, collection of these data will help construct population PK models for VWD patients in the near future.

MATERIALS AND METHODS

This multicentre retrospective observational cohort study was conducted in five Academic Haemophilia Treatment Centres in the Netherlands (Erasmus University Medical Centre Rotterdam (n=51); Academic Medical Centre Amsterdam (n=15); University Medical Centre Groningen (n=14); Leiden University Medical Centre (n=12), Radboud university medical centre (n=11). This study was not subject to the Medical

Research Involving Human Subjects Act, as retrospective anonymized data were analysed, and therefore, according to Dutch law, review by the Ethical Committee and informed consent were not required.

Subject selection

Patients with a clinical and laboratory diagnosis of VWD (historically lowest levels of VWF:Ag ≤0.30 IU/mL and/or VWF:Act ≤0.30 IU/mL and/or FVIII ≤0.40 IU/mL) were included. Patients who underwent a minor or major surgical procedure as defined by Koshy et al. (25), under replacement therapy with a plasma derived VWF/FVIII concentrate between January 1st 2000 and January 1st 2015 were eligible. Only patients treated with Haemate® P were included, the most widely used concentrate for treatment of VWD in the Netherlands. Monitoring of minimally two VWF:Act and FVIII levels was obligatory for inclusion. Patients with other known haemostatic disorders and patients lacking accurate documentation were excluded.

Study objective

The study objective was to evaluate current perioperative management with a specific VWF/FVIII factor concentrate (Haemate® P) in VWD patients by specification of concentrate administration and analysis of subsequently achieved peak and trough levels of VWF:Act and FVIII in comparison to target VWF and FVIII levels as prescribed by national guidelines (Figure 1) (18). In this study, both potential predictors of low and high levels of VWF:Act and FVIII as well as variables associated with VWF/FVIII concentrate consumption were collected and evaluated.

Laboratory assessment

VWF:Act and FVIII were generally monitored daily during hospitalization. Immediately before surgery, peak levels were assessed and in the days after surgery trough levels were measured once or twice daily. In all cases, perioperative dosing was based on FVIII levels, as VWF:Act results were generally not or not rapidly available. FVIII was measured by one-stage clotting assays in all participating centres. In various centres, different VWF activity (VWF:Act) assays were performed according to local protocol.

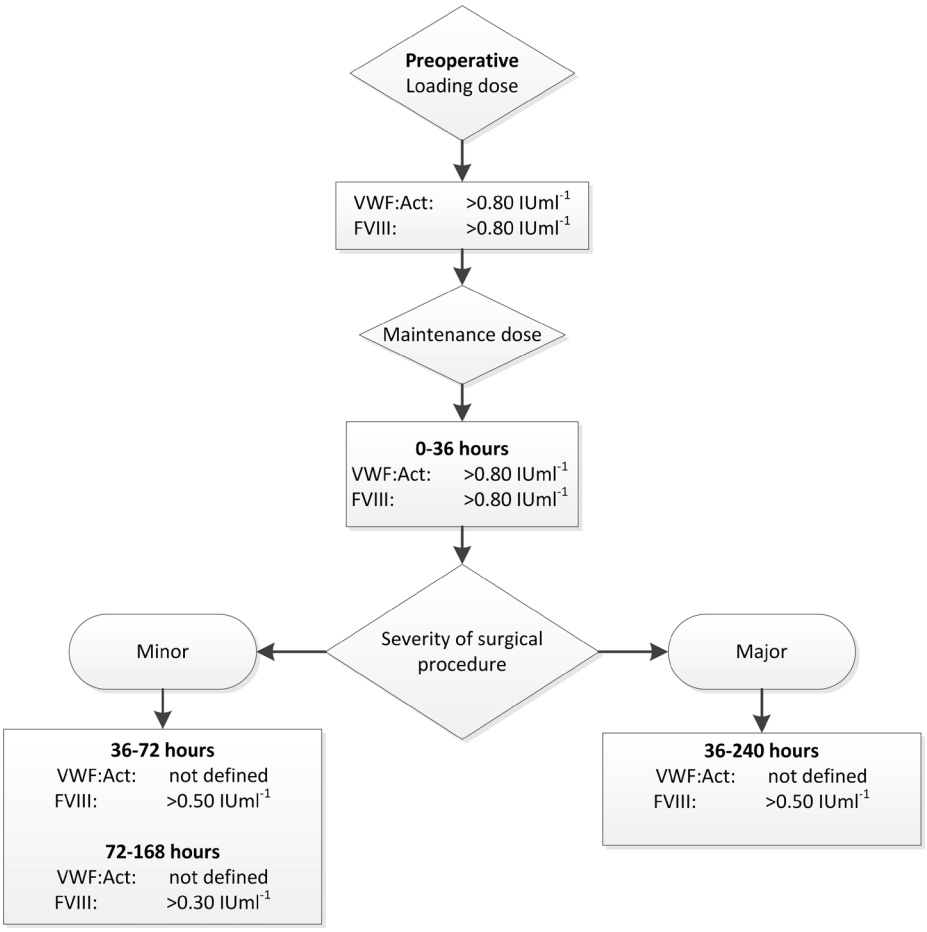

Figure 1. Target VWF:Act and FVIII in VWD patients in the perioperative setting. According to National guidelines[3]. Guidelines describe a standard perioperative dosing regimen of VWD patients undergoing minor and/or major surgery. A loading dose of VWF/FVIII factor concentrate of 50IUkg^{-1} FVIII (30-50 IUkg^{-1} in case of minor surgery) followed by maintenance doses of 15-25IUkg^{-1} FVIII twice daily, depending on FVIII measurements. Both, VWF:Act and FVIII are targeted at trough and/or steady state levels.

Data collection

Patient, surgical and treatment characteristics during the hospitalization period were collected retrospectively. Patient characteristics included age, body weight, gender, type of VWD, baseline VWF:Ag, VWF:Act and FVIII (historically lowest level), ABO blood group, and VWF gene mutation if available. Surgical characteristics consisted of procedure severity as classified by surgical risk score (25), duration of surgery, perioperative blood loss and postsurgical bleeding complications. Bleeding complications were assessed according to definition by the International Society

of Thrombosis and Haemostasis (26) and defined as necessity of second surgical intervention, hemoglobin decrease of ≥1.24 mmol/L and/or requiring red blood cell transfusion, or bleeding prolonging patient hospitalization. A clinically relevant bleeding complication was defined as a bleeding complication requiring a second surgical intervention and/or red blood cell transfusion. Treatment characteristics included: timing and dosing of VWF/FVIII concentrate administration and achieved VWF:Act and FVIIIduring and after surgical procedure, mode of infusion (continuous or bolus infusion) of VWF/FVIII concentrate and co-medication with effect on hemostasis (desmopressin, tranexamic acid, low molecular weight heparin, non-steroidal anti-inflammatory drugs) as well as duration of hospitalization. Duration of hospitalization was defined as day of discharge minus day of surgical procedure and initiation of replacement therapy with VWF/FVIII concentrate.

National guideline and evaluation of perioperative VWF/FVIII concentrate management

National guidelines prescribe a FVIII-based regimen with a loading dose of VWF/FVIII concentrate (ratio of 2.4:1) of 50 $IUkg^{-1}$ FVIII for major surgery and 30-50 $IUkg^{-1}$ FVIII for minor surgical interventions followed by maintenance doses of 15-25 $IUkg^{-1}$ FVIII twice daily with regular monitoring of VWF:Act and FVIII, although no definition of regular monitoring is given. Frequency and timing of monitoring is left to the expertise of the treating physician and depends on VWD type, type and severity of surgery and bleeding phenotype. Dosing is adjusted according to VWF:Act and FVIII target levels specified in guidelines and depicted in Figure 1 (18). In general, patients are treated 7-10 days in case of a major surgical procedure, and 4-7 days in case of a minor surgical procedure. This is in accordance with the UKHCDO and Nordic guidelines (27, 28). Perioperative dosing was left to the discretion of treating physician. When patients were prescribed thromboprophylaxis, in the majority of patients low molecular weight heparin was used. Thromboprophylaxis was given at the discretion of the treating physician, taking type of surgery, duration of hospitalization and patient risk factors for thrombosis, such as age, body mass index, history of thrombosis and genetic predisposition for thrombosis into account.

Perioperative management with VWF/ FVIII concentrate after first peak values was evaluated by comparing achieved VWF:Act and FVIII trough and steady state levels to target VWF:Act and FVIII levels. Trough levels were defined as measurements prior to bolus infusion or measurements at least 12 hours after infusion, when no subsequent factor concentrate infusion was given. Redundantly, no peak levels after bolus infusion were included in these analyses. Steady state samples were defined as VWF and FVIII levels sampled when concentrate substitution is expected to equal elimination of

VWF/FVIII concentrate when administered by continuous infusion. In general, it is assumed that steady state will be reached after a loading dose has been administered and continuous infusion has started.

Analysis of predictors of low and high levels of VWF:Act/FVIII could only be performed in type 1 and type 2 VWD disease patients, due to limited numbers of patients with type 3 VWD. A stepwise backward and forward logistic regression analysis was performed with low levels defined as VWF:Act or FVIII below predefined target levels stated by guidelines, and high levels as all VWF:Act or FVIII levels above the predefined target level with a deviation of ≥0.20 IU/mL. Potential predictors for low and high VWF:Act or FVIII levels in the analysis were severity of surgical procedure, blood group O versus non-O, body weight, age, mode of infusion and treatment centre.

Statistical analysis

Descriptive data are presented as numbers with percentages for categorical variables and as medians with an interquartile range (IQR) for continuous variables, as data were not normally distributed. The non-parametric Mann-Whitney U test was used to compare VWF/FVIII concentrate consumption between surgical procedures of different severity. If a patient was subjected to two or more surgeries, calculations were only performed for the first surgical procedure. Potential predictors of lower and higher VWF:Act/FVIII levels than aimed for were analyzed by stepwise backward and forward logistic regression analysis with elimination of variables with P>0.10. A linear regression analysis was performed to calculate if FVIII accumulation occurred after repetitive dosing of VWF/FVIII concentrate, whereby regression coefficients were compared between both VWF:Act and FVIII. Data management and statistical analysis were performed with IBM SPSS statistics for Windows, version 23.0 (IBM Corp, Armonk, NY, USA). A P-value of <0.05 was considered statistically significant.

RESULTS

The study population consisted of 103 patients undergoing a total of 148 surgical procedures; 54 type 1 VWD patients (73 surgical procedures), 43 type 2 VWD patients undergoing 67 procedures in total: 24 type 2A patients (34 procedures), 7 type 2B patients (8 procedures), 3 type 2N patient (8 procedures) and 9 type 2M patients (17 procedures) and 6 type 3 VWD patients (8 surgical procedures) (Table 1). Half of patients had blood group O (51%). Median historical lowest measured VWF:Ag level and VWF:Act level was 0.30 and 0.22 IU/mL for type 1 VWD patients; 0.29 and 0.10 IU/mL for type 2 VWD and 0.05 and <0.10 IU/mL (lower than detection limit) for type 3

VWD patients. Median historical lowest measured FVIII level was 0.54 IU/mL for type 1, 0.42 IU/mL for type 2 and 0.03 IU/mL for type 3 VWD patients. Some patients in the study population underwent multiple surgical procedures (Table 1). Procedures were mainly orthopaedic (n=36; 24%), general (n=26; 18%) and gynaecological (n=24; 16%). No differences in number and type of surgical procedures between VWD types were observed. Almost all patients received replacement therapy by bolus infusion (90%). Median duration of hospitalization was six days (Table 1). Eleven (29%) and 52 (47%) patients with respectively a minor and major surgical procedure received thromboprophylaxis with low molecular weight heparin. In 51 surgical procedures, patients received tranexamic acid.

Actual VWF: Act and FVIII levels compared to predefined target levels

No differences were observed in achieved VWF:Act and FVIII levels between type 1, type 2 and type 3 VWD patients (Figure 2) after replacement therapy. In all VWD types, most perioperative VWF:Act and FVIII levels were well above predefined target levels. Postoperatively, accumulation of FVIII was observed after repetitive dosing of VWF/FVIII concentrate, resulting in increased FVIII in comparison to VWF:Act ($p<0.01$) (Figure 3). No differences in FVIII accumulation were observed between type 1 and type 2 (data not shown). Thirteen (8%) FVIII trough levels were above 2.70 IU/mL.

In the 54 type 1 VWD patients, *in the first 36 hours* after surgery, median trough *VWF:Act* was 1.48 IU/mL(IQR 1.03-1.87). Eighty-four percent of trough and steady state levels were above predefined target level with a median deviation of 0.80 IU/mL (IQR 0.38-1.11). Seven levels were below target level (median deviation: 0.24 IU/mL [IQR 0.03-0.38]). All these patients underwent a major surgical procedure, and received an additional bolus infusion with VWF/FVIII concentrate to correct lower levels. With regard to *FVIII*, median trough and steady state was 1.46 IU/mL (IQR 1.14-1.82) in this time period. Ninety-two percent of measured levels were above predefined target level, with a median deviation of 0.70 IU/mL (IQR 0.43-1.07). Only in five patients (9%) *FVIII* was below the predefined target level. All received additional treatment: in four patients this consisted of VWF/FVIII concentrate, and in one patient of intravenous desmopressin. In the period from *36 hours until 72 hours after surgery*, all trough and steady state *FVIII* levels were above FVIII target level (median FVIII 1.80 IU/mL [IQR 1.35-2.11]).

Overall, no differences in achieved VWF:Act and FVIII were observed for minor versus major surgical procedures, blood group non-O versus O, adults versus children and between modes of infusion (data not shown). Moreover, high VWF:Act and FVIII levels (defined as >0.20 IU/mL above target) were predominant as illustrated by the fact that 65% of trough and steady state VWF:Act levels and 91% of FVIII values were above target.

Table 1. General characteristics of the study population

	N (%) or median [IQR]			
Patients	**Total**		**Type 1**	
No. of patients	103	(100)	54	(100)
Sex (females)	69	(67)	38	(70)
Age (years)	51	[36-62]	52	[40-61]
Height (cm)	175	[167-180]	173	[166-179]
Body weight (kg)	77	[65-85]	79	[68-89]
Body mass index (kg/m^2)	24.9	[22.7-28.1]	25.4	[23.6-29.1]
Blood group O	51	(50)	32	(59)
Baseline VWF/FVIII levels*				
VWF antigen (IU/mL)	0.28	[0.21-0.38]	0.30	[0.22-0.38]
VWF activity (IU/mL)	0.14	[0.10-0.25]	0.22	[0.13-0.30]
FVIII (IU/mL)	0.44	[0.28-0.60]	0.54	[0.34-0.69]
Surgery				
No. of surgical procedures	148	(100)	73	(100)
Total number of patients undergoing				
1 procedure	75	(73)	41	(76)
2 procedures	16	(15)	7	(13)
3 procedures	10	(10)	6	(11)
≥ 4 procedures	2	(2)	0	(0)
Severity of surgical procedure				
Minor	38	(26)	13	(18)
Major	110	(74)	60	(82)
Treatment				
Duration of hospitalization (days)	6	[4-8]	6	[4-8]
Type of infusion				
Bolus infusion	133	(90)	64	(88)
No. of complications				
Bleeding	20	(14)	12	(16)
Re-operation	1	(5)	0	(0)
Hemoglobin drop ≥1.24 mmol/L and/or RBCTF	19	(95)	12	(100)
Prolonged hospitalization	0	(0)	0	(0)
Thrombosis	0	(0)	0	(0)

^24 type 2A, 7 type 2B, 3 type 2N and 9 type 2M patients. *Historically lowest measured VWF/FVIII levels. #VWF:Act measurements were lower than the limit of detection 0.10 IU/mL in a number of VWD type 3 patients. No.= number (percentages); Median [IQR = Interquartile range 25-75%]; cm = centimeter; kg = kilogram; kg/m^2 = kilogram per square meter; VWF = von Willebrand factor; IU/mL= international units per milliliter; mmol/L= millimol per liter RBCTF = red blood cell transfusion

	Type 2		Type 3	
	43	(100)^	6	(100)
	27	(63)	4	(67)
	53	[36-66]	22	[16-33]
	175	[165-183]	179	[168-180]
	75	[62-83]	74	[65.7-78.5]
	24.2	[21.7-25.7]	22.5	[21.2-26.2]
	16	(37)	3	(50)
	0.29	[0.22-0.39]	0.05	[0.01-0.06]
	0.10	[0.05-0.15]	0.10	[0.04-0.10]#
	0.42	[0.24-0.57]	0.03	[0.02-0.08]
	67	(100)	8	(100)
	29	(67)	5	(83)
	9	(21)	0	(0)
	3	(7)	1	(17)
	2	(5)	0	(0)
	18	(27)	7	(88)
	49	(73)	1	(12)
	6	[3-9]	7	[3-8]
	62	(93)	7	(88)
	8	(12)	0	(0)
	1	(11)	0	(0)
	7	(89)	0	(0)
	0	(0)	0	(0)
	0	(0)	0	(0)

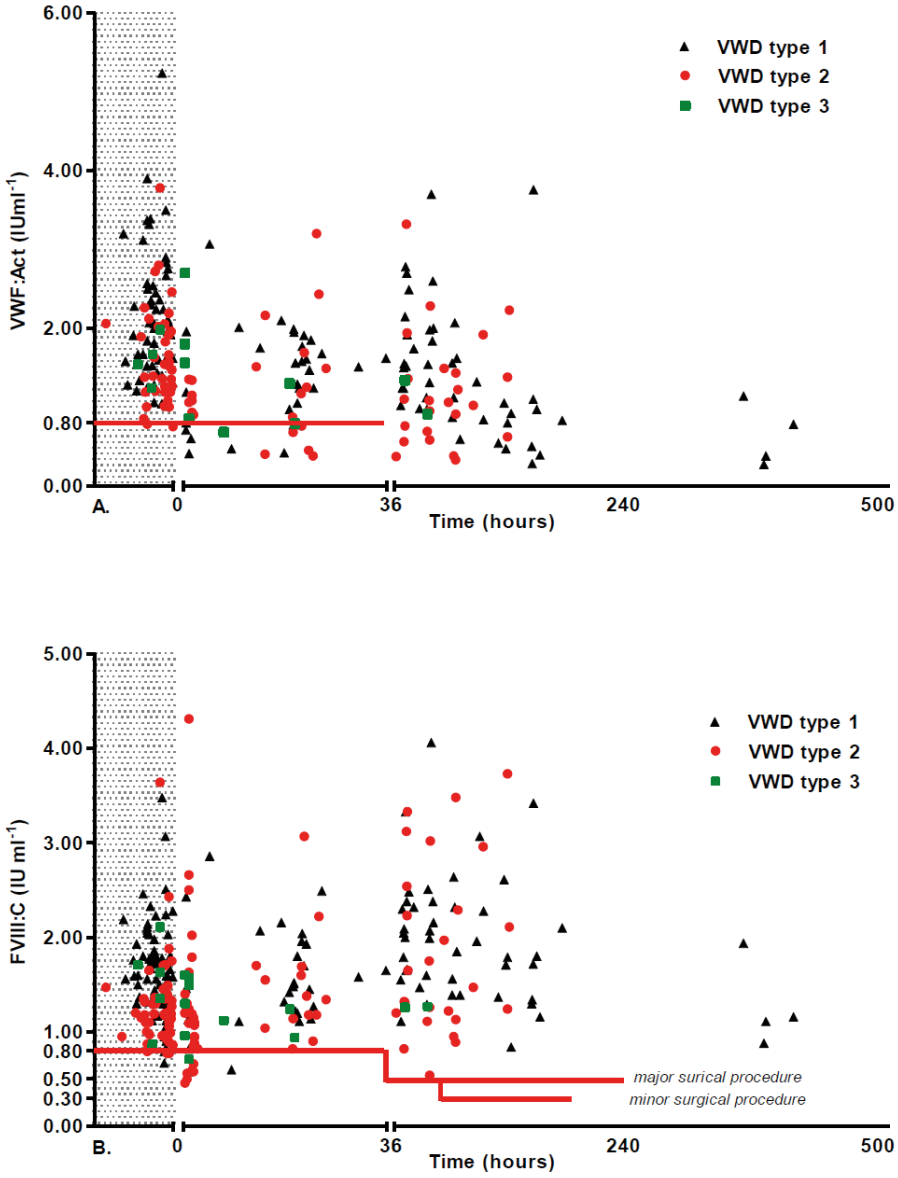

Figure 2. Achieved VWF: Act and FVIII levels in the perioperative period. The red lines indicate predefined target VWF:Act and FVIII according to National guidelines[3]. Preoperative peak VWF:Act and FVIII levels are shown < 0 hours. Postoperative trough and steady state VWF:Act and FVIII measurements are shown after surgery. Time of surgical procedure was defined as t=0 hours. A) Achieved VWF:Act and B) Achieved FVIII levels. No differences in achieved VWF:Act and FVIII are observed between types of VWD.

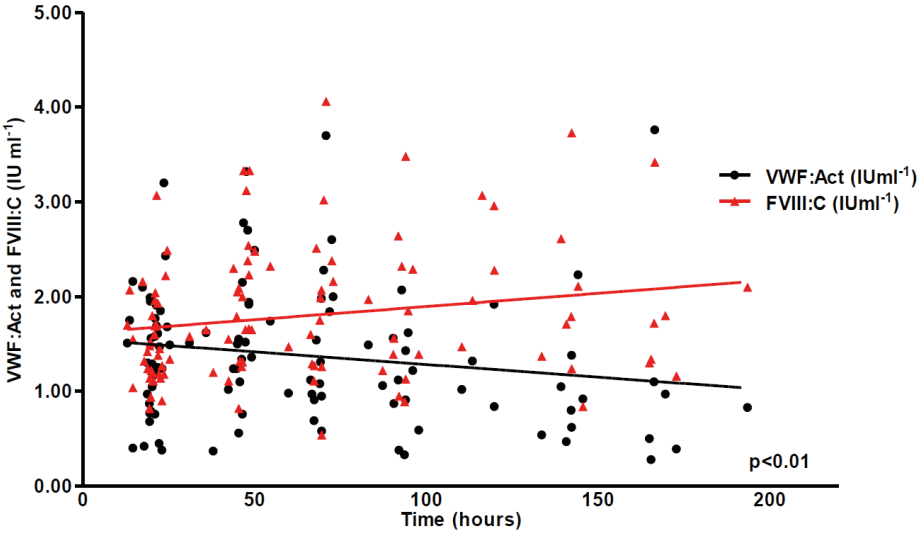

Figure 3. Accumulation of FVIII after repetitive dosing of VWF/FVIII concentrate*. Accumulation of FVIII was present after repetitive dosing of VWF/FVIII concentrates, resulting in increased FVIII in comparison to VWF:Act (p<0.01) (F=6.90 DFn=1, DFd=209); *Haemate P®

In the 43 type 2 and 6 type 3 VWD patients, 62% and 71% of trough *VWF:Act* levels were above predefined target level in the *first 36 hours after surgery* (not significantly different from type 1 VWD). Median *VWF:Act* in this period was 1.07 IU/mL [IQR 0.68-1.50] and 1.30 IU/mL [IQR 0.82-1.68], respectively. Eighty-six percent and 89% of trough *FVIII* were above target in the first 36 hours with a median deviation of 0.40 IU/mL [IQR 0.26-0.85] and 0.47 IU/mL [IQR 0.28-0.71], respectively for type 2 and type 3 VWD patients. In addition, all *FVIII* were above target *after 36 hours* of hospitalization for both minor and major surgical procedures. High VWF:Act and FVIII (≥0.20 IU/mL) were present in 53% and 57% of VWF:Act and in 72% and 73% of FVIII for type 2 and type 3 VWD patients respectively.

Bleeding complications

Overall, occurrence of bleeding complications was not associated with a low trough VWF:Act and/or low FVIII (p=0.95 and 0.25 respectively). Exception was one patient, undergoing a craniotomy with excessive blood loss with need for blood cell transfusions and presenting with lower trough VWF:Act (0.40 IU/mL) and FVIII (0.60 IU/mL) levels (Table 2). Clinically relevant bleeding only occurred in 5 (3.4%) surgical procedures, as four surgical procedures required red blood cell transfusion postsurgery and only one a second surgical intervention (Table 2). Despite excessive FVIII levels, no thrombotic

complications were reported. Of the 18 patients reaching very high (>2.70 IU/mL) FVIII levels, 61% received thromboprophylaxis with low molecular weight heparin.

Treatment

Two type 1 VWD patients received only desmopressin prior to surgery in order to achieve VWF:Act and FVIII target levels. After surgery, trough VWF:Act and FVIII were 0.56/0.55 and 0.59/0.48 IU/mL, respectively. Consecutively, the treating physician administered VWF/FVIII concentrate on following postoperative days. Four type 1 VWD patients received desmopressin as well as Haemate P before start of surgery. In the postoperative period, desmopressin was administered in 7 type 1 VWD patients and 1 type 2A VWD patient.

In type 1 and type 2 VWD patients, median loading dose for minor and major surgical procedures did not differ (Figure 4). In type 1 VWD patients, maintenance dose on day 1 (0-24 hours) after surgery differed between minor and major procedures with a significantly higher dose in cases of minor surgery (33 $IUkg^{-1}$ and 26 $IUkg^{-1}$ respectively, p=0.048). No differences between minor and major surgical procedures were observed for loading and maintenance doses in type 2 VWD. Loading dose and maintenance doses did not differ between type 1 and type 2 VWD patients, as median for loading doses were 36 $IUkg^{-1}$ [IQR 27-49] and 43 $IUkg^{-1}$ [IQR 37-52], p=0.12, and median maintenance doses ranged from 22-27 $IUkg^{-1}$ and 21-35 $IUkg^{-1}$. Patients who underwent a minor procedure were generally treated with VWF/FVIII concentrate for a median duration of 48 hours. Median duration of hospitalization for patients undergoing a minor or major surgical procedure did not differ significantly (respectively 4 [IQR 4-8] versus 6 [IQR 4-8] days, p=0.88).

Predictors of low and high VWF:Act and FVIII levels

It was only possible to evaluate predictors in type 1 and type 2 VWD patients, due to a limited number of type 3 patients. This was performed for both VWF:Act and FVIII by both stepwise backward logistic regression analysis as well as stepwise forward logistic regression analysis. In type 1 VWD, in the total postoperative period, only blood group O was predictive of high VWF:Act levels (VWF:Act levels ≥0.20 IU/mL above target) (OR 2.9; 95%CI [1.3-6.6]); not of high FVIII levels. No other predictors were found for low and high VWF:Act and FVIII levels in both type 1 and type 2 VWD patients.

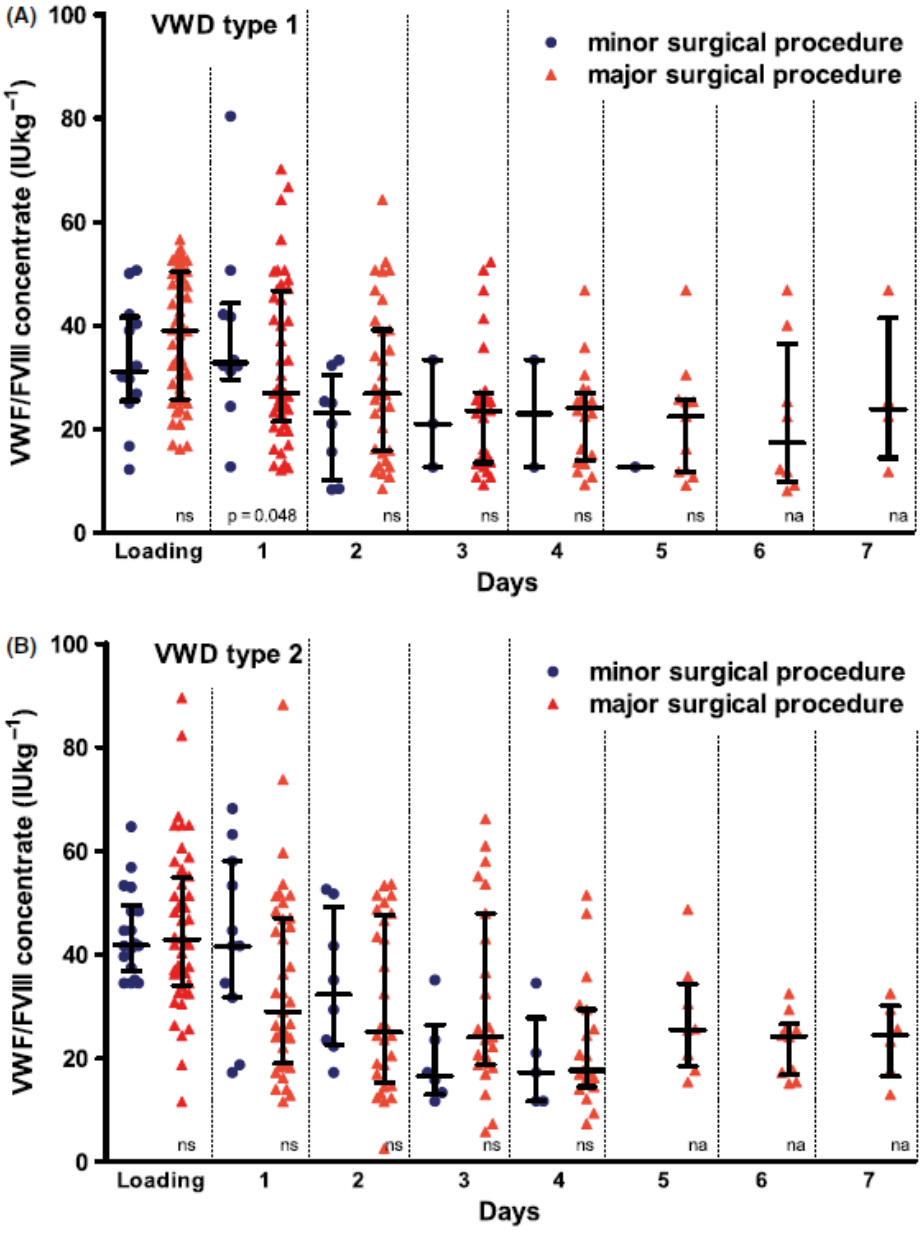

Figure 4. Loading and maintenance doses in minor and major surgical procedures in type 1 and type 2 VWD patients. Loading and maintenance doses in minor and major surgical procedures are shown using a scatter dot plot with median and 5-95% quartile ranges for A. Type 1 and B. Type 2 VWD patients. The non-parametric Mann-Whitney U test was used to compare VWF/FVIII concentrate consumption between minor and major surgical procedures.

Table 2. Characteristics of perioperative VWD patients with a clinically relevant bleeding complication

Patient	VWD type	Age (years)	Surgical procedure	Complication (No.)	Day of complication	Hb drop (mmol/L)
1	2A	50	Cervical conisation	Reoperation	Day 1	8.0 - 6.7
2	1	14	Craniotomy	RBCTF (6)	Day 0	7.5 - 4.8
3	1	46	Adrenalectomy	RBCTF (5)	Day 0	6.1 - 4.5
4	1	34	Aortic valve replacement & resection aneurysm	RBCTF (3)	Day 0	7.4 - 4.3
5	1	61	Total hip replacement	RBCTF (1)	Day 2	7.4 - 5.6

No. = number; mmol/L = millimol per liter; IU/mL = international units per milliliter; VWF: Act = VWF activity; RBCTF = red blood cell transfusion; NA = not applicable; TXA = tranexamic acid; DDAVP = desmopressin; *Trough VWF:Act and FVIII measurements at time of occurrence of the bleeding complication.

DISCUSSION

This study is the largest so far evaluating perioperative management of VWD patients in a resource rich country. We present data that underline the complexity of VWF/FVIII concentrate dosing in this patient population, as illustrated by the fact that in type 1 VWD patients, 65% of trough and steady state VWF:Act and 91% of FVIII levels were ≥0.20 IU/mL above predefined target levels. In type 2 and type 3 VWD respectively 53% and 57% of VWF:Act and 72% and 73% of FVIII were ≥0.20 IU/mL above predefined target levels. In contrast to results in perioperative severe and moderate hemophilia A patients (29), only a small percentage of VWD patients experienced low levels in the first 36 hours after surgery, as only 16% of VWF:Act levels in type 1 VWD and 38 and 29% of VWF:Act levels in type 2 and 3 VWD patients respectively and only 8%, 14% and 11% of FVIII levels in respectively type 1, type 2 and type 3 VWD were below prescribed target level. This is probably due to FVIII-based dosing performed according to the Dutch national guidelines applied in this study. Although both VWF:Act and FVIII were measured perioperatively, VWF:Act was not directly available in most cases and could not be used to monitor perioperative VWF/FVIII concentrate management. In our cohort, prevalence of clinically relevant bleeding complications was low (3.4%) and was not associated with achieved VWF:Act and/or FVIII. This is supported by others (9, 13, 15, 16,30) and confirms that other causal factors for bleeding than VWF:Act and FVIII, either haemostatic or surgical must be involved. In this study, no predictors of bleeding could be identified. Strikingly, blood group O was predictive of high VWF:Act levels (≥0.20 IU/mL above target) in type 1 VWD in the total postoperative period.

Preoperative peak VWF:Act (IU/mL)	Preoperative peak FVIII (IU/mL)	Trough* VWF:Act (IU/mL)	Trough* FVIII (IU/mL)	Surgical blood loss (mL)	Other medication	Blood group
1.63	1.19	NA	NA	0	TXA	Non-O
NA	3.07	0.40	0.60	2800	DDAVP	Non-O
1.6	1.12	NA	NA	4000	Heparin	O
NA	NA	1.84	1.14	0	TXA, DDAVP	Non-O
1.04	1.18	1.99	1.51	425	None	O

Most probably this is explained by lower endogenous baseline VWF:Act and FVIII levels resulting in administration of higher dosages of VWF/FVIII concentrates. A limitation of this retrospective study, depicting real-life data, is that in the different centres, different assays were used and may have been altered during the study period. Therefore, one should keep in mind that inter-assay variability may have influenced the generalizability of the results in terms of plasma FVIII and VWF levels. Furthermore, as no clear definition of regular monitoring is given in the guidelines, amount and timing of FVIII and VWF:Act measurements differed between occasions. When evaluating only major surgical procedures, VWF:Act and/or FVIII was measured <24 hours before surgery in 89% of occasions, with emergency surgery as a partial explanation for the missing measurements. In 78% of occasions, VWF:Act and/or FVIII was measured at least once within 24 hours after start of the procedure, and in 57% of occasions within 24-48 hours after start of surgery, if the patient was still hospitalized. There were no clear differences in the amount or timing of the measurements between centres.

Analyses were performed for the total VWD population as well as separately for each type of VWD, as it has been shown that clearance mechanisms of the endogenous VWF differ between VWD types (2, 5). However, no differences were found in achieved VWF:Act and FVIII level after preoperative loading and subsequent maintenance doses between type 1 and type 2 VWD. Also, VWF/FVIII consumption did not differ between types of VWD. Counterintuitively, on day 1 (0-24 hours) after surgery, a significantly higher VWF/FVIII concentrate consumption was observed for minor surgical procedures when compared to major surgical procedures. This is probably explained by the fact that patients undergoing a minor surgical procedure received less

frequent but higher dosed bolus infusions within a shorter period of time. This finding is supported by a previous study in 29 type 1, 2A, 2M and 3 VWD patients in which no differences in concentrate consumption between patients undergoing minor or major surgical procedures was observed (13).

In this perioperative study, accumulation of FVIII was observed after repetitive dosing of VWF/FVIII concentrate, with median FVIII values increasing with time (Figure 3). Increasing FVIII levels, due to concomitant increase of both endogenous and exogenous FVIII, were significantly higher than VWF:Act levels ($p<0.01$). This may be partly explained by findings by Kahlon et al. (31) who observed an intraoperative decrease and postoperative increase of VWF and FVIII levels in 30 individuals without a bleeding disorder undergoing surgery. In these healthy individuals, mean VWF:Act and FVIII levels were greater than 1.00 IU/mL at all intra- and postoperative time points. This physiological response to surgery may reflect an increased need of VWF in the perioperative period. Current guidelines are not in line with these physiological responses to surgery, as perioperative target VWF:Act and FVIII levels are >0.80 IU/mL (0-36 hours postoperatively) and >0.30/0.50 IU/mL (36-240 hours postoperatively) and thus below 1.00 IU/mL.

Although we observed high FVIII levels that confer a possible risk for thrombosis (22, 32, 33), no thrombo-embolic complications were observed. Previously, Wells et al. demonstrated that FVIII levels above 2.70 IU/mL are associated with a higher risk of thrombosis in non-surgical patients (21). In our study, 8% of trough levels of FVIII were above 2.70 IU/mL. Also, observed postoperative VWF:Act and FVIII levels were increased for only a brief period of time and coincide with physiological levels in healthy individuals without a bleeding disorder (31). Mannucci et al. also reported this scarcity of thrombosis in perioperative VWD patients on replacement therapy (32). In our study, it must also be taken into account that almost half of patients undergoing a major surgical procedure received thromboprophylaxis with low molecular weight heparin.

As reported, plasma derived VWF/FVIII concentrate in this study (Haemate P ®), has a VWF:Act/FVIII ratio of 2.4:1 and contains large amounts of high molecular-weight multimers, which are thought to be the most hemostatically potent multimers. Earlier, in vivo recovery (IVR) studies have demonstrated a median IVR of 2.0 for VWF:Act and FVIII, implying a rise of approximately 0.02 IU/mL in VWF:Act and FVIII for each infused IUkg^{-1} for VWF:Act or FVIII. Theoretically, for each infused IUkg^{-1} of FVIII an increase of approximately 0.05 IU/mL VWF:Act will be observed (2.4*0.02 IU/mL). Currently, it is common practice to apply IVR to dose and monitor replacement therapy (13, 15).

However, dosing based on body weight and IVR does not take interindividual differences in clearance and volume of distribution into account that are associated with half-life of VWF/FVIII concentrates. Personalized perioperative dosing based on IVR deducted from a preoperative PK profile is not possible, as PK profile is not representative for clearance during surgery as shown by Di Paola et al. (12). In this study, only a weak correlation was shown between IVR values of VWF:Act obtained one week prior to surgery and IVR values obtained directly after surgery (n=41; r=0.41) (12). Both the changes of IVR following surgery and differences in half-life between VWD types demonstrate the complexity and importance of development of alternative dosing algorithms to individualize treatment for each VWD patient. Hypothetically, VWD population PK models will be able to incorporate these differences between VWD types due to: mutational variation, differences in baseline values of endogenous VWF:Act and FVIII, higher FVIII levels with a longer half-life (34), differences in clearance of endogenous and exogenous VWF:Ag and VWF:Act, and differences in composition of administered VWF/FVIII concentrates. Also, other known and unknown modifying factors that influence clearance and volume of distribution in an on demand-, perioperative setting can be incorporated. The development of such models will lead to Bayesian adaptive dosing to predict VWF:Act and FVIII and effects of treatment more precisely. In the long run, we believe such an approach will optimize patient care and potentially reduce overall costs of treatment by reduction of the amount of total infused clotting factor concentrate (13, 32, 35-37). Therefore, PK-guided dosing forms a promising approach for more efficient and individualized replacement therapy in VWD with considerable clinical and economic impact due to the frequency of this bleeding disorder.

CONCLUSION

Although perioperative replacement therapy in VWD patients is successful with few bleeding complications, it can be optimized as patients are currently over treated with accumulation of FVIII as a consequence, fortunately without thrombotic complications. Due to the complexity of treatment in VWD, we hypothesize that population PK models, which incorporate known and unknown modifying factors of clearance and other PK parameters of VWF/FVIII concentrates, may be promising tools for personalization of replacement therapy in all VWD patients.

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Part II

Individualizing treatment by population pharmacokinetic modelling


5

Chapter 5

Quantification of the relationship between desmopressin concentration and von Willebrand factor in von Willebrand disease type 1: a pharmacodynamic study

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ABSTRACT

Introduction

Desmopressin can be used to prevent bleeding in von Willebrand disease (VWD), but the relationship between desmopressin and von Willebrand factor activity (VWF: Act) has yet to be quantified.

Aim

To quantify the relationship between desmopressin dose, its plasma concentration and the VWF: Act response in type 1 VWD patients.

Methods

Forty-seven VWD patients (median age 25 years, IQR: 19-37; median body weight 71 kg, IQR: 59-86) received an IV desmopressin dose of 0.3 mcg/kg. In total, 177 blood samples were available for analysis. We developed an integrated population pharmacokinetic-pharmacodynamic (PK-PD) model using nonlinear mixed effect modelling. Subsequently, we performed Monte Carlo simulations to investigate the efficacy of the current dosing regimen.

Results

A one-compartment PK model best described the time profile of the desmopressin concentrations. In the PD turnover model, the relationship between desmopressin plasma concentration and release of VWF: Act from the vascular endothelium was best described with an Emax model. Typically, VWF: Act increased 452% with an EC50 of 0.174 ng/ml. Simulations demonstrated that after 0.3 mcg/kg desmopressin intravenously, >90% patients with a VWF: Act baseline of ≥0.20 IU/mL attain a VWF: Act >0.5 IU/mL up to ≥4 hours after administration. A capped dose of 30 mcg was sufficient in patients weighing over 100 kg.

Conclusion

The relationship between desmopressin and VWF: Act was quantified in a PK-PD model. The simulations provide evidence that recently published international guidelines advising an intravenous desmopressin dose of 0.3 mcg/kg with a capped dose of 30 mcg >100 kg gives a sufficient desmopressin response.

INTRODUCTION

Von Willebrand disease (VWD) is the most common inherited bleeding disorder and is caused by a deficiency or qualitative defect of von Willebrand factor (VWF) (1). VWF is a plasma glycoprotein which plays a crucial role in primary haemostasis by promoting platelet adhesion to the subendothelium at sites of vascular injury and by initiating platelet aggregation. Subsequently it also plays a role in secondary haemostasis by protecting factor VIII (FVIII) from proteolysis in the circulation, safeguarding thrombin and fibrin generation (2). VWD is classified into three main types based on a partial or complete quantitative defect of VWF (type 1 and 3) or a qualitative defect in VWF (Type 2) (2). Type 1 consists of patients with VWF lower than 0.30 IU/mL or between 0.30 and 0.50 IU/mL, with abnormal bleeding (3).Type 2 is further divided into the subtypes 2A, 2B, 2M and 2N. Risk of bleeding as well as treatment choice depends on VWD type, although inter-individual variation in bleeding tendency and response to treatment is notably large in VWD.

Desmopressin (1-deamino-8-d-arginine vasopressin) is a synthetic analogue of the antidiuretic hormone l-arginine vasopressin (4). Desmopressin binds to V2 receptors and thereby induces the release of endogenous VWF from vascular endothelial cells (5, 6). Desmopressin can be used to prevent bleeding during surgical procedures in most type 1 VWD patients and in some patients with type 2A, 2M, and 2N VWD (7). The most recent advice is to always perform a desmopressin test in VWD patients with baseline VWF activity <0.30 IU/mL, in order to quantify the VWF response (3). The use of desmopressin is contraindicated in type 2B VWD as it may induce thrombocytopenia. Desmopressin is not effective in type 3 VWD.

Recently published international guidelines recommend an intravenous desmopressin dose of 0.3 mcg/kg, with a capped dose of 20-30 mcg (3, 8). This recommendation is, however, solely based on empirical evidence. It is unclear if the variability in pharmacokinetics (PK) of desmopressin contributes to the consecutive observed variability in VWF response, or pharmacodynamic (PD) effect. Furthermore, proposed capping of dosing, i.e. applying a fixed dose independent of body weight when 0.3 mcg/kg exceeds 20-30 mcg, has never been substantiated by pharmacological evidence. Population PK-PD modelling can be used to establish this concentration-effect relationship (9, 10). We developed a population PK-PD model to evaluate and quantify the concentration-effect relation of desmopressin on the VWF activity (VWF: Act) response in type 1 VWD. The aim of this study was to investigate if current treatment guidelines -including capped dosing- can be substantiated with this novel PK-PD model.

PATIENTS AND METHODS

Patients

VWD patients (historical lowest VWF antigen (VWF: Ag) and/or VWF: Act < 0.50 IU/mL) with abnormal bleeding and/or a family history of VWD were included if a desmopressin test was performed at the Erasmus MC or Erasmus MC - Sophia Children's Hospital Rotterdam, the Netherlands, between April 1st 2011 and July 1st 2014. The 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 Centre Rotterdam. All patients provided written informed consent.

Blood sampling

Residual stored plasma samples from a prospective single-center cohort study, investigating desmopressin side effects, were obtained (11). All patients signed informed consent before data and samples were collected.

Desmopressin test protocol

In all patients, desmopressin was administered intravenously in a dose of 0.3 µg/kg dissolved in 30 or 50 mL of NaCl 0.9% in children and adults respectively and infused in 30 minutes. In children, blood was sampled prior to (T0) desmopressin infusion, and at 1 (T1), 2 (T2), 4 (T4) and 6 (T6) hours after infusion. In adults, blood was sampled at T0, T1, T3, T6 and T24.

Laboratory measurements

Venous whole blood was collected in 0.105M sodium citrate tubes and centrifuged twice at 2.200 g for 10 minutes at room temperature and stored at -80°C. Coagulation factor measurements were performed within a few days after sample collection. VWF: Ag was measured by ELISA and VWF: Act was measured by GpIbα binding assay (HemosIL™ von Willebrand Factor Activity; Instrumentation Laboratory BV, Breda, the Netherlands). FVIII activity (FVIII: C) was measured by one-stage clotting assay. Desmopressin plasma concentrations were assessed in the Amsterdam UMC using LC-MS/MS in positive ionisation mode on a Shimadzu LC-30 (Nishinokyo-Kuwabaracho, Japan) UPLC system coupled to an ABSciex (Framingham, MA, USA) API5500Q LC-MS[12]. The method was validated over a range of 0.0200 – 4.00 ng/mL. The accuracy ranged from 89.2% to 111.8% across the validated range, with intra-day and inter-day imprecision below 17.6% and 13.8%, respectively.

Software

Nonlinear mixed-effects modelling software (NONMEM 7.3 ICON Development Solutions, Hanover, MD, USA) and Pirana (version 2.9.4), R (version 3.6.1) and PsN version (version 4.6.0) were used for the PK-PD analysis.

Pharmacokinetic modeling

We performed a sequential PK-PD analysis. During PK model development, both one- and two-compartment models were evaluated. *A priori* allometric scaling of PK parameters by body weight was included in the structural PK model. Inter-individual variability (IIV) was estimated for each population PK model parameter. Various residual error models were evaluated. Next, associations between specific covariates and PK parameters were tested in order to explain the IIV in these parameters, by using a stepwise approach. The following covariates were evaluated: age, sex, height, baseline FVIII, baseline VWF: Act, baseline VWF: Ag and blood group (O, non-O). The supplement contains more details about the development of the PK model.

Pharmacodynamic modeling

We used individual post-hoc PK parameter estimates as input for the PD model. In literature, the maximum effect of desmopressin occurs approximately 1 hour after the end of intravenous administration (13). We modelled the time lag using a turn-over model (Figure 1) (14). The turn-over model consists of a zero-order rate constant describing the constant release of VWF from the vascular endothelium (K*in*) and a first-order rate constant for loss of VWF (K*out*) from plasma. The baseline VWF: Act (BASE) of each patient is determined by the equilibrium of K*in* and K*out* and was fixed at the VWF: Act level as determined before the desmopressin administration.

In the PD analysis, the relationship between the increase in VWF release (K*in*) and desmopressin plasma concentration was quantified by a linear function, Emax function and sigmoidal Emax function. IIV was estimated for the PD parameters, and various residual error models were evaluated. The covariates as mentioned under the PK analysis were tested for correlation with the PD parameters. The supplement contains more details on the development of the PD model.

Pharmacokinetic-pharmacodynamic model evaluation

Model selection criteria were based on the change in the objective function value (OFV), goodness-of-fit (GOF) plots, precision of parameter estimates, decreases in IIV and residual variability, condition number, shrinkage and a successful convergence step, with at least three significant digits in parameter estimates (16).

Visual predictive checks (VPCs) with 1000 simulated data sets were used to assess the predictive performance of the model. The 5th, 50th, and 95th percentiles of the predictions from the simulations and observations from the original dataset were derived and plotted against time. A non-parametric bootstrap was performed to assess parameter precision and to calculate confidence intervals (CI) for both the population PK and PD parameters. The 5th and 95th percentiles of the bootstrap parameter distribution constitute the 90% CI.

Monte Carlo simulations

Using the final population PK and PD models, Monte Carlo simulations were performed for 1000 patients (females and males) with body weights of 50, 70, 100 and 130 kg to investigate if recently published international desmopressin guidelines (3) can be substantiated by the constructed PK-PD model. Moreover, we investigated whether dosing can be simplified by capping of desmopressin dosing when 0.3 mcg/kg dosing exceeds the 20-30 mcg cap in patients >100 kg.

All virtual patients had a baseline VWF: Act of 0.20 IU/mL. VWF: Act time profiles were simulated and desmopressin doses of 5, 10, 15, 21, 25, 30, 35 and 39 mcg were administered in all patients. A patient was considered a responder if VWF: Act levels were greater than 0.50 IU/mL at 4 hours after desmopressin administration. For each body weight and dose, the percentage of responders was calculated. Treatment was considered effective when >90% of the simulated patients of each body weight were responders.

RESULTS

Patients

The study population consisted of 47 patients, 15 males and 32 females with type 1 VWD. The median age was 25 years and body weight was 71 kg. Further characteristics are summarized in Table 1.

Pharmacokinetic analysis

A total of 177 desmopressin plasma concentrations were available. A one-compartment model adequately described the PK of desmopressin. IIV could be estimated for clearance (CL) and volume of distribution (V), which resulted in a significant (p <0.05) decrease in OFV. The residual variability was described by a combined (proportional + additive) error model.

During covariate model selection, inclusion of the following covariates significantly improved the fit of the PK model to the data (p <0.05): sex on CL and sex, baseline FVIII, baseline VWF: Ag and baseline VWF: Act on V. The association between sex and V produced the largest improvement in model fit (p < 0.001): V was 22% higher in females compared to males. After inclusion of sex in the model, the remaining significant covariates were added one-by-one. However, no improvement of the model was observed (p > 0.05).

The goodness-of-fit plots showed sufficient agreement between predicted and observed desmopressin concentrations (Figure S1). The VPC of the final model is presented in Figure S2. Overall, the 2.5th, 50th and 97.5th percentiles of observed concentrations were mostly within the predicted 95% confidence interval (CI) of the predicted percentiles. The median values of the parameter estimations of the bootstraps were approximately equal to the final model's respective values (Table 2).

Table 1. Patient characteristics

	N=47 **Number or median [IQR]**
Sex (female)	32
Age (years)	25 [19 – 37]
Body weight (kg)	71 [59 – 86]
Height (cm)	167 [160 – 177]
Historical lowest VWF: Act (IU/mL) Historical lowest VWF: Ag (IU/mL)	0.46 [0.34 – 0.51] 0.43 [0.35 – 0.49]
Baseline (T0) VWF: Act (IU/mL) Baseline (T0) FVIII (IU/mL) Baseline (T0) VWF: Ag (IU/mL)	0.48 [0.41 – 0.60] 0.59 [0.51 – 0.71] 0.45 [0.39 – 0.0.59]
Blood group (n)* Non O O	 13 32
Bleeding score (ISTH-BAT) at diagnosis Blood group non O Blood group O	 5 [2 – 6] 4 [1 – 6]

IQR = interquartile range; VWD = Von Willebrand disease; VWF = Von Willebrand factor; FVIII = factor VIII. *Blood group data were unknown in 2 patients.

Table 2. Desmopressin population pharmacokinetic parameters

Parameter	Final model Values (RSE%) [Shrinkage %]	Bootstrap Median value [95% CI]
CL (L/h/70 kg)	9.43 (5)	9.48 [8.48 – 10.3]
V (L/70 kg)	25.9 (11)	26.1 [21.1 – 32.5]
(%) Increase V in females	22.0 (10)	20.6 [4.11 – 49.2]
Inter-individual variability		
CL(CV%)	31.7 (16) [4]	30.7 [21.3 – 41.7]
V (CV%)	36.3 (18) [11]	35.0 [20.4 – 46.7]
Covariance CL~V	0.0705	0.0683 [0.0128 – 0.0131]
Residual variability		
Proportional error (CV%)	1.22 (12)	1.18 [0.869 – 2.00]
Additive error (ng/mL)	0.146 (13)	0.145 [0.0890 – 0.184]

CL = clearance; V = central volume of distribution; CV = coefficient of variation; RSE = relative standard error; CI = confidence interval; CV was calculated as: CV = sqrt(exp(variance)-1) x 100%; RSE was calculated as: RSE = 100 × standard error/parameter estimate.

Pharmacodynamic analysis

A total of 177 VWF: Act levels were available. The time profile of VWF: Act was described using the turn-over model shown in Figure 1. In the modelling procedure BASE (baseline VWF: Act) was fixed to individual baseline VWF: Act values (Table 1). The performance of several PD functions describing the relationship between VWF release and desmopressin concentration was tested (i.e. a linear function, Emax function, and sigmoid Emax function): The relationship between the VWF release and desmopressin concentration was best described with an Emax function (supplement eq. 10). We attempted to estimate the value of BASE, but this did not result in successful convergence of the model. Implementation of IIV on Emax significantly improved the model (p <0.001). Residual variability was best described by an additive error model. No significant relationship was found between covariates and PD parameters. Baseline VWF release (K*in*) was typically increased by 452% with an EC50 of 0.174 ng/ml (Table 3). The IIV of Emax was modest with a value of 29.1%. In the concentration-effect curve, the EC90 was reached at a desmopressin concentration of 0.314 ng/mL. Figure 2 displays the time profile of the desmopressin plasma concentration, PD effect and VWF: Act for a typical patient of 70 kg receiving 0.3 mcg/kg desmopressin.

Goodness-of-fit plots showed good agreement between predicted and observed VWF: Act concentrations (Figure S1). The VPC plots in Figure S2 show that the observed VWF: Act values are well-centred around the predicted median of the PD model. The bootstrap median and confidence intervals are comparable to the parameter estimates (Table 3).

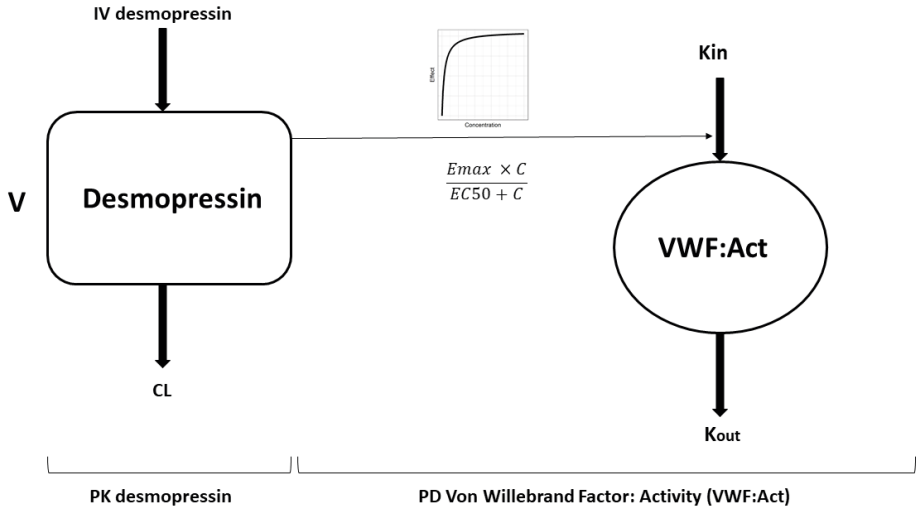

Figure 1. A schematic representation of the PK-PD model relating desmopressin concentration to VWF: Act. V represents the volume of distribution, CL represents the clearance, C the plasma concentration of desmopressin, Emax the maximum effect, EC50 the concentration at half maximal effect, *Kin*, the zero-order constant for release of VWF: Act by the endothelium and *Kout* the first-order rate constant for loss of VWF: Act, IV = intravenous.

Table 3. Population pharmacodynamic parameters

Parameter	Final parameter Values (RSE%) [Shrinkage %]	Bootstrap median [95% CI] of parameter value
Kout (h^{-1})	5.66 (4)	5.66 [4.71 – 6.81]
EC50 (ng/mL)	0.174 (26)	0.178 [0.107 – 0.277]
Emax	4.52 (10)	4.54 [3.80 – 5.55]
Inter-individual variability		
Emax (CV%)	29.1 (10) [11]	28.8 [22.2 – 34.1]
Residual variability		
Additive error (IU/mL)	0.238 (11)	0.235 [0.183 – 0.282]

Kout = first-order rate constant for loss of VWF: Act; Emax = maximum effect; EC50 = drug concentration which produces 50% of the maximal effect; CV, coefficient of variation; RSE = relative standard error; CI = confidence interval; CV was calculated as: CV = sqrt(exp(variance)-1) x 100%; RSE was calculated as: RSE = 100 × standard error/parameter estimate.

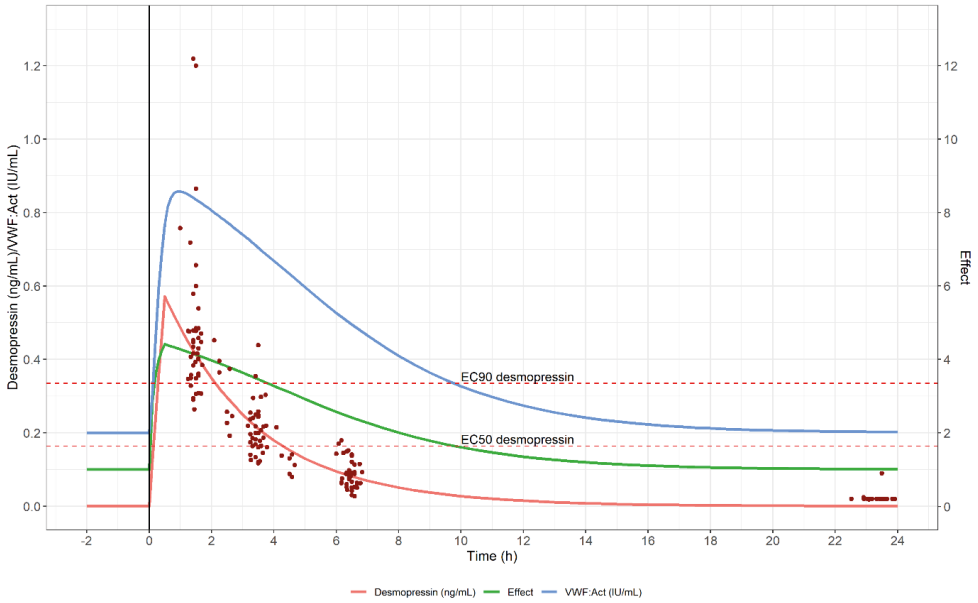

Figure 2. Time profiles of desmopressin plasma concentration, the PD effect and VWF: Act for a typical patient weighing 70kg with a VWF: Act baseline of 0.20 IU/mL. The red line represents the typical plasma desmopressin concentration, the red dots represent the observed concentration in all individual patients. The green line depicts the effect of desmopressin starting at unity (no effect) with a maximum value of 5.8. The blue line depicts the VWF: Act response on basis of the turnover model.

Monte Carlo simulations

The simulated dosage regimens targeting VWF: Act levels above 0.50 IU/mL at 4 hours after desmopressin administration are shown in Figure 3. Figure 3 displays the percentage responders against various dosage regimens for patients with a body weight of 50, 70, 100 and 130 kg. For patients weighing 50 kg, a dose of 15 mcg was necessary to attain a sufficient response in 92% of patients. For patients weighing 70 kg, a dose of 21 mcg was necessary to attain a sufficient response in 93% of patients. Patients with a body weight of 100 kg needed a dose of 25 mcg to attain a sufficient response in 92% of patients. Finally, Patients with a body weight of 130 kg needed a dose of 30 mcg to attain a sufficient response in 91% of patients.

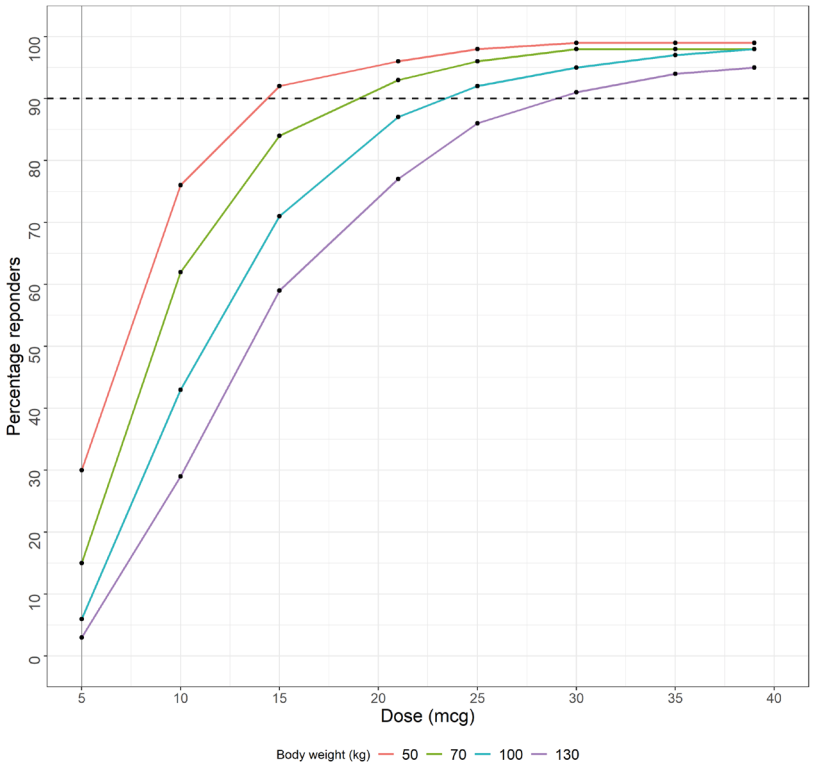

Figure 3. Percentage of VWF: Act responders 4 hours (T4) after desmopressin administration. Desmopressin dosages (5, 10, 15, 21, 25, 30, 35, 39 mcg) given to virtual patients with various body weights (50, 70, 100 or 130 kg). Responders demonstrated VWF: Act greater than 0.50 IU/mL at 4 hours after desmopressin administration. The y-axis denotes the percentage of virtual patients that demonstrated a response. The dashed horizontal black line denotes the 90% responders threshold.

DISCUSSION

An innovative and novel turn-over PK-PD model was developed characterizing the relationship between desmopressin dose, desmopressin plasma concentration and VWF: Act response. We demonstrate that a maximum increase in VWF: Act can be established by capped dosing with a fixed dose when body weight exceeds a certain maximum. By performing simulations based on the developed PK-PD model, we confirm the feasibility and efficacy of the recently published guidelines for treatment of VWD with desmopressin of the ASH ISTH NHF WFH 2021 (3).

Our simulations demonstrate that an adequate response is reached when patients weighing 50 to 100 kg receive a dose of 0.3 mcg/kg desmopressin intravenously. Although administration of 25 mcg resulted in an adequate response in patients weighing 100 kg, this dose may be insufficient for patients over 100 kg (Figure 3). For practical considerations we therefore suggest a capped dose of 30 mcg desmopressin in all patients above 100 kg and 0.3 mcg/kg for all patients below 100 kg, to ensure an adequate VWF: Act response.

In our PK model describing desmopressin concentrations, the volume of distribution (V) was 22% higher in females compared to males. V was 25.9 L/70kg in males which may reflect limited distribution of desmopressin to tissues other than plasma, which could be explained by the higher body fat percentage in females compared to males (15). Due to a higher V, females exhibited lower peak concentrations than males. When we stratified our simulations for sex, a slightly higher peak in desmopressin concentration in males was observed in comparison to females [data not shown]. However, this has no implications for the attained VWF: Act levels, as VWF: Act levels at T1 and T4 were similar in both males and females. The median peak desmopressin concentration for females is 0.52 ng/mL and for males 0.63 ng/mL, which is more than adequate to produce the maximum effect, as the EC50 is 0.174 ng/mL. Therefore, dose adjustments based on sex are not necessary. In addition, simulations were performed for patients with a VWF: Act baseline of 0.20 IU/mL. Patients with either a higher or lower baseline will attain higher and lower VWF: Act values after receiving 0.3 mcg/kg. Nevertheless, in our study population, only four patients had a baseline lower than 0.20 IU/ml. In usual clinical practice, patients with a VWF: Act baseline lower than 0.30 IU/mL always undergo a desmopressin test to check their responsiveness. If a patient fails to achieve an adequate VWF: Act response, a VWF-containing factor concentrate should be administered to achieve sufficient VWF: Act levels (16).

Based on figure 2, desmopressin is eliminated from the body after approximately 14h in a typical patient of 70kg. Still, in most patients, it is advised to administer a subsequent desmopressin dose only after 24h due to potential side effects, such as fluid retention due to its antidiuretic effects (17).

It is well known that patients with blood group O have lower VWF: Act levels (18). During population PK-PD model development, we tested blood group O and non-O as a covariate. In our PD model, *Kout* reflects the CL of VWF: Act. We investigated if the *Kout* differs between blood group O and non-O, but this did not improve the model. Therefore, we did not include blood group O as a covariate in our models.

Argenti et al. explored the relationship between desmopressin concentrations and VWF: Act in healthy volunteers (6). In this study, the temporary delay in VWF response was described by a hypothetical effect compartment model. A value of 0.237 ng/mL was reported for EC50 and 367% for Emax, which is comparable to the values observed for VWD patients in our study. Furthermore, this study reported a value of 2.16 h^{-1} for rate constant *Ke0*, which corresponds to a half-life of ca. 20 minutes and a delay of ca. 80 minutes before desmopressin changes in plasma are completely reflected in VWF: Act. This also corresponds with the results of our simulations.

A strength of this study is that we have included patients from a real-life population, including a wide range of ages. We included patients in our study if they had abnormal bleeding symptoms and either a historical lowest VWF: Ag or VWF: Act below 0.50 IU/mL. In some patients, there was a difference between historical lowest VWF: Act and VWF: Act at T0. A few patients were diagnosed with VWD 10-30 years before the desmopressin test. In these patients, the higher VWF: Act at T0 could be explained by an age-related increase of VWF (19). We however also observed differences in some patients who underwent a desmopressin test shortly after diagnosis. It is well known that VWF may also increase due to stress and a desmopressin test can be a stressful event for some patients, especially children (1).

We acknowledge some limitations of our study. Our analysis was limited to only type 1 VWD. Therefore, the concentration-effect relationship could not be established for other types of VWD. Also, we did not observe extremely fast clearance as observed in type 1 Vicenza in any of the patients. Furthermore, our dataset contained only six patients with a body weight over 100 kg. Therefore, simulations may be less precise in this category of patients. V of desmopressin was 25.9 L/70kg and we assumed that desmopressin has a limited distribution to the other tissues. This is important for obese patients, since they have more adipose tissue compared to non-obese patients.

The total body weight in obese patients is mainly increased because of the adipose tissue, but lean body weight would increase much less (20). Based on this, we assumed that 30 mcg would be adequate for more severely obese patients based on the finding for the 130 kg patients.

In conclusion, our novel turn-over PK-PD model successfully characterized the relationship between desmopressin dose, desmopressin plasma concentration and VWF: Act response. Simulations confirm that current international desmopressin dosing guidelines in which an intravenous dose of 0.3 mcg/kg and a capped dose of 30 mcg desmopressin is recommended are effective for the treatment of VWD patients. The developed PK-PD model can be applied to further investigate the relationship between specific patient characteristics and VWF response, thereby potentially eliminating the necessity of desmopressin testing in the near future.

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SUPPLEMENT

SUPPLEMENTARY FIGURES

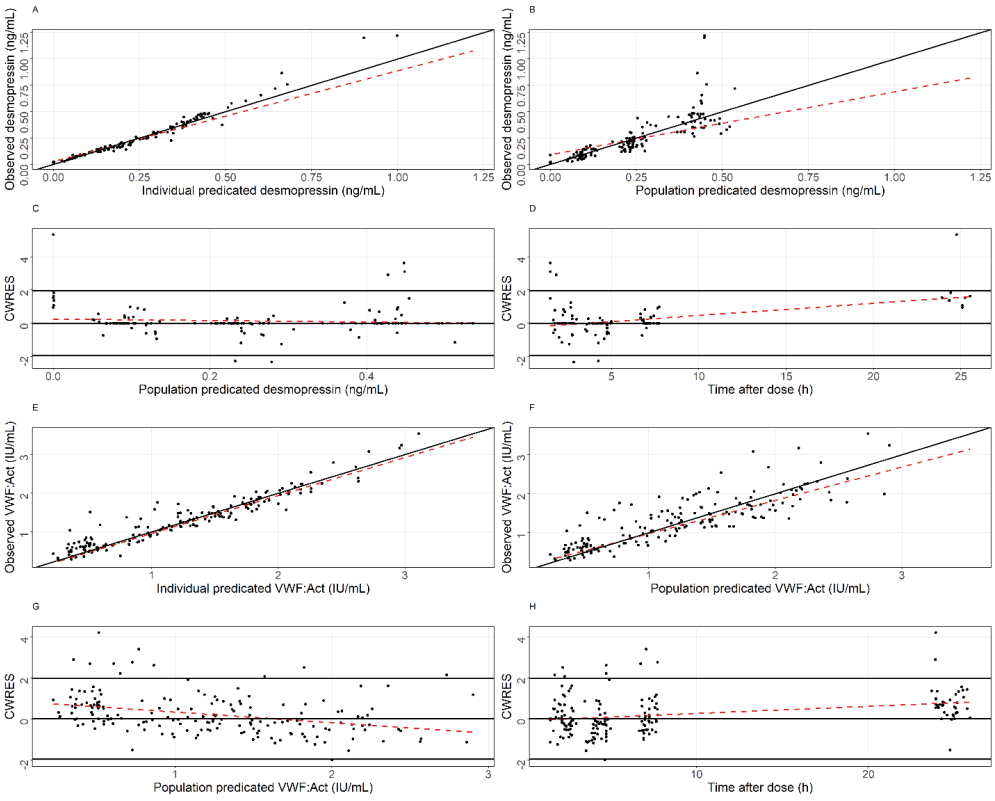

Figure 1. Goodness-of-fit plots of the final pharmacokinetic model of desmopressin (top) and population model of VWF: Act (bottom). A and E) Individual predicted (IPRED) vs observed concentrations, B and F) Population predicted (PRED) vs observed concentrations, C and G) Conditional weighted residuals (CWRES) vs PRED, D and H) Time after administration vs CWRES. The solid line is the line of identity. The dashed line represents the local regression smooth line (loess smooth).

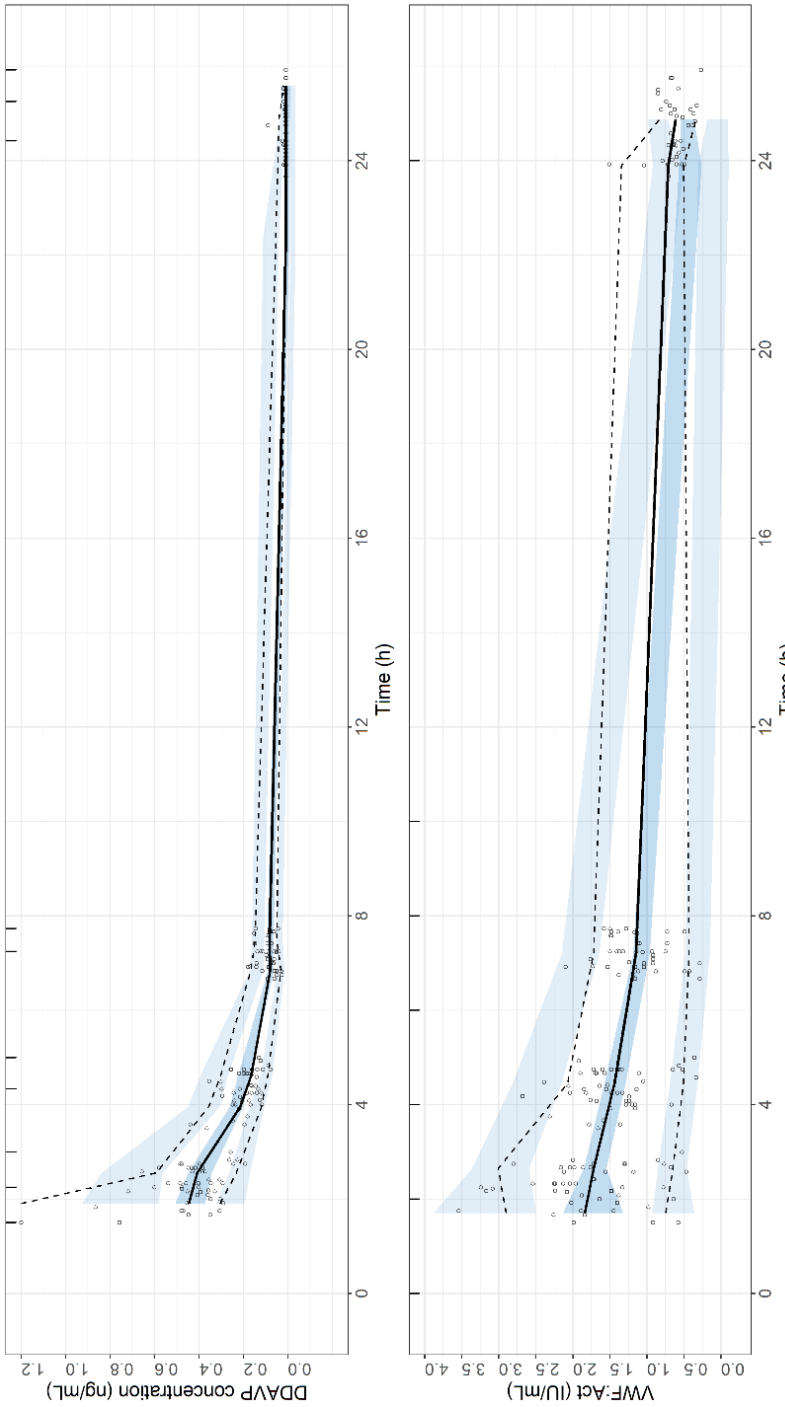

Figure 2. Visual predictive check of the final pharmacokinetic model of desmopressin and pharmacodynamic model of VWF: Act. Dots represent observed desmopressin concentrations (top) and VWF: Act (bottom); the solid black line represents the 50th percentile of observed data; the dashed black lines represent the 5th and 95th percentiles of the population model. Shaded areas depict the model predicted 95% confidence intervals of the simulated percentiles.

SUPPLEMENTARY METHODS SECTION

Population PK model development

A sequential PK-PD analysis method was performed. A population PK model was developed using NONMEM subroutines ADVAN1, TRANS2 and the Laplacian estimation method.

A priori allometric scaling of bodyweight on clearance (CL) and central volume of distribution (V) was included in the structural model (equation 1):

$$\theta_{pop\ PK} = \theta_{pk} \times \left(\frac{Bodyweight}{70}\right)^{\theta_{exp}} \quad (1)$$

In which, is the typical population value for a PK parameter dependent on bodyweight, is the typical PK value for a patient with a body weight of 70 kg, and is an exponent fixed at 0.75 for CL and 1 for V.

The individual PK parameters were described by using equation 2.

$$\theta_i = \theta_{pop\ PK} \times exp^{\eta i} \quad (2)$$

In which, is the estimated individual PK parameter of the i^{th} individual, is the typical population value for a PK parameter, ηi is the inter-individual variability from normal distribution with a mean of zero and estimated variance of ω^2 of the i^{th} individual. A full omega variance–covariance block matrix was tested on the PK parameters

For the residual error model, an additive (equation 3), proportional (equation 4) and combined error models (combination of equation 2 and 3) were tested, in which Y_{ij} is the prediction of the concentration of individual *i* at time *j* is the individual concentration prediction at time *j* and ε is the residual error originating from a normal distribution with a mean of zero and estimated variance of σ^2.

$$Y_{ij} = f\left(\theta, \eta i, x_{ij}\right) + \varepsilon_{ij} \quad (3)$$

$$Y_{ij} = f\left(\theta, \eta_i, x_{ij}\right) \times (1 + \varepsilon_{ij}) \quad (4)$$

The following covariates were evaluated: age, sex, height, baseline FVIII, baseline VWF: Act, baseline VWF: Ag and blood group (O, non-O). Blood groups of two patients were missing, which were excluded during covariate analysis. Continuous covariates were included by a power model, in which the covariate was centred around its

median value and the exponent was estimated (equation 5). Categorical covariates were modelled with the use of flag variables (1 and 0 for "true" and "false"; equation 6).

$$\theta_{pop} = \theta_{pk} \times \left(\frac{Cov}{Cov_{median}}\right)^{\theta_{exp}} \quad (5)$$

$$\theta_{pop} = \theta_{pk} \times (\theta_1^{Flag1} \times \theta_2^{Flag2} \times \theta_3^{Flag3} \cdots) \quad (6)$$

A stepwise forward inclusion and backward exclusion process was used to evaluate covariates, in which a reduction in the objective function value (OFV) of 3.84 ($p = 0.05$) or more was considered significant during forward inclusion and a reduction in OFV of 7.88 ($p = 0.005$) or more in the backward exclusion process. The covariate that resulted in the largest decrease in OFV was first implemented in the model. The remaining covariates that significantly decreased OFV were then sequentially added to the covariate model, and repeated until all significant covariates were included.

Plasma concentrations below the lower limit of quantification (LLOQ) were taken in consideration in the analysis, but were flagged and treated as categorical data in the population PK analysis using the M3 method; a likelihood-based approach which maximizes the likelihood of the data being below LLOQ with respect to the model parameters[1].

Population PD modelling

For the population PD model development, the first-order estimation method with the interaction option (FOCE-I) and NONMEM subroutine ADVAN6, TOL3 was used.

A turnover model was used to describe the release of VWF: Act. The turnover model consists of a zero-order constant describing release of VWF: Act from the vascular endothelium (Kin) and a first-order rate constant for loss of VWF: Act (Kout). The baseline VWF: Act (BASE) is determined by the equilibrium of Kin and Kout (equation 7).

$$BASE = \frac{K_{in}}{K_{out}} \quad (7)$$

The differential equation for the turnover-model is displayed in equation 8.

$$\frac{dVWF{:}Act}{dT} = BASE \times K_{out} \times E - K_{out} \times VWF{:}Act \quad (8)$$

where E is the effect as a function of the individual predicted desmopressin concentration. A linear and a sigmoidal Emax concentration-effect relationship were tested (equation 9,10).

$$E = 1 + \theta_{slope} \times C \quad (9)$$

$$E = 1 + \frac{\theta_{Emax} \times C^n}{\theta_{EC50} + C^n} \quad (10)$$

where *C* is the desmopressin plasma concentration and *slope* is the change of effect per ng/mL of desmopressin, *Emax* is the maximum effect, *EC50* is the desmopressin concentration which produces 50 % of the maximal effect, *n* is the hill coefficient which was both estimated and fixed at 1. The hill coefficient determines the steepness of the sigmoidal concentration-effect curve. Model comparison using equation 9 and 10 was done using the Bayesian information criterion (BIC). Afterwards, IIV was estimated for the PD parameters to obtain individual PD parameters by using equation 11.

$$\theta_i = \theta_{pop\ PD} \times exp^{\eta i} \quad (11)$$

The following covariates were tested for correlation with the PD parameters: bodyweight, age, sex, height, VWD type, baseline FVIII, baseline VWF: Act, baseline von Willebrand factor antigen, von Willebrand factor-multimers and blood group (equations 5 and 6).


6

Chapter 6

Population pharmacokinetic modelling of von Willebrand factor activity in von Willebrand disease patients after desmopressin administration

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ABSTRACT

Objective

Most von Willebrand disease (VWD) patients can be treated with desmopressin during bleeding or surgery. Large interpatient variability is observed in von Willebrand factor (VWF) activity levels after desmopressin administration. The aim of this study was to develop a pharmacokinetic (PK) model to describe, quantify, and explain this variability.

Methods

Patients with either VWD or low VWF, receiving an intravenous desmopressin test dose of 0.3 μg kg^{-1}, were included. A PK model was derived on the basis of the individual time profiles of VWF activity. Since no VWF was administered, the VWF dose was arbitrarily set to unity. Interpatient variability in bioavailability (*F*), volume of distribution (*V*), and clearance (*Cl*) was estimated.

Results

The PK model was developed using 951 VWF activity level measurements from 207 patients diagnosed with a VWD type. Median age was 28 years (range: 5–76), median predose VWF activity was 0.37 IU/mL (range: 0.06–1.13), and median VWF activity response at peak level was 0.64 IU/mL (range: 0.04–4.04). The observed PK profiles were best described using a one-compartment model with allometric scaling. While *F* increased with age, Cl was dependent on VWD type and sex. Inclusion resulted in a drop in interpatient variability in *F* and Cl of 81.7 to 60.5% and 92.8 to 76.5%, respectively.

Conclusion

A PK model was developed, describing VWF activity versus time profile after desmopressin administration in patients with VWD or low VWF. Interpatient variability in response was quantified and partially explained. This model is a starting point toward more accurate prediction of desmopressin dosing effects in VWD.

INTRODUCTION

Von Willebrand disease (VWD) is the most common inherited bleeding disorder (1). This autosomal inherited disease is characterized by quantitative or qualitative defects of von Willebrand factor (VWF), with often reduced factor VIII (FVIII) levels (2). VWF is essential for primary hemostasis as it contributes to platelet adhesion and aggregation at sites of injury, resulting in platelet plug formation. Additionally for secondary hemostasis, it acts as a chaperone protein for FVIII, protecting it from proteolysis in the circulation (3).

VWD can be categorized into several subtypes, each having their specific characteristics and treatment strategies. Type 1 VWD is defined as a partial- and type 3 VWD as a complete quantitative VWF deficiency, whereas type 2 VWD consists of several subtypes in which VWF is qualitatively affected (1). Type 2 VWD can be divided into four subtypes: type 2A, characterized by impaired platelet adhesion caused by selective deficiency of high-molecular-weight VWF multimers; type 2B, characterized by enhanced platelet glycoprotein Ib affinity; type 2M, characterized by impaired platelet adhesion despite a normal size distribution of VWF multimers; and type 2N, characterized by decreased affinity for FVIII (4). Patients with low VWF are defined as having a bleeding phenotype and VWF levels between 0.30 and 0.50 IU/mL. Treatment options in patients with VWD include desmopressin, which mobilizes VWF from endothelial cells with both VWF and subsequent FVIII increase in plasma, various plasma-derived VWF/FVIII and VWF concentrates, and a recombinant VWF concentrate (5). VWF-containing concentrates are used for treatment of bleedings and surgical prophylaxis when desmopressin is not (sufficiently) effective, or contraindicated for instance due to VWD subtype or cardiovascular risk profile (6, 7).

Desmopressin is usually administered intravenously or intranasally. Potential side effects are headaches, facial flushing, and circulatory overload (8). Costs of desmopressin are low in comparison to alternative treatments. Based on controlled prospective studies in healthy volunteers, maximum VWF response is achieved with a desmopressin dose between 0.2 and 0.3 $\mu g\ kg^{-1}$, whereas maximum FVIII response is observed at a dose of 0.3 $\mu g\ kg^{-1}$ (9-13). Therefore, it is generally accepted that a standard intravenous dose of desmopressin is 0.3 $\mu g\ kg^{-1}$ (10). It should be noted that desmopressin has a relatively short duration of effect and also tachyphylaxis after repeated dosing. The latter is caused by VWF depletion from the endothelial compartment when administered repeatedly within a short time period (14, 15). The interpatient variability in VWF and FVIII response is considered a disadvantage due to patient burden and unpredictability of a clinical relevant response (16). Several studies

have identified pathophysiological differences underlying VWD, more specifically variation in VWF mutations, potentially affecting VWF production, secretion, or clearance in and between individuals as potential causes of these differences (8, 17). Therefore, we hypothesize that desmopressin testing and treatment can be improved if its response can be predicted by specific patient characteristics and/or other modifying factors. In this study, a population pharmacokinetic (PK) model was developed in which the interpatient variability in VWF response profile was quantified in patients, diagnosed with VWD or low VWF, after administration of a desmopressin test dose.

METHODS

Data

Data of patients diagnosed with VWD or low VWF (historically lowest VWF antigen or VWF activity level of 0.30–0.50 IU/mL), tested between January 1, 2000 and January 1, 2017 at the Erasmus University Medical Centre Rotterdam in the Netherlands, were collected from electronic patient files according to Dutch rules and regulations for Good Clinical Practice. VWD was diagnosed according to current International Society on Thrombosis and Haemostasis classification (4). All study patients received a single intravenous desmopressin test dose of 0.3 μg kg^{-1}, administered over 30 minutes.

The dataset contained patient demographics describing weight, height, age, sex, blood group type, VWD type, baseline levels of VWF antigen, VWF activity and FVIII (lowest levels ever measured), renal function and hepatic function (characterized by aspartate aminotransferase, alanine aminotransferase, gamma glutamyl transferase, alkaline phosphatase, lactate dehydrogenase, albumin, urea, and creatinine), and VWF multimer status (18).

VWF antigen, VWF activity, VWF collagen binding, and FVIII levels were measured for routine diagnostics in the hemostasis laboratory at the Erasmus University Medical Centre, before and after desmopressin administration. VWF antigen was measured with an in-house enzyme-linked immunosorbent assay using polyclonal rabbit antihuman VWF antibodies and horseradish peroxidase-conjugated antihuman VWF antibodies (DakoCytomation) for detection. VWF activity was measured using different assays over the years: a VWF ristocetin cofactor (VWF:RCo) assay from 2000 to 2005, a monoclonal antibody (VWF:Ab) assay from 2005 to 2012, and a VWF glycoprotein 1b binding (VWF:GP1bM) assay from 2012 onwards. In the VWF:RCo assay, agglutination of fixed thrombocytes using ristocetin as a cofactor was measured on the PAP-4 or Chrono-log (lower limit of quantification [LLOQ]: 0.10 IU/mL, calibration range: 0.25–1.00). The

VWF:Ab was measured with a latex immune assay on an automated coagulometer with monoclonal antibodies against the GP1bα binding site of VWF (HemosIL VWF activity; Instrumentation Laboratory BV, Breda, The Netherlands) (LLOQ: 0.12 IU/mL, calibration range: 0.25–2.00 IU/mL).(204) VWF:GP1bM was measured with the INNOVANCE VWF Ac reagent (Siemens Healthcare Diagnostics) on a Sysmex CS-5100 analyzer using the manufacturer's protocol (LLOQ: 0.04 IU/mL, calibration range: 0.12–1.80 IU/mL). VWF collagen binding was measured with an in-house enzyme-linked immunosorbent assay, for which collagen type 1 (MilliporeSigma) was used for capture and horseradish peroxidase-conjugated antihuman VWF antibodies (DakoCytomation) for detection. FVIII was measured by using different one-stage clotting assays. VWF activity, FVIII, VWF antigen, and VWF collagen binding levels were determined at baseline and at approximately 1, 3, 6, and 24 hours after administration of desmopressin. A detailed overview of patient and data characteristics is provided in Table 1 (20, 21).

Table 1. Characteristics of the available data

Data	Children		Adults		Total population	
Number of patients	64	(30.9)	143	(69.1)	207	(100)
Age (y)	10.5	(5-17)	38	(18-76)	28	(5-76)
Weight (kg)[a,b]	39	(18-79)	75	(41-123)	69	(18-123)
Female sex	34	(53.1)	91	(63.6)	125	(58)
Height (cm)[b]	152.5	(109-182.4)	167	(137-200)	167	(109-200)
Blood group O[b]	41	(64.1)	82	(57.3)	123	(57)
Baseline VWF activity (IU/mL)	0.12	(0.01-0.61)	0.1	(0.01-0.43)	0.1	(0.01-0.61)
Baseline FVIII (IU/mL)	0.605	(0.07-1.36)	0.48	(0.07-1.22)	0.51	(0.07-1.36)
Baseline VWF antigen (IU/mL)	0.34	(0.03-0.83)	0.35	(0.03-1.34)	0.35	(0.03-1.34)
Hepatic function abnormalities[b]	0	(0.0)	4	(3)	4	(2)
Renal function abnormalities[b]	0	(0.0)	3	(2)	3	(2)
Number of VWD-type diagnosis[b]						
1	31	(48.4)	77	(53.8)	108	(52.2)
2[c]	2	(3.1)	3	(2.1)	5	(2.4)
2A	8	(12.5)	29	(20.3)	37	(17.9)
2M	3	(4.7)	12	(8.4)	15	(7.2)
2N	2	(3.1)	3	(2.1)	5	(2.4)
Low VWF activity[d]	18	(28.1)	19	(13.3)	37	(17.9)

Abbreviations: FVIII, factor VIII; VWD, von Willebrand Disease; VWF, von Willebrand factor.Note: Data expressed as frequency (%) or median (range).[a] Using linear regression missing weight values are estimated and included in the data.[b] Missing data were present in 6% weight, 22% height, 11% blood group, 43% hepatic function abnormalities, and 37% renal function abnormalities.[c] VWD type 2 represents a group with unknown diagnosed subtype.[d] Low VWF activity definition is VWF antigen or VWF activity <0.50 IU/mL and not classified as one of other VWD types.

Population PK Modeling

VWF activity data of all patients were analyzed simultaneously by nonlinear mixed effect modeling (NONMEM). This method can be applied in studies with sparse data with random sampling times. The time profiles of VWF activity were described with compartmental PK models using NONMEM software package version 7.4.1 (ICON Development Solution, Gaithersburg, Maryland, United States). Manipulation, visualization, and statistical evaluation of the data and the developed empirical model were achieved using R v3.5.1 and PsN v4.8.1 in combination with Piraña v2.9.9 (22-24). VWF activity levels were analyzed, resulting in typical PK parameters, as well as interpatient variability and remaining residual variability between observed and predicted VWF activity. As patients' endogenous VWF is released from the endothelium after desmopressin, the exact amount delivered is unknown. Therefore, the VWF dose was arbitrarily set to a value of 1. Subsequently, the model was parameterized in terms of an absorption rate constant (K_a), clearance (Cl), volume of distribution (V), and bioavailability (F). F can be regarded as bioavailability of VWF activity after desmopressin administration. We allowed F to vary between patients. For example, patients with a low F will have a lower exposure than patients with a higher F. In the evaluated models the typical (median) value of F was fixed to unity as only the apparent Cl (Cl/F) and V (V/F) were estimated. Despite the fact that the typical value of F was fixed to unity, interpatient variability for this parameter could be estimated as well as interpatient variability for Cl and V. Consequently, e.g., patients with a low F have a large apparent Cl and a low VWF activity exposure.

While the historically measured baseline values are defined as the lowest levels ever measured in a patient, the predose VWF activity baselines were moreover higher than historically measured baseline values. It is well known that intraindividual VWF variability is caused by physiological variability, preoperative anxiety, increasing age, or presence of comorbidity, and therefore this phenomenon was expected (21). For modeling purposes, an additional fixed virtual dose was administered to these patients, in combination with a separate varying F, prior to the time of measurement of the predose VWF activity level. This virtual dose was administered to produce the predose levels in patients which have higher pretest levels than historical baseline levels. The F of the virtual dose (F_{VD}) and its interpatient variability were estimated without influencing the estimations of other parameters (K_a, Cl, V, F) as the height of this virtual dose was estimated through the parameter F_{VD} in the model. Detailed information regarding the population PK modeling can be found in the supplementary material (available in the online version).

Covariate Modeling

For a possible association with interpatient variability in PK parameter estimates, the following covariates were evaluated: weight, height, age, sex, VWD type, blood group, FVIII activity and VWF antigen levels, VWF multimer pattern, and renal and hepatic functions. A forward inclusion (p-value < 0.05) and backward elimination (p-value < 0.01) method was performed as covariate analysis. Potential covariates were identified with a univariate method and subsequently included in a multivariate analysis. As data from pediatric patients were included in the analysis, allometric scaling on the basis of weight was used to scale size-related changes in PK parameters. Detailed information regarding the covariate modeling and allometric scaling can be found in the supplementary material.

Model Evaluation and Validation

Predictive performance of the model was evaluated by visual inspection of the goodness-of-fit plots. Visual predictive checks were performed to internally validate the model using (n = 1,000) simulations of observed data, after which the simulated data were compared with the observed data. Finally, nonparametric statistics of the estimated parameters obtained from a bootstrap method were compared with estimates obtained from the final model.

RESULTS

A total of 951 VWF activity measurements were collected from 207 patients and used for development of population PK models describing the time profiles of the individual patients. A group of patients consisting of 143 adults, with a median age of 38 years (range: 18–76 years), and 64 children with a median age of 10 years (range: 5–17 years) were included in the analysis. The median age of the total population was 28 years. After intravenous administration of desmopressin, VWF activity levels ranged from 0.04 to 4.04 IU/mL over time (Fig. 1) Historical VWF activity baseline levels ranged from 0.01 to 0.61 IU/mL (Table 1). Each patient received one test dose of desmopressin and was monitored up to a maximum of 27.75 hours after dosing. A total of 123 patients received an additional virtual dose. The median number of measured VWF activity levels within 24 hours after dosing was five for every patient, ranging from two to six measurements.

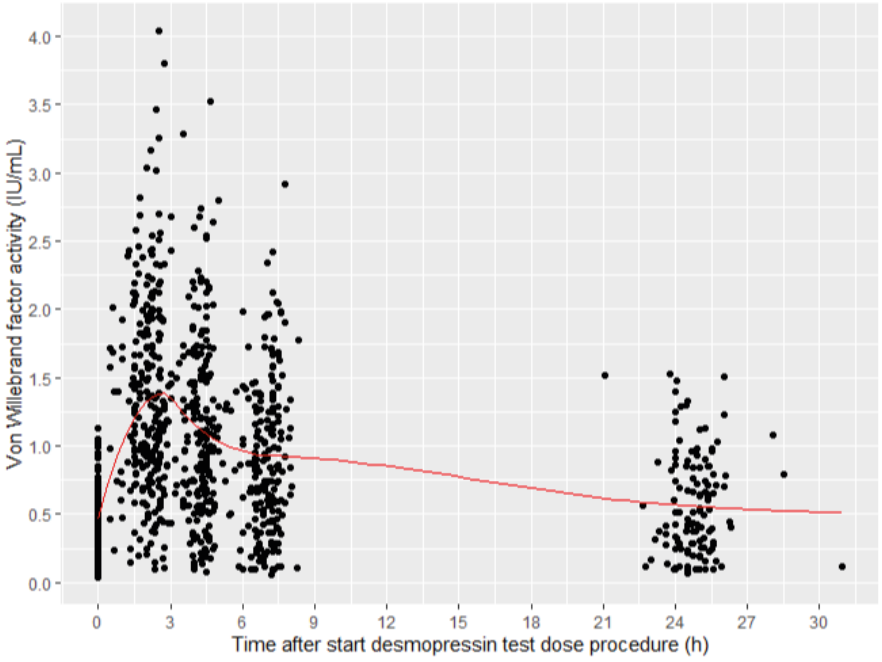

Figure 1. Profiles of all individuals ($n = 207$) described by the 951 von Willebrand factor activity measurements over time after start of the desmopressin test. The *red line* indicates the mean VWF activity profile of the population. VWF, von Willebrand factor.

Structural Model

A one-compartment model best described the PK of VWF activity after intravenous administration of desmopressin. A two-compartment model was evaluated but parameter estimates were less robust. The K_a was fixed to a random high value, thereby creating a VWF response curve similar to an intravenous VWF bolus. Cl and V were allometrically scaled, resulting in a better data fit of the model. Body weight was unknown for 2% of all 207 patients. The remaining missing values were imputed as described in the supplementary material (25). F_{VD} successfully corrected for the difference in baseline level just before administration of desmopressin and historical level of the VWF activity, without influencing any other parameter estimations. The interpatient variability of Cl, V, and F were estimated to be 92.8, 28.7, and 81.7%, respectively. A correlation coefficient was estimated; however, no convergence was found. Estimated values of the structural empirical PK model are depicted in Table 2.

Table 2. Parameter estimates of the structural desmopressin response model, final desmopressin response model, and bootstrap analysis

Parameter	Structural model		Final model		Bootstrap	
	Estimate	95% CI	Estimate	95% CI	Estimate	95% CI
Ka (h^{-1})	1830 (fixed)	-	1830 (fixed)	-	1830 (fixed)	-
Clearance (L/70 kg/h)	0.147	0.125 – 0.169	0.152	0.116 – 0.188	0.146	0.116 – 0.188
Volume of distribution (L/70 kg)	0.907	0.826 – 0.988	0.994	0.906 – 1.082	0.963	0.915 – 1.074
F	1 (fixed)	-	1 (fixed)	-	1 (fixed)	-
$F_{Virtual\ dosing}$	0.0714	0.001 – 0.193	0.0780	0.031 – 0.125	0.0823	0.005 – 0.151
Age on F	-	-	0.57	0.44 – 0.7	0.574	0.426 – 0.713
VWD type 1 on clearance	1 (fixed)	-	1 (fixed)	-	1 (fixed)	-
VWD type 2 on clearance	-	-	1.99	0.273 – 3.707	1.08	0.034 – 4.015
VWD type 2A on clearance	-	-	2.51	1.781 – 3.239	2.40	1.873 – 3.140
VWD type 2M on clearance	-	-	1.9	0.965 – 2.835	2.17	0.987 – 2.823
VWD type 2N on clearance	-	-	0.885	0.591 – 1.179	0.906	0.576 – 1.195
Low VWF activity on clearance	-	-	0.915	0.666 – 1.164	0.938	0.575 – 1.255
Sex on clearance	-	-	0.715	0.553 – 0.877	0.728	0.591 – 0.840
IIV clearance (%)	0.621 (92.8)	0.417 – 0.825	0.461 (76.5)	0.323 – 0.599	0.444 (74.8)	0.362 – 0.560
IIV volume of distribution (%)	0.0794 (28.7)	0.003 – 0.156	0.0697 (26.9)	0.009 – 0.130	0.0689 (26.7)	0.010 – 0.130
IIV $F_{virtual\ dosing}$ (%)	3.83 (671.3)	3.53 – 4.13	3.35 (524.4)	0.959 – 5.74	2.67 (366.6)	0.013 – 7.97
IIV F (%)	0.511 (81.7)	0.424 – 0.598	0.312 (60.5)	0.222 – 0.402	0.306 (59.8)	0.223 – 0.401
Additive residual variability	0.0519	0.028 – 0.075	0.0517	0.023 – 0.08	0.0635	0.032 – 0.071
Proportional residual variability	0.147	0.127 – 0.167	0.146	0.124 – 0.168	0.147	0.128 – 0.165

Abbreviations: K_a: apparent first-order absorption rate constant, F: bioavailability, VWD: von Willebrand Disease, VWF: von Willebrand Factor, IIV: interindividual variability, CI: confidence interval. Note: Bootstrap results are based on 1,000 data subsets sampled from the original data with resampling and were successful for 96.7%. Residual error is fixed to 1. Type in subscript becomes applicable once patient is diagnosed with similar type; other types will be set to 1. Female in subscript becomes applicable once patients sex is determined as female; males will be set to 1.

Table 2. Continued

$$\text{Clearance } (L/h) = 0.152 * \frac{\text{Body weight}^{0.75}}{70} * 1_{\text{type 1}} * 1.99_{\text{type 2}} * 2.51_{\text{type 2A}} * 1.9_{\text{type 2M}} * 0.885_{\text{type 2N}} * 0.915_{\text{Low VWF activity}} * 0.715_{\text{female}} * e^{\text{Interindividual variability}_{\text{clearance}}}$$

$$\text{Volume of distribution } (L) = 0.994 * \frac{Body\ weight}{70} * e^{\text{Interindividual variability}_{\text{volume of distribution}}}$$

$$F = 1 * \frac{\text{Age}^{0.57}}{28} * e^{\text{Interindividual variability}_{F}}$$

$$F_{\text{virutal dosing}} = 0.0714 * e^{\text{Inter-individual variability}_{F_{\text{virtual dosing}}}}$$

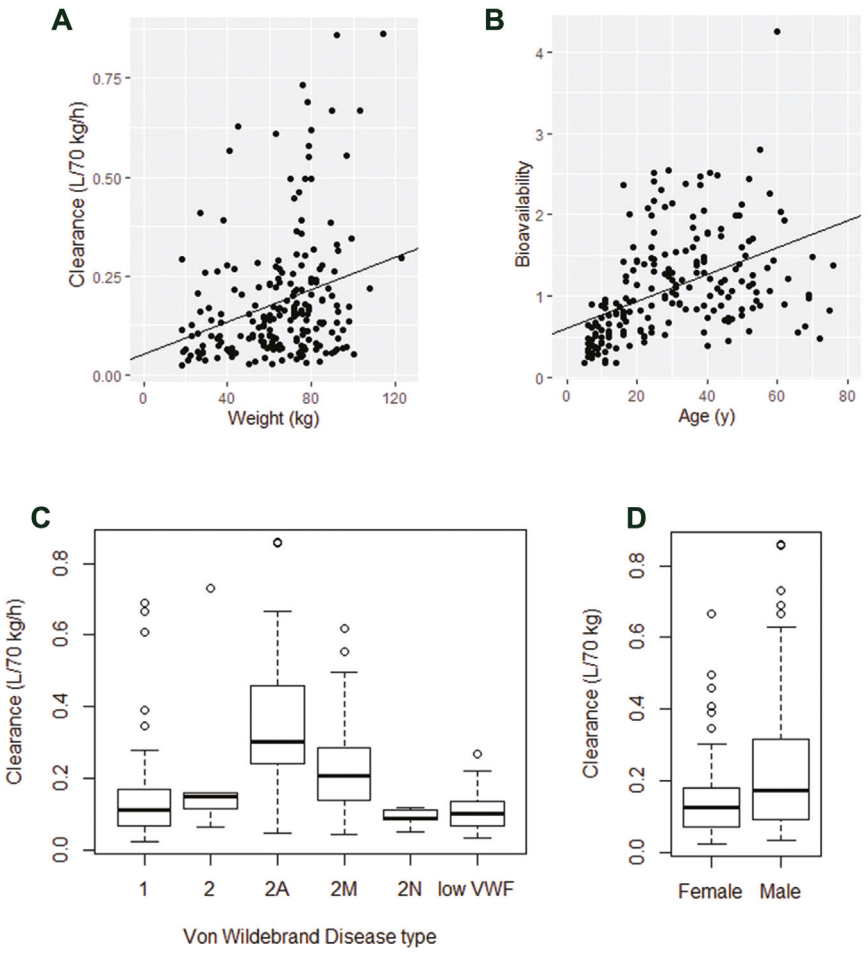

Figure 2. Relation between clearance and weight (**A**); age and bioavailability (**B**); disease type (**C**); and sex and clearance (**D**).

Covariate Model

The forward inclusion of the covariate analysis identified the following covariates as statistically significant ($p < 0.05$): VWD type, blood type, height and age with *F*; sex, VWD type and VWF multimer pattern with Cl; and height, age, baseline FVIII levels and baseline VWF antigen levels with *V*. Backward exclusion confirmed age with *F* and VWD type and sex with Cl as most statistically significant ($p < 0.01$) associations with interpatient variability. When the weight increased from 53 to 79 kg (respectively 25th and 75th percentile of interquartile range [IQR]), apparent Cl increased from 0.16 L/70 to 0.21 L/70 kg/h (Figure 2A). Likewise, when the age increased from 15 to 44 years (respectively 25th and 75th percentile of IQR), *F* fraction increased from 0.86 to 1.34 resulting in increased VWF activity (Figure 2B). The changes in *F* are all relative as the typical value was fixed to unity. Furthermore, for VWD types, Cl ratios were 1 (type 1): 1.99 (type 2, without known subtype): 2.51 (type 2A): 1.9 (type 2M): 0.885 (type 2N): 0.915 (low VWF), which indicates that patients with qualitative VWF defects leading to aberrant platelet binding show higher Cl of VWF. The relationship between Cl and VWD type is illustrated in Figure 2C. Finally, sex affected Cl, resulting in a better model fit; women have a 28.5% lower Cl of VWF activity compared to men (Figure 2D), resulting in higher VWF activity levels post-desmopressin. Implementation of these covariates in the final population PK model resulted in a drop in interpatient variability of *F*, Cl, and *V* of 81.7 to 60.5, 92.8 to 76.5, and 28.7 to 26.9%, respectively. Parameter and covariate estimates of the final VWF activity response model after intravenous desmopressin administration are shown in Table 2.

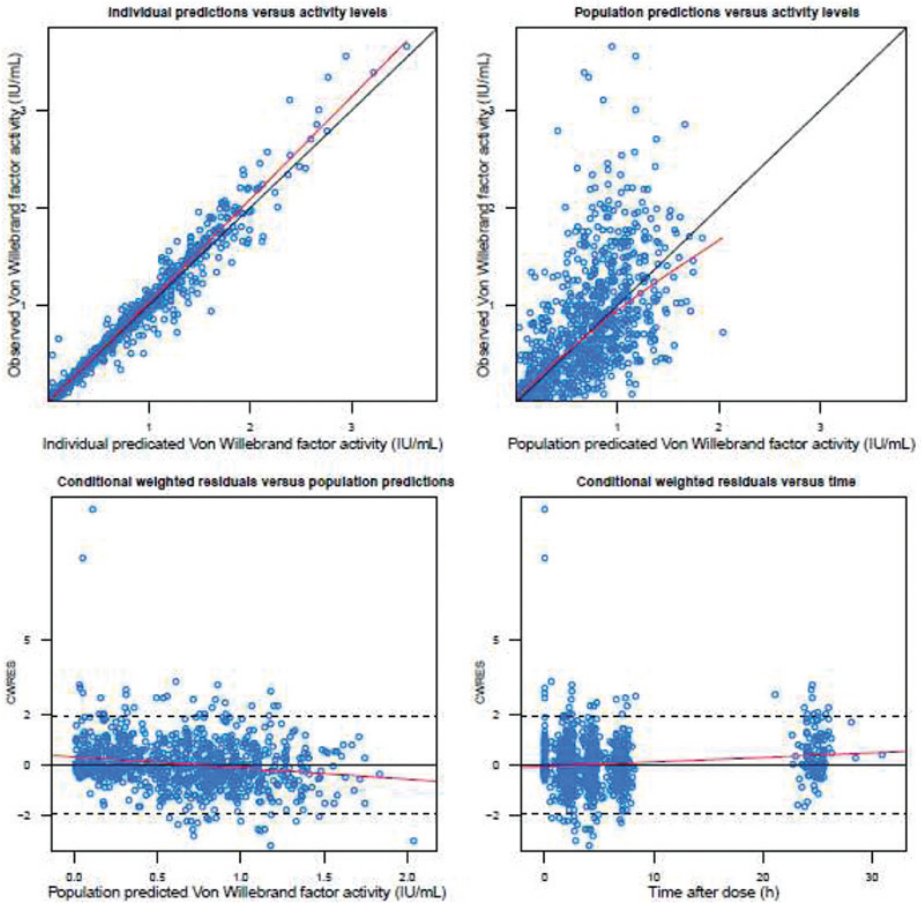

Figure 3. The goodness-of-fit plots for the final model, including individual predicted VWF activity obtained using the final model versus the observed VWF activity (**A**), population predicted VWF activity obtained using the final model versus the observed VWF activity (**B**), conditional weighted residual (CWRES) versus the population predicted VWF activity (**C**), and CWRES versus the time after dose administration (**D**). The *black line* indicates the line of identity (line $y = x$), whereas the *red line* depicts the local repressor line, following the densest part of the data. VWF, von Willebrand factor.

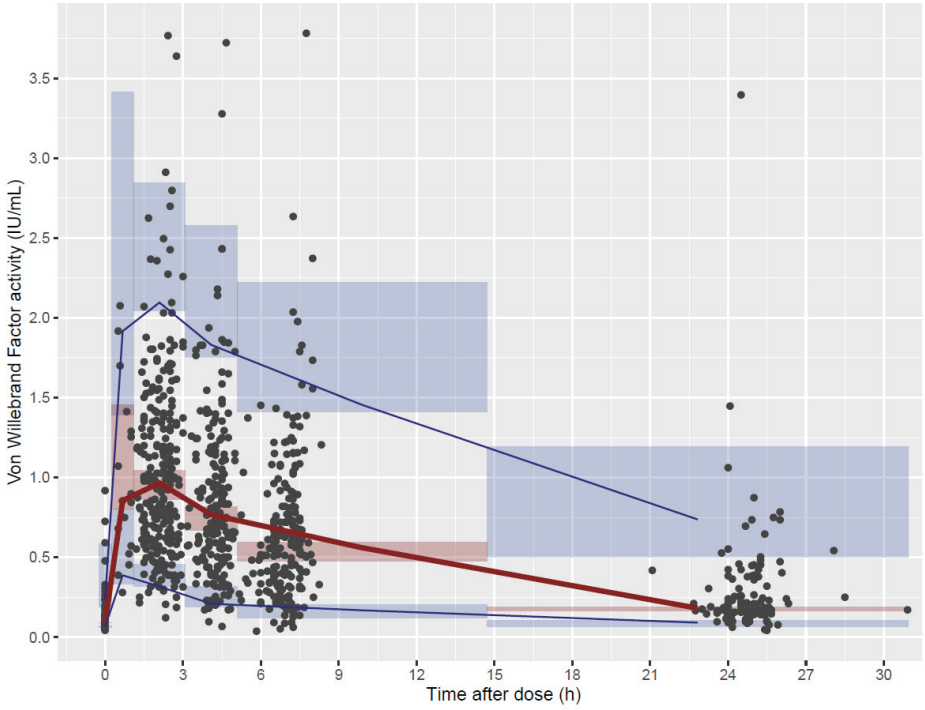

Figure 4. Prediction-corrected visual predictive check of the final empirical pharmacokinetic model of the VWF activity response after intravenous administration of desmopressin. The median (*red line*) and the 2.5th and 97.5th percentile of the observations at each bin (*blue lines*) of the observed data are plotted against the simulated data ($n = 1{,}000$) indicated as highlighted areas (*red area*: median; *blue area*: the 2.5th and 97.5th percentile of the simulated prediction corrected data at each bin). Individual observations in the data are shown as *black dots*. A model predicts the concentrations adequately when the *blue* and *red lines* run through the corresponding areas.

Model Validation and Evaluation

The goodness-of-fit of the final model is presented in Figure 3. Although a small deviation of the population predictions of VWF activity was shown, the main part of the population predications was distributed symmetrically around the line of identity, demonstrating adequacy of the model to describe the measured VWF activity. The individual profiles were well described as all predications were presented on the line of identity. Adequate model performance of the final population PK model of VWF activity response after intravenous desmopressin administration is visualized using a prediction-corrected visual predictive check (Figure 4). Bootstrap confirmed robustness of parameter estimates obtained in the final empirical population PK model (Table 2). The robustness indicates that the standard deviation of the sampling distribution given by the procedure reflects better what is understood by standard deviation of its sampling distribution under repeated sampling.

DISCUSSION

The aim of this study was to develop a population PK model in which interpatient variability in VWF profiles is quantified and explained in VWD and low VWF patients receiving desmopressin. Patient characteristics explaining variability in the population PK model were weight, age, VWD type, and sex.

Predose VWF activity levels were sometimes higher than historically measured VWF activity levels. It is well known that in type 1 VWD patients, VWF and FVIII levels increase with age and are dependent on varying circumstances (26). Exercise, stress, time of day, menstrual cycle, comorbidities, and inflammatory states may also lead to varying VWF activity levels (21, 27-29). Data with regard to these potential covariates are often difficult to determine and were unfortunately not available in the applied dataset.

In the final population PK model, age was also positively correlated with *F*, relating to a larger release of endogenously stored VWF with increasing age. This is comparable to clinical practice, as older patients show more elevation of VWF and FVIII than children (30). Pathophysiologically, desmopressin binds to vasopressin receptors on the endothelial cell surface, ultimately resulting in observed endogenous release of VWF and FVIII (26, 31). Hypothetically, age may influence number or sensitivity of vasopressin receptors, resulting in a higher increase in VWF activity after desmopressin administration. Moreover, although not validated in humans, adult rats have been shown to have more vasopressin receptors than young rats, potentially resulting in a larger increase in VWF activity after desmopressin administration (32).

As expected, VWF activity Cl was dependent on the VWD type. Type 2A and type 2M VWD both showed a higher Cl than type 1, resulting in overall lower VWF responses. The confidence interval of type 2M (0.965–2.835) included unity, indicating that the relationship was not statistically significantly different. This may be due to the low number of patients ($n=15$) diagnosed with type 2M that are included in the study. Patients with type 2A and 2M have a qualitative deficiency of VWF, resulting in a shortened VWF half-life and therefore also a higher elimination rate (33). Type 2A VWD is characterized by loss of high-molecular-weight VWF multimers, probably due to abnormal VWF synthesis and packaging prior to endothelial cell secretion or due to an increased susceptibility to ADAMTS13 after exocytosis. Type 2M VWD is characterized by a decreased interaction of VWF with platelet GP1bα due to VWF gene mutations, and normal VWF multimers, which may explain the decreased desmopressin response. Although VWF multimer distribution is normal, it is important to realize that multimers

remain intrinsically dysfunctional in type 2M VWD as they cannot bind platelets properly (34). Furthermore, no difference in Cl was observed when comparing low VWF patients to type 1 VWD as both are characterized by a partial quantitative plasmatic deficiency of an otherwise structurally and functionally normal VWF. Unfortunately, because the pathophysiology of underlying low VWF levels is largely unknown, difficulties remain in diagnosis and therapeutic management of these patients (35).

Finally, sex was identified as a significant covariate leading to a 28.5% lower VWF activity Cl in women after desmopressin administration, compared to men. This effect increases once a patient becomes older. Although no studies have yet been reported on causes of sex differences, it has been suggested in other diseases that both female humans and animals are more sensitive to desmopressin as there are indications that desmopressin sensitivity may be regulated by genetic and hormonal differences (28, 36-38). Future studies should at least include genetic analyses of the VWF gene and ideally also explore effects of other potential modifiers of VWF Cl such as CLEC4M (C-type lectin domain family 4, member M), lipoprotein receptor 1, and exocytosis (e.g., STXBP5 syntaxin-binding protein 5) (39-41). The final population PK model used a large dataset and was able to identify estimated variability in desmopressin response. However, the interindividual variability estimate of *V* is joined by an increased residual squared error and shrinkage, indicating inaccuracy and imprecision of that estimate. While the PK of desmopressin is yet unknown, the response of desmopressin regarding the VWF activity could be identified. Covariates identifying age, disease type, and sex have been found to influence the desmopressin response. The developed model can therefore be seen as a starting point toward a more accurate prediction of effects of desmopressin dosing. Unfortunately it should be noted that the number of patients per type of disease was not equally distributed in the used dataset. Furthermore, as the PK of desmopressin is yet unknown, inclusion of the variability of the desmopressin PK might improve the applications of this model.

In conclusion, we are the first to present a population PK model of VWF activity response after intravenous desmopressin administration, constructed of data obtained from patients with VWD or low VWF. This population PK model was able to identify estimated variability in desmopressin response and can therefore help predict the effects of desmopressin dosing. This may reduce in the near future the necessity of individual desmopressin testing with multiple samples in VWD patients.

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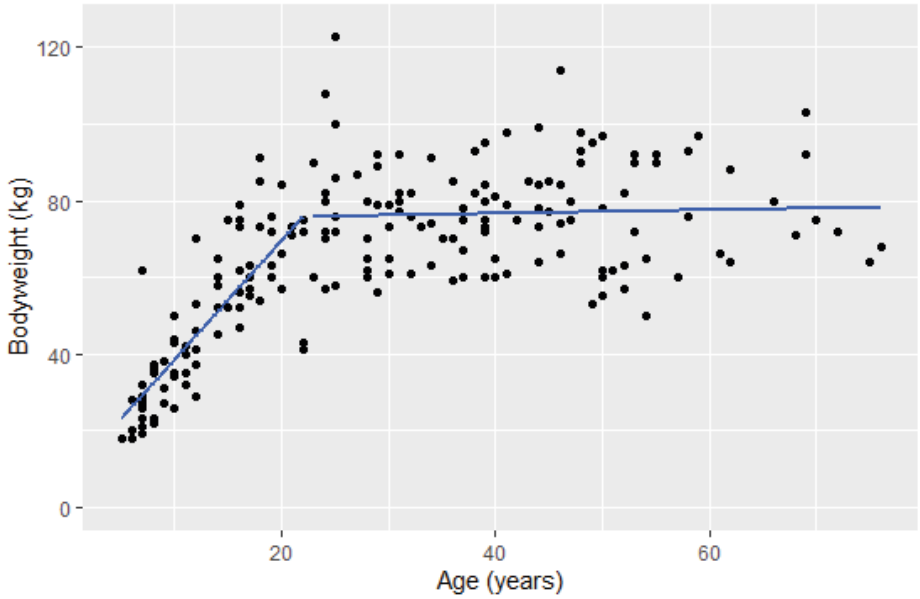
SUPPLEMENT

Estimation of missing body weight values

In order to estimate missing body weight values most accurately, a piecewise linear regression model was developed. The relationship between known age and body weight is depicted in Supplementary Fig. S1. The blue line indicates the weight predications for all ages, based on collected data.



Supplementary Fig. S1 Linear regression using body weight versus age. Black dots indicate individual known body weights of 197 patients. The blue line indicates the predicated body weight with age as a predictor and is described in the following manner: body weight = 7.7049 + 3.0952 * age - 3.0544 * (age - 22) * K_{age}. K_{age} is considered 1 when a patient's age is 22 years or older; otherwise K_{age} is 0.

Population PK modelling

A one-compartment model with first order absorption and elimination, including administration of a fixed virtual VWF dose, was used to fit VWF:actover time data. A two-compartment model was considered, as it resulted in a decrease of the objective function value (OFV). However, a single compartment model was joined with improved model robustness and more accurate and precise PK estimations. First-order conditional estimation method with interaction was used to derive population (θ) PK parameters and their variability (η) regarding observed and predicted VWF activity. The interindividual variability in PK model parameters were characterised in an exponential manner (equation 1), where p_{ij} is the estimate of the j^{th} PK parameter in

individual i, θ_j is the typical value of the j^{th} PK parameter, and η_{ij} is a random variable for the i^{th} individual and the j^{th} PK parameter distributed with mean zero and variance ω^2.

1. $p_{i,j} = \theta_j * e^{\eta_{i,j}}$

Covariate modelling

Potential covariates were one-by-one included into the model and set in relation to the PK parameters (equation 2), where $\theta_{covariate}$ represents the impact of the relevant covariate.

2. $p_{i,j,final} = p_{i,j} * \theta_{covariate}$

The tested covariates were continuous or categorical. Continuous covariates are numeric variables that have an infinite number of values. The covariates in PK model parameters were characterised in an exponential manner (e.g. equation 3), where $covariate_i$ is the covariate value for i^{th} individual, $covariate_{median}$ is the median value of the population and $\theta_{(continuous)\ covariate}$ is the estimated covariate effect. Categorical covariates contain a finite number of categories or distinct groups (e.g. equation 4). Note that for 'sex' 1 is applicable for women and 0 for men.

3. $\theta_{Effect\ age\ on\ F} = \frac{Age_i}{28}^{0.57}$
4. $\theta_{Effect\ female\ sex\ on\ Cl} = 0.715^{sex}$

All covariates were statistically tested by the using the OFV. During the forward inclusion, covariates that significantly (P<0.05) influence desmopressin response were included in the final model, as a change in OFV of >3.84 was considered statistically significant (based on a χ^2 distribution with 1 degree of freedom). During backward covariate deletion, a P value of <0.01, corresponding to a change in OFV of >6.64 was considered statistically significant.

Allometric scaling

Allometry is the change in organisms in relation to changes in body size. As altering body size influences drug metabolism, PK parameters V and Cl are scaled using body weight of each individual (equation 5 and 6).

1. $V_{i,j,final} = V_{i,j} * \frac{Body\ weight_i}{70}$
2. $Cl_{i,j,final} = Cl_{i,j} * \frac{Body\ weight_i}{70}^{0.75}$

NONMEM control stream

```
$PROBLEM DDAVP in vWD patients, VWFRCo model
  $INPUT COMMENT ID MDV EVID TIME DOSE_DUMMY = AMT ;Dose LOAD ;Tag
  virtual dose DV ;VWF activity WT_ADJ AGE
GENDER DISEASE LOWEST_VALUE_ADJ ;Baseline VWF activity
  $DATA ….csv IGNORE = C
  $SUBROUTINES ADVAN6 TOL = 4
  $MODEL
  COMP = (DEPOT,DEFDOS) ;dummie dose of 1
  COMP = (CENTRAL) ;comp for VWFRCO = CVWFRCO
  $PK
  EAGE = (AGE/28)** THETA(8)
  FLAG2 = 0
  FLAG21 = 0
  FLAG23 = 0
  FLAG24 = 0
  FLAG4 = 0
  IF(DISEASE.EQ.2)FLAG2 = 1
  IF(DISEASE.EQ.21)FLAG21 = 1
  IF(DISEASE.EQ.23)FLAG23 = 1
  IF(DISEASE.EQ.24)FLAG24 = 1
  IF(DISEASE.EQ.4)FLAG4 = 1
  EDISEASE = (THETA(9)**FLAG2)*(THETA(10)**FLAG21)*(THETA(11)**FLAG23)*
  (THETA(12)**FLAG24) *(THETA
(13)**FLAG4)
  200204FLAG = 0
  IF(GENDER.EQ.0)FLAG = 1
  EGENDER = THETA(14)**FLAG
  IIVK12 = ETA(3)
  TVK12 = THETA(3)
  K12 = TVK12*
  EXP(IIVK12)
  IIVCL = ETA(4)
  TVCL = THETA(4)*
  ((WT_ADJ/70)**0.75)
  CL = TVCL*EXP(IIVCL)*EDISEASE*EGENDER
  IIVV = ETA(5)
  TVV = THETA(5)*(WT_ADJ/70)
```

```
V = TVV*EXP(IIVV)
S2 = V
K20 = CL/V
IF(LOAD.EQ.1)THEN
IIVF1 = ETA(1)
TVF1 = THETA(6)
F1 = TVF1*EXP(IIVF1)
ELSE
IIVF2 = ETA(2)
TVF2 = THETA(7)
F1 = TVF2*EXP(IIVF2)*EAGE
ENDIF
$DES
DADT(1) = -K12 * A(1)
DADT(2) = K12 * A(1) - K20 * A(2)
$ERROR
CVWFRCO = A(2)/S2 + LOWEST_VALUE_ADJ
W = SQRT(THETA(1)**2*CVWFRCO**2 + THETA(2)**2)
Y = CVWFRCO + W*EPS(1)
IPRED = CVWFRCO
IRES = DV-IPRED
IWRES = IRES/W
$THETA
(0.0517) ;Proportional error
(0.146) ;Additive error
(1830) FIX ;K12
(0.152) ;CL
(0.994) ;V
(0.0780) ;F1 LOAD
1 FIX ;F1 apparant
(0.57) ;EAGE
(1.99) ;EDISEASE II
(2.51) ;EDISEASE II.I
(1.9) ;EDISEASE II.III
(0.885) ;EDISEASE II.IV
(0.915) ;EDISEASE IV
(0.715) ;EGENDER
$OMEGA
3.35 ;IIV F1 LOAD
```

```
0.312 ;IIV F1 apparant
0 FIX ;IIV K12
0.461 ;IIV CL
0.0697 ;IIV V
$SIGMA
1 FIX
$EST MAXEVAL = 9999 PRINT = 1 METHOD = 1 INTERACTION POSTHOC
$COV PRINT = E UNCONDITIONAL
$TABLE ID EVID TIME AMT IPRED IWRES CWRES K12 CL V F1 EAGE EDISEASE
EGENDER NOPRINT ONEHEADER FILE = …
```


7

Chapter 7

One piece of the puzzle: population pharmacokinetics of FVIII during perioperative Haemate® P / Humate P® treatment in von Willebrand disease patients

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ABSTRACT

Introduction

Many patients with von Willebrand Disease (VWD) that are treated on demand with von Willebrand factor and factor VIII (FVIII) containing concentrates present with VWF and/or FVIII plasma levels outside set target levels. This carries a risk for bleeding and potentially, thrombosis. Development of a population pharmacokinetic (PK) model based on FVIII levels is a first step to more accurate on demand, perioperative dosing of this concentrate.

Methods

Patients with VWD undergoing surgery in Academic Haemophilia Treatment Centers in the Netherlands between 2000-2018 treated with a FVIII/VWF plasma-derived concentrate (Haemate® P/Humate P®) were included in this study. Population PK modelling was performed based on measured FVIII levels using nonlinear mixed-effects modelling (NONMEM).

Results

The population PK model was developed using 684 plasma FVIII measurements of 97 VWD patients undergoing 141 surgeries. Subsequently, the model was externally validated and re-estimated with independent clinical data from 20 additional patients undergoing 31 surgeries and 208 plasma measurements of FVIII. The observed PK profiles were best described using a one-compartment model. Typical values for volume of distribution and clearance were 3.28 L/70 kg and 0.037 L/h/70 kg. Increased VWF activity, decreased physical status according to ASA classification (ASA class >2), and increased duration of surgery were associated with decreased FVIII clearance.

Conclusion

This population PK model derived from real world data adequately describes FVIII levels following perioperative administration of the FVIII/VWF plasma-derived concentrate (Haemate® P/Humate P®) and will help to facilitate future dosing in VWD patients.

INTRODUCTION

Von Willebrand disease (VWD) is the most common inherited bleeding disorder diagnosed in humans (1). This autosomally inherited disorder is characterised by quantitative or qualitative defects of Von Willebrand Factor (VWF), and concomitant lower factor VIII (FVIII) levels. VWF is essential for both primary and secondary haemostasis as it contributes to platelet adhesion and aggregation at sites of injury, resulting in platelet plug formation. Moreover, it acts as a chaperone protein for FVIII, protecting it from proteolysis in the circulation (2, 3).

The current VWD classification is based on observed VWF abnormalities. Whereas type 1 VWD describes a partial and type 3 VWD a complete quantitative VWF deficiency, type 2 VWD is comprised of several qualitative VWF defects. VWD is mainly characterised by mucocutaneous bleeding and bleeding after trauma or surgery. Available treatment focuses on normalisation of VWF and FVIII levels in cases of acute bleeding, when trauma occurs or during surgery. VWF and FVIII levels can be increased by administration of desmopressin (DDAVP), which stimulates endogenous release, or by replacement therapy with intravenously administered exogenous VWF concentrate with or without FVIII (4). Prophylactic treatment is rarely necessary and usually restricted to type 3 VWD patients.

A widely used plasma-derived VWF concentrate in patients with VWD is Haemate P® or Humate P® (5). This concentrate contains both VWF and FVIII in a ratio of 2.4:1. Inter-individual variability in achieved levels after infusion of this VWF/FVIII containing concentrate has been reported by several investigators, both in the on demand treatment of bleeding and in the surgical setting (6–9). This variability can be explained by both the interindividual differences in pharmacokinetics (PK) of the exogenous VWF/FVIII containing concentrate and the interindividual differences in residual endogenous VWF and FVIII levels. Moreover, endogenous FVIII levels which are known to vary unpredictably due to FVIII release from the endothelium after induced stress, trauma or surgery can differ significantly within an individual patient and between individuals. This variability hampers adequate dosing of VWF/FVIII concentrate leading to achieved levels that may be higher or lower than targeted (6). Subsequently this may lead to an increased risk of thrombosis or bleeding, respectively. In addition, patient and societal burden of treatment are unnecessarily high due to frequent monitoring of plasma FVIII and VWF levels and more consumption of concentrate than necessary (6).

The current challenges to achieve the required target levels in VWD patients using this specific VWF/FVIII concentrate call for additional tools to dose more adequately. Population PK modelling and subsequent maximum a posteriori (MAP) Bayesian analysis could be promising tools to reach individualize care in VWD patients that need to undergo surgery.

Historically, perioperative dosing of VWD patients with VWF/FVIII concentrates has been based on FVIII levels for a variety of reasons. Firstly, generally FVIII plasma levels were presumed more important in preventing perioperative bleeding (10). Secondly, product labels only contained information on FVIII potency. And finally more practically, the more rapid availability of FVIII level results in most laboratories, made FVIII-based dosing a more feasible guide for replacement therapy with VWF/FVIII concentrate. However, nowadays some researchers recommend that especially during the first 36 postoperative hours, VWF activity also needs to be measured because the presence of sufficient VWF activity can be important for the aggregation of platelets during primary hemostasis, and therefore initial wound closure (3, 11). Sufficient FVIII levels are subsequently required for complete wound healing and are therefore often monitored during the whole perioperative period (12-14). Dutch national guidelines have adopted these general principles and describe FVIII and VWF targets for the first 36 hours after the surgery and FVIII targets for the further monitored postoperative period (13).

The aim of the study is to assess the population PK of FVIII activity levels after perioperative administration of a specific VWF/FVIII concentrate and to identify any patient, surgical or treatment factors correlating with the PK parameters of FVIII. The population model can be a starting point for the individualization of replacement therapy during the perioperative period in VWD patients, and may be especially useful when only FVIII targets apply.

METHODS

Data

The data used to construct this population PK model was obtained from a multicenter retrospective cohort study performed by the OPTI-CLOT study group, conducted in five Academic Haemophilia Treatment Centers in the Netherlands (6). This first data set is referred to as the *index data set* and was used for the development of this FVIII-based population PK model. Additionally, an extra data set from the Erasmus University Medical Center Rotterdam (n=20) was collected, which was used for external

validation of the developed FVIII-based population PK model. This data set will be referred to as the *validation data set*. The combination of both data sets was used to build the final FVIII-based population PK model. All data were collected between 2000-2018 and where acquired in accordance with the Dutch rules and regulations for Good Clinical Practice.

All VWD patients included in this study underwent a surgical intervention requiring replacement therapy with VWF/FVIII concentrate (Haemate P®). The data consisted of FVIII plasma levels, patient demographics, surgical characteristics and treatment information. Patient demographics included: sex, age, height, weight, blood group, haemoglobin levels, baseline VWF antigen (VWF:Ag), VWF activity (VWF:Act) and FVIII activity levels (lowest levels ever measured in the patient), renal- and hepatic function (characterized by aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), albumin, urea and creatinine), type of VWD as diagnosed following the national guidelines and surgical risk classification based on the American Society of Anaesthesiologists physical status classification system (ASA) (13). Surgical characteristics consisted of type, severity and duration of surgery (15). Treatment information described timing and dosing of the concentrate and/or comedication with effect on haemostasis (NSAID, tranexamic acid or heparin) and achieved FVIII, VWF:Act, VWF:Ag and VWF: Collagen binding (VWF:CB) levels. Perioperative dosing of the VWF/FVIII concentrate was based on FVIII levels, which were measured by one-stage clotting assays (13). Dosages and levels obtained after additional desmopressin use were excluded, as FVIII pharmacokinetics after desmopressin were expected to deviate due to excessive endogenous FVIII release (16). The included patients did not receive prophylactic treatment and when occasionally a dose was given before the loading dose of the surgery this dose was included in the database. A more detailed overview of data characteristics is documented in table 1.

The population PK modelling approach analyses the data from all patients simultaneously instead of modelling individual patients separately. An analysis provides typical (median) values of PK parameters and the corresponding inter- and intra-individual variability . With this method sparse data with random sampling times can be analysed, which is usually the case during clinical data collection.

Table 1. Characteristics of the index data, validation data and combination of all available data.

Demographics	**Subset**					
	Index data		**Validation data**		**All available data**	
Number of patients	97	-	20	-	117	-
Female sex	66	(68%)	12	(60%)	78	(67%)
Age (years)	50	(0.5 – 82)	48.5	(6.0 – 76.0)	50	(0.5 – 82)
Height (cm)*	173	(69 – 194)	170	(120 – 183)	172	(69 – 194)
Weight (kg)	76.0	(8.8 – 118.0)	83.0	(24.0 – 112.0)	77.0	(8.8 – 118.0)
Blood group O*	49	(51%)	9	(45%)	58	(50%)
Baseline FVIII level ($IUmL^{-1}$)	0.41	(0.01 – 0.97)	0.40	(0.1 – 0.7)	0.41	(0.01 – 0.97)
Baseline VWF:Act level ($IUmL^{-1}$)	0.16	(0.0 – 0.58)	0.11	(0.05 – 0.31)	0.15	(0.0 – 0.58)
Baseline VWF:Ag level ($IUmL^{-1}$)	0.28	(0.0 –0.93)	0.22	(0.07 – 0.56)	0.28	(0.0 – 0.93)
Liver function disorders*	18	(19%)	1	(5%)	19	(16%)
Surgical characteristics						
Number of patients undergoing						
1 surgery	69	(71%)	13	(65%)	82	(70%)
2 surgeries	16	(16%)	5	(25%)	21	(18%)
3 surgeries	10	(10%)	1	(5%)	11	(9%)
4 surgeries	0	(0%)	0	(0%)	0	(0%)
5 surgeries	1	(1%)	1	(5%)	2	(2%)
6 surgeries	1	(1%)	0	(0%)	1	(1%)
Duration of procedure (min)	71	(7 –470)	48	(10 –387)	65	(7 – 470)
Number of occasions/surgeries	141	-	31	-	172	-
Diagnosis per occasion						
Number of VWD type diagnoses						
1	66	(47%)	15	(48%)	81	(47%)
2A	34	(24%)	12	(39%)	46	(27%)
2B	8	(6%)	2	(6%)	10	(6%)
2M	17	(12%)	2	(6%)	19	(11%)
2N	8	(6%)	0	(0%)	8	(5%)
3	8	(6%)	0	(0%)	8	(5%)

Table 1. Continued

	Index data		Validation data		All available data	
Number of ASA classifications*						
II	99	(82%)	27	(87%)	126	(83%)
III	21	(17%)	4	(13%)	25	(16%)
IV	1	(1%)	0	(0%)	1	(1%)
Severity of surgical procedure						
Minor	37	(26%)	12	(39%)	49	(28%)
Major	104	(74%)	19	(61%)	123	(72%)
Treatment information						
Haemate P® dosages per occasion	5	(1 – 30)	7	(2 – 20)	5	(1 – 30)
FVIII dose (IU/kg)	22.1	(5.5 – 66.1)	16.7	(5.6 – 50.0)	20.8	(5.5 – 66.1)
Tranexamic acid use during occasion	59	(42%)	9	(29%)	68	(40%)
NSAID use during occasion	6	(4%)	3	(10%)	9	(5%)
Heparin use during occasion	58	(41%)	12	(39%)	70	(40%)

Data expressed as frequency (%) or median (range). *Missing data was present in 4.3% height-, 4.3% blood group-, 18.8% altered hepatic functioning-, and 11.6% ASA classifications of all available data.

Population PK modelling

A compartmental population PK model describing the PK of FVIII levels after administration of this specific VWF/FVIII concentrate in the perioperative setting was developed using nonlinear mixed effect modelling (NONMEM), as implemented in software package NONMEM version 7.4.2 (ICON Development Solution, Gaithersburg, MD, USA). Visualisation and evaluation of the data and the developed FVIII PK model was achieved using R v3.4.1 and PsN v4.7.0 in combination with Piraña v2.9.6 (17–20). FVIII levels were log transformed and after analysis the PK parameters, their inter-individual variability (IIV) and residual variability between observed and predicted FVIII were derived. In order to determine what number of compartments produced the best fit of the data, single and multiple compartment linear models were used to fit the FVIII versus time data. The PK parameters, volume of distribution (V) and clearance (CL), were estimated. When using e.g. a two compartment model, estimation of the peripheral volume of distribution and inter-compartmental clearance were included.

Baseline FVIII was estimated in the PK analysis and subtracted from the observed FVIII level in the modelling process. Though, in 92 of the 180 surgeries, FVIII was measured before administration of the VWF/FVIII concentrate and these values did not always coincide with the measured baseline FVIII, i.e. FVIII before administration was often higher than the lowest value ever measured in the patient. This difference is most likely caused by physiological variability in FVIII levels or by preoperative anxiety, increasing age or presence of co-morbidity (21-23). For modelling purposes, a correction was introduced by administration of a fixed virtual dose with varying bioavailability to these patients prior to the time of measurement of the pre-dose FVIII level. Application of this technique causes FVIII estimation to return to the lowest value ever measured instead of FVIII level before administration. The rationale of the use of this technique was strengthened by the presence of lower FVIII levels at the end of perioperative treatment than pre-dose FVIII measured in ten occasions. It was possible to estimate the bioavailability (F) and its variability as a correction without influencing estimations of other PK parameters.

Finally, as a wide variatiety of ages and weights was present in the data the PK parameters were a priori scaled to a bodyweight of 70 kg using the allometric scaling principle (24).

Covariate modelling

In order to test the capability of the factors sex, age, height, blood group, duration and severity of surgical procedure, VWD type, ASA classification, (baseline) VWF:Act, (baseline) VWF:Ag, VWF:CB, use of NSAIDs, tranexamic acid and/or heparin and altered hepatic and/or -renal function to explain the IIV or inter-occasion variability (IOV) in PK parameter estimates, a covariate analysis using a forward inclusion and backwards elimination method was performed. Using a univariate analysis, potential covariates could be identified, and subsequently be included in a multivariate analysis (25). Factors to be included in the covariate analysis were selected when respective data was available in ≥50% of patients. Therefore, in our study haemoglobin was ultimately excluded from the covariate analysis. For the time-varying covariates VWF:Act, VWF:Ag and VWF:CB, the last observation carried forward (LOCF) method was applied. This method assumed the last measured observation until a new observation is known. Periods where a virtual loading dose was estimated were handled separately, as no VWF/FVIII had been administrated yet. A more in-depth overview of the population pharmacokinetic modelling can be found in supplement 1.

Model evaluation and validation

The predictive performance of the model was evaluated by visual inspection of the goodness-of-fit (GOF) plots. Furthermore, visual predictive checks (VPC) were

performed in order to internally validate the model. The evaluated model generates (n=1000) simulations of the observed data, where after the simulated data is compared with the observed data.

Subsequently, this intermediate PK model based on 97 patients was externally validated in 20 other patients by fitting the validation data set without re-estimating model parameter estimates. Visual inspection of GOF-plots was performed and the predictive performance of the intermediate FVIII PK model was determined by calculating the mean percentage error (MPE) (equation 8) and mean absolute percentage error (MAPE) (equation 9), respectively, representing bias and inaccuracy.

Equation 8: $$MPE(\%) = \frac{1}{n}\sum_{j=1}^{n}\left(\frac{C_{pred}-C_{obs}}{C_{obs}}\right) * 100\%$$

Equation 9: $$MAPE(\%) = \frac{1}{n}\sum_{j=1}^{n}\left|\frac{C_{ipred}-C_{obs}}{C_{obs}}\right| * 100\%$$

Where Cpred represents the population predication, Cipred the individual predication and Cobs the observed FVIII for a total number of observations (n). The bias is regarded as non-significant when 0 is included in the confidence interval. Inaccuracy below the arbitrary chosen 25% was accepted.

Subsequently, the FVIII PK model was fully developed after re-estimation of all parameter values using all data resulting in the final FVIII PK model. Finally, a bootstrap method was applied, using 1000 data subsets resampled from the complete original data.

RESULTS

From a total of 97 patients, 684 FVIII measurements were collected and used for model building, while the remaining 208 FVIII samples of 20 patients were used for external validation of the developed model. FVIII levels after administration of the VWF/FVIII concentrate ranged from 4.70 IU/mL as highest top level to 0.01 IU/mL over time. Bolus infusion dosages ranged from 5.5 to 66.1 IU FVIII per kg body weight, while 4.7 % of the dosages were given as continuous infusion with doses ranging from 0.19 to 4.2 IU/h per kg body weight. Samples were collected within a period of 146 hours before surgery and 524 hours postoperatively; the majority of the samples was collected up to 168 hours after the surgery. Each patient received at least one bolus or continuous infusion and was monitored for a period ranging from 1 to 22 days after surgery. The median number of FVIII measurements during hospitalisation was five (ranging from 1 to 14). Younger patients were under represented, as only 7 children with a median age of 14 years (range:

0.5 - 16 years) and median body weight of 54 kg (range: 8.8 – 107 kg) were included. None of the FVIII samples were below the lower limit of quantification (0.01 IU/mL). Haemostatic complications during surgery were limited, as no thrombotic events were reported and in only five surgeries a clinically relevant bleeding occurred. Additional information can be found in the article describing the data (6).

Structural model

A one compartment linear model best described FVIII PK after administration of the VWF/FVIII concentrate in a perioperative setting. Allometric scaling for bodyweight was applied to V and CL. Parameter F successfully corrected for the difference in the baseline FVIII level and the FVIII level observed prior to the surgical procedure without influencing the estimation of the other PK parameters. The IIV was identified in PK parameters V and CL, whereas the inter-occasion variability was identified in F. Furthermore, a correlation coefficient was estimated between the variability of V and CL. Estimated values of this structural FVIII PK model can be found in table 2.

Covariate modelling

During the forward inclusion of the covariate analysis, statistically significant ($P<0.05$) associations were identified between covariates surgery duration, ASA classification and VWF:Act levels over time and the PK parameter CL. Backwards exclusion revealed all associations to be statistically significant ($P<0.01$). When surgery duration increased from 45 to 106 min (interquartile range (IQR)), CL decreased with 38%. Additionally, when the VWF:Act increased from 0.78 to 2.21 U/mL (IQR of all measured VWF:Act levels) CL decreased with 29%, presumably caused by prevention of degradation of FVIII by binding to VWF. The associations between these exponentially modelled covariates and CL are visualised in figure 2A and 2B. In figure 2C and 2D inter-individual variability in CL is plotted against VWF activity level and surgery duration. These plots should show no trend, as this indicates that the covariates explain the variability well. Finally, patients in ASA class III or IV exhibited a 44% decrease of CL in comparison to patients in ASA class II.

Model validation and evaluation

The intermediate PK model based on the index data set was validated with an external data set. The bias and inaccuracy, described by the MPE and MAPE, were found to be -10.2% (95% CI: -14.3 - -6.2) and 13.0% (95% CI: 11.6 – 14.4). Therefore, the predictive performance of the model in the validation data set showed a small bias and acceptable inaccuracy. The GOF-plots of the validation (supplement 1) depict the same results and visualize the small bias seen in population prediction versus the observed levels plot and

acceptable inaccuracy in the population prediction as well as the individual prediction versus observed levels plot.

Following re-estimation of the parameters using all data, GOF plots (figure 1) indicated that the final FVIII population PK model adequately describes FVIII levels of the total study population. In these plots the trend lines are close to the line of identity indicating that no bias is present and the data are randomly distributed around the line y=x. Figure 1A shows the predicted FVIII levels based on the population PK parameters with covariate adjustment. Since IIV is not taken into account large deviations from the line y=x are observed. Figure 1B displays the individual predicted FVIII levels compared to the observed levels. The individual predicted levels are calculated by using the individual PK parameters estimated by Bayesian analysis. Smaller deviations around the line y=x are observed as IIV of the PK parameters is taken into account. However, residual error is still present. In figure 1C and 1D the conditional weighted residuals (CWRES), representing the difference between the observed and predicted FVIII level, versus population prediction or time after dose are shown. The vast majority of the points are between -2 and +2 SD without a trend, indicating sufficient model performance.

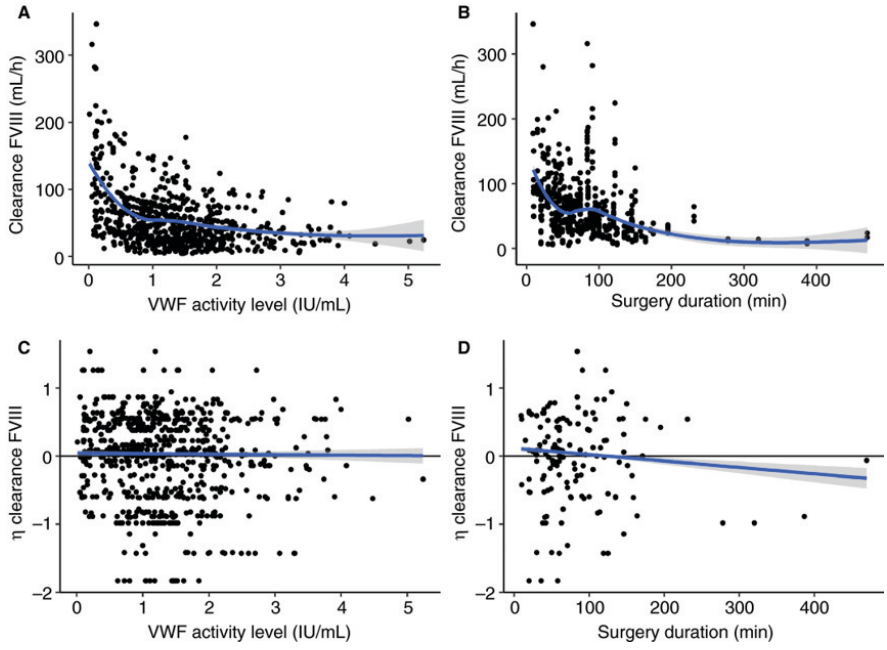

Figure 1. Relation between clearance and A, the VWF activity level and B the duration of surgery in the population PK model for a specific VWF/FVIII concentrate (Haemate P®/ Humate P®). The interindividual random effects for interindividual variability (η) show no trend when plotted against VWF activity level **C**, and duration surgery **D**, demonstrating the appropriateness of the covariates to explain variability. FVIII, factor VIII; PK, pharmacokinetics; VWF, von Willebrand factor

Adequate model performance of the final FVIII PK model is visualized using a prediction-corrected VPC (figure 2). Bootstrap confirmed the robustness of the parameter estimates obtained in the final FVIII PK model. Estimated parameters of the intermediate and final validated FVIII PK model parameters and bootstrap values can be found in table 2.

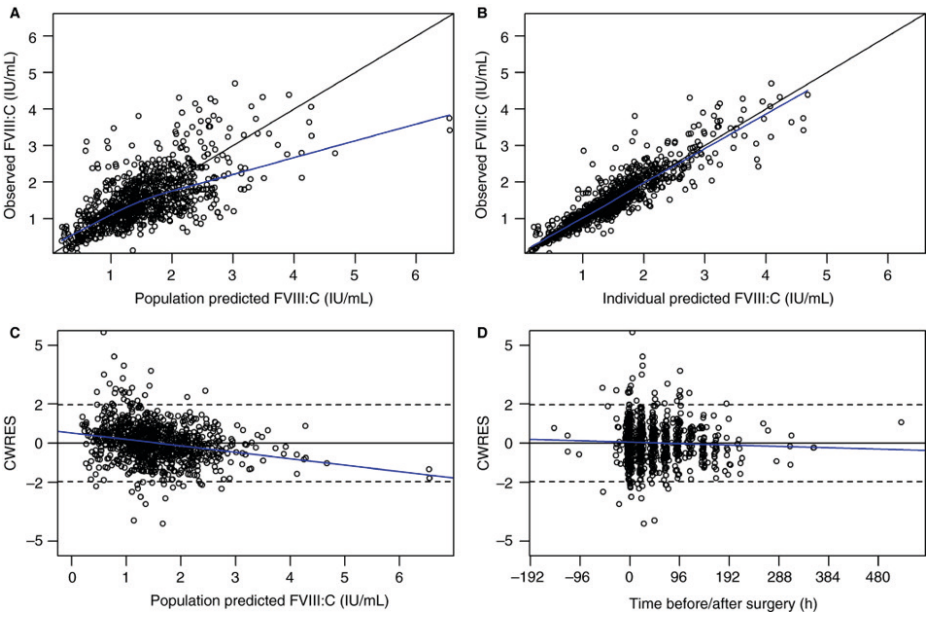

Figure 2. The goodness-of-fit plots of the final FVIII population pharmacokinetic model for a specific VWF/FVIII concentrate (Haemate P®/Humate P®). A, Population predicted and **B**, individual predicted FVIII levels are compared to observed FVIII levels. Conditional weighted residuals (CWRES) representing the difference between the observed and predicted FVIII levels are compared to the **C**, population predicted levels and **D**, time before/after surgery. The individual data (black circles) are visualized as a trend line (blue solid line) that approximates the line of identity (black solid line). The blue line should be close to the line of identity, indicating that no bias is present in the pharmacokinetic model. FVII, factor VIII; VWD, von Willebrand factor

Table 2. Parameter estimates of the structural, intermediate and final FVIII-based PK model of a specific VWF/FVIII concentrate (Haemate P®/Humate P®)

Parameter	Structural FVIII PK model			Intermediate FVIII PK model			Final FVIII PK model			Bootstrap	
	Estimate	RSE (%)	Shr. (%)	Estimate	RSE (%)	Shr. (%)	Estimate	RSE (%)	Shr. (%)	Estimate	95% CI
Volume of distribution (L/70 kg)	3.31	4.4		3.27	4.4		3.28	3.8		3.28	(3.05 – 3.55)
Clearance (L/70 kg/h)	0.044	9.8		0.044	9.8		0.037	10.9		0.038	(0.029 – 0.048)
Bioavailability virtual dose	0.193	21.3		0.221	21.5		0.200	16.2		0.203	(0.138 – 0.288)
Surgery duration on CL	-	-		-0.435	24.6		-0.416	25.2		-0.419	(-0.669 – -0.236)
ASA class III or IV on CL	-	-		0.553	15.4		0.555	14.1		0.581	(0.437 – 0.822)
VWF activity on CL	-	-		-0.303	43.2		-0.263	47.1		-0.253	(-0.578 – -0.001)
Inter-individual variability (%CV)											
IIV Volume of distribution	33.1	13.4	19.6	34.6	12.6	18.1	30.9	12.1	19.3	30.39	(22.34 – 38.32)
IIV Clearance	82.0	11.4	16.8	65.1	12.7	17.7	84.1	12.7	16.6	84.37	(59.85 – 114.53)
Correlation between V and CL	53	-		51	-		47	-		56.27	(25.27 – 85.20)
IOV Bioavailability virtual dose	172.5	21.9		160.0	24.3		154.5	19.0		146.69	(67.35 – 280.65)
Proportional residual variability (%)	22.5	7.2		21.3	7.6		20.3	6.6		20.1	(17.6 – 22.9)

V: volume of distribution, CL: clearance, CV: Coefficient of variation calculated as √(exp(ω2)-1) * 100, RSE: relative standard error, CI: confidence interval, Shr: Shrinkage, IIV: inter-individual variability, IOV: inter-occasion variability. Bootstrap results are based on 1000 data subsets sampled from the original data with resampling.

$$CL = \theta_{CL} * \left(\frac{Weight_i}{70}\right)^{0.75} * \left(\frac{Surgery\ duration_i}{81}\right)^{-0.416} * \left(\frac{VWF{:}Act_{ij}}{1.65}\right)^{-0.263} * 0.555_{ASA\ class\ 3,4} * e^{\eta_{CL}}.$$

$$V = \theta_V * \left(\frac{Weight_i}{70}\right)^{1} * e^{\eta_V}.$$

DISCUSSION

The aim of this study was to develop a population PK model describing FVIII levels after administration of a specific VWF/FVIII concentrate (Haemate P®/Humate P®) in a perioperative setting. Additionally, using covariate analysis, any patient, surgical or treatment factors correlating with the PK parameters of the developed model were identified.

A one compartment PK model was able to fit the available data describing FVIII levels after administration of the VWF/FVIII concentrate in the perioperative setting. Almost all achieved FVIII levels of included study patients were well above predefined targets as stated by national guidelines, specifically 95.2% during first 36 hours and 98.9% in the subsequent period (13). Twenty-five of the included patients showed excessive FVIII levels (>2.5 IU/ml) during the perioperative period, indicating the potential benefit of PK guided dosing. Some studies have already examined application of PK guided dosing of this specific VWF/FVIII concentrate following surgery (14, 26). The prospective multicenter trial of Lethagen et al. demonstrated feasibility in selection of the loading dose prior to elective surgery based on the PK profile of the patient. However, the study of Di Paola et al. observed a poor correlation between the presurgical and postsurgical IVR values, questioning the potential profit of PK guided dosing. However, our approach is likely superior to the study by Di Paola et al. in which PK guided dosing of this VWF/FVIII concentrate with a standard two compartment PK model was evaluated without taking the prior information of the population and influences of covariates into account (26). A covariate analysis is important as various international guidelines recommend specific FVIII target levels depending on the type and extent of the surgical procedure (11, 13, 27). Unfortunately, correlation between the presurgical and postsurgical IVR values could not be estimated in this study as presurgical PK profiles were not available.

The effects observed in this study; that increasing surgery duration is linked to decreased CL of FVIII, is possibly indicative of an enhanced production or –release, or decreased clearance of FVIII (and possibly primarily of VWF) to safeguard haemostasis during longer lasting haemostatic challenges with larger tissue damage. Patients in ASA class III or IV showed a decreased FVIII CL compared to patients in ASA class II. This can possibly be linked to earlier findings that patients with comorbidities exhibit higher VWF and FVIII levels (23). However, as FVIII baseline levels are included in this population PK model, a decreased FVIII clearance for these patients with more comorbidities would mean that their FVIII levels would rise more during the surgery than patients without comorbidities. This has not yet been observed. In the data used

for the covariate analysis no patients were classified in ASA class V (moribund patient not expected to survive 24 hours with or without an operation) and therefore this class could not be included in the final FVIII population PK model (28).

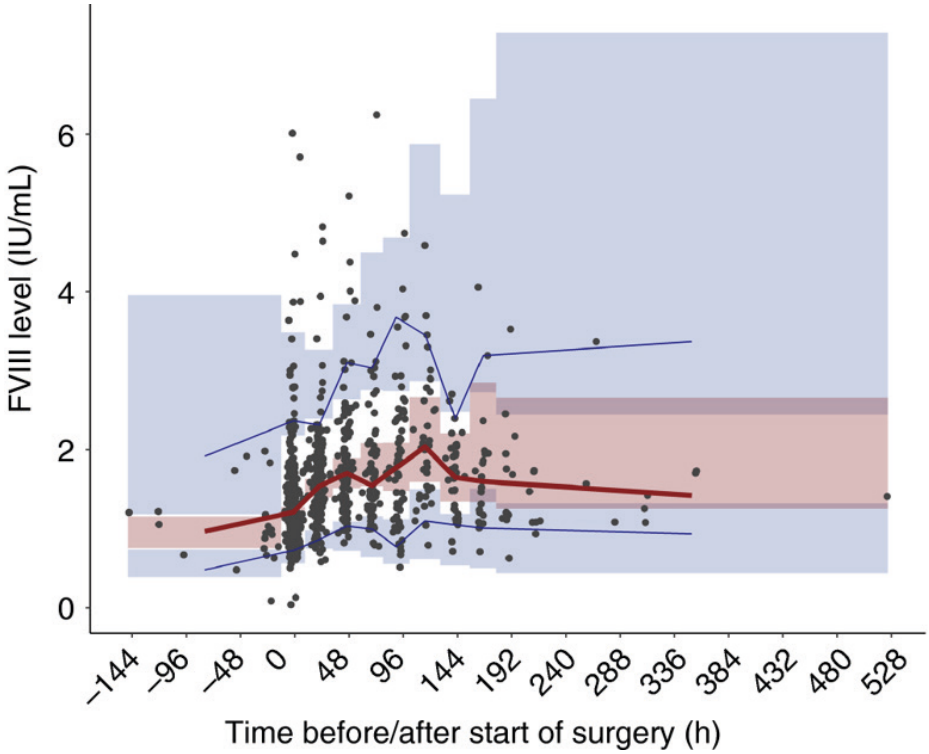

Figure 3. Prediction-corrected visual predictive check (VPC) of the final FVIII-based pharmacokinetic model of a specific VWF/FVIII concentrate (Haemate P®/Humate P®). The median (red line) and 95% CI (blue lines) of the observed data are plotted against the simulated data (n = 1000) indicated as highlighted areas (red area: median; blue area: 95% prediction interval). Individual observations in the data are shown as black dots. A model predicts the concentrations adequately when the blue and red lines run through the corresponding areas CI, confidence interval; FVIII, factor VIII; VWF, von Willebrand factor.

The interaction between VWF and FVIII is complex, considering the variations in the VWF-interactive region located on the light chain of FVIII and possible underlying genetic mutations (29, 30). Since VWF acts as a chaperone for FVIII, the observed effect of higher VWF:Act levels resulting in decreased FVIII clearances seems logical (31). Nonetheless, it should be noted that the influence of VWF:Act on FVIII in this PK model is only based on the measured VWF:Act levels which were assumed to be constant until the next measured level, while in fact VWF:Act levels are expected to constantly change over time after the administration of the VWF/FVIII concentrate.

Furthermore, the high relative standard error (RSE= 51%) of the parameter estimate describing the relationship implies that this observation may be inaccurately estimated. This inaccuracy can be caused by the heterogeneity of VWD types or the absence of sufficient data to fully describe this association. The effect of VWF:Ag on FVIII PK was also evaluated, however, against expectations this influence was insignificant (OFV -3.54, p=0.05).

Remaining covariates included in the covariate analysis showed no significant associations with PK parameters present in the final FVIII PK model. Minor or major surgery severity was identified as a significant covariate; however, the ASA classification system and surgery duration achieved a higher statistical significance in the multivariate analysis. VWD type was also expected to have a significant influence on the PK parameters. During univariate analysis, this covariate showed a significant association with CL, as type 2 and type 3 respectively showed a 54 and 74% higher clearance relative to type 1 patients. However, this effect was not significant when the other covariates were also included in the model. An earlier study evaluating the PK of the VWF/FVIII concentrate in elective surgery also showed no difference between VWD types and the PK of individual patients (14). However we cannot directly compare this study with our current study, as a different PK approach was used, and a different loading dose was administrated. One possible explanation could be that VWD type has less effect on the FVIII clearance than expected after administration of VWF/FVIII concentrate as (functional) VWF is simultaneously administrated. On the other hand, it should be noted that the majority of the patients included in this population PK model were type 1 and 2A and 2M patients and that the model contains fewer data on other VWD types e.g. the data of only data of 8 VWD type 2B, 8 type 2N, and 8 type 3 patients. Therefore, the model is expected to be less applicable in these patients. Patient characteristics height, age, sex, blood group, and renal- and hepatic functioning were not associated with any PK parameters in the final FVIII-based PK model for this VWF/FVIII concentrate.

The large estimated IIV in CL indicates a clinically relevant variability in FVIII clearance after administration of this specific VWF/FVIII concentrate between VWD patients. The estimated IIV of CL became smaller when inter-occasion variability (IOV) was taken into account. The latter quantifies the intra-patient variability of CL. Unfortunately, inclusion of inter-occasion variability on CL resulted in an unstable model and was therefore excluded. The large IIV on CL could however be partially explained by introduction of the statistically significant covariates. However, after re-estimation of the PK parameters using both subsets, IIV on CL increased again. This can be explained by the fact that the *validation data set* differed from the *index*

data set as the *validation se*t was not composed randomly from all data, but solely included data from one center during a certain time period. Differences between the data sets included lower average surgery durations, a higher percentage of patients in ASA class II, less tranexamic acid administration and less patients with blood group O in the validation data set. Moreover, one patient with a genetically proven VWD type 1 Vicenza, which is associated with a high clearance, was present in this data set. Overall, clearance in this validation subset was highly variable.

A limitation of the study is that the developed PK model could not distinguish endogenous FVIII from exogenous FVIII, as it is not possible yet to detect endogenous FVIII as a separate entity. The terminal half-life calculations can be misleading, due to subsequent increases in endogenous FVIII after increase of exogenous and endogenous VWF after administration of this specific VWF/FVIII concentrate in the perioperative period (32, 33). The median calculated FVIII half-life of 57.7 hours is compatible with a rise in endogenous FVIII, as this is longer than the generally reported FVIII half-life of approximately 12 hours.

The population PK of FVIII after perioperative dosing of the specific VWF/FVIII concentrate in patients diagnosed with VWD can be adequately described by the model outlined in this paper. Increased VWF activity or surgery duration and classification in a higher ASA class are correlated with a decrease in FVIII CL. As individual predicted FVIII over time profiles can be established using this model, this could be a first step into the direction of PK-guided dosing in VWD patients undergoing surgery treated with this specific VWF/FVIII concentrate. With the developed model the FVIII levels can be tailored to the individual patient, which is especially useful when only FVIII targets apply. Development of new population PK models for the various other VWF/FVIII concentrates is necessary as the PK of these concentrates differs, due to varying VWF/FVIII ratios and multimer patterns. Furthermore, a VWF-based population PK model for this specific concentrate is currently under development, and the ultimate goal is to provide a model describing both VWF and FVIII and the VWF and FVIII interaction, to facilitate PK-guided dosing based on VWF as well as FVIII levels. Eventually this overall approach may result in more accurate individualized therapy and therefore in increased quality and cost-effectiveness of care for patients with VWD.

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SUPPLEMENT

SUPPLEMENT 1

First-order conditional estimation with interaction was applied to derive population mean (ϑ) PK parameters, their variability (η) and the residual variability (ε) between observed and predicted FVIII. Inter-individual variability (IIV) in PK parameter estimates was described using an exponential function (equation 1), where P_{ij} is estimate P of the j^{th} parameter of the i^{th} individual; ϑ_j is the typical mean value of the j^{th} parameter; η_{ij} is the random variability for the i^{th} individual and the j^{th} parameter distributed with mean zero and variance ω^2. Using ε, a proportional error model on linear scale was modelled (equation 2), where $Y_{ij,\ obs}$ and $Y_{ij,\ pred}$ are the observed and predicted FVIII of the i^{th}individual at time point j, respectively. Baseline FVIII is the measured endogenous FVIII level of the i^{th}individual; and ε is the residual unexplained variability with mean zero and variance σ^2.

Equation 1: $$P_{ij} = \theta_j * e^{\eta_{ij}}$$

Equation 2: $$Y_{ij,\ obs} = (baseline\ FVIII{:}\ C_i + Y_{ij,\ pred}) * (1 + \varepsilon)$$

The effect of F was described as shown in equation 3, where ϑ_F represents impact of this virtual dose and η_F describes inter-occasion variability.

Equation 3: $$F = \theta_F * e^{\eta_F}$$

As a wide variation of ages and weights were present in the data, a priori allometric principles were applied to all involved PK parameters as described in equation 4 (physiological volumes like V) and equation 5 (metabolomics like CL).

Equation 4: $$V_i = \theta_V * \left(\frac{Weight_i}{70}\right) * e^{\eta_{V_i}}$$

Equation 5: $$CL_i = \theta_{CL} * \left(\frac{Weight_i}{70}\right)^{0.75} * e^{\eta_{CL_i}}$$

Covariate modelling

Potential continuous covariates were introduced to the FVIII structural PK model as described by equation 6, where Cov_i is the value of the covariate of the i^{th} individual, Cov_{med} is the median covariate value in the population and ϑ_{cov} is an exponent describing the relationship between covariate and PK parameter. The introduction of categorical covariates is described by equation 7, where $\vartheta_{Cov\ i,n}$ is the fractional increase of the covariate of category n.

Equation 6: $P_j = \theta_j * (\frac{Cov_i}{Cov_{med}})^{\theta_{cov}}$

Equation 7: $P_j = \theta_j * \theta_{Cov_i,n}$

Initially, potential significant covariate relations were identified by plotting η of every PK parameter against the potential covariate. Improvement of the FVIII PK model by introduction of a covariate was statistically tested on the basis of the drop in objective function value (OFV). During this forward inclusion method a drop of OFV>3.84 (based on 1 degree of freedom) as a result of inclusion of a covariate was considered a statically significant ($p<0.05$) improvement of the model. Backwards elimination considers a raise of OFV>6.64 (based on 1 degree of freedom) after deletion of a covariate as a statistically significant ($p<0.01$) deterioration of the model. Above all, as a covariate should describe the random variability, a drop in IIV of the respective PK parameter should be observed. The significant covariates were added to the structural model, resulting in the intermediate FVIII PK model.

SUPPLEMENT 2

Goodness of fit plots of the validation of the intermediate FVIII population pharmacokinetic model.

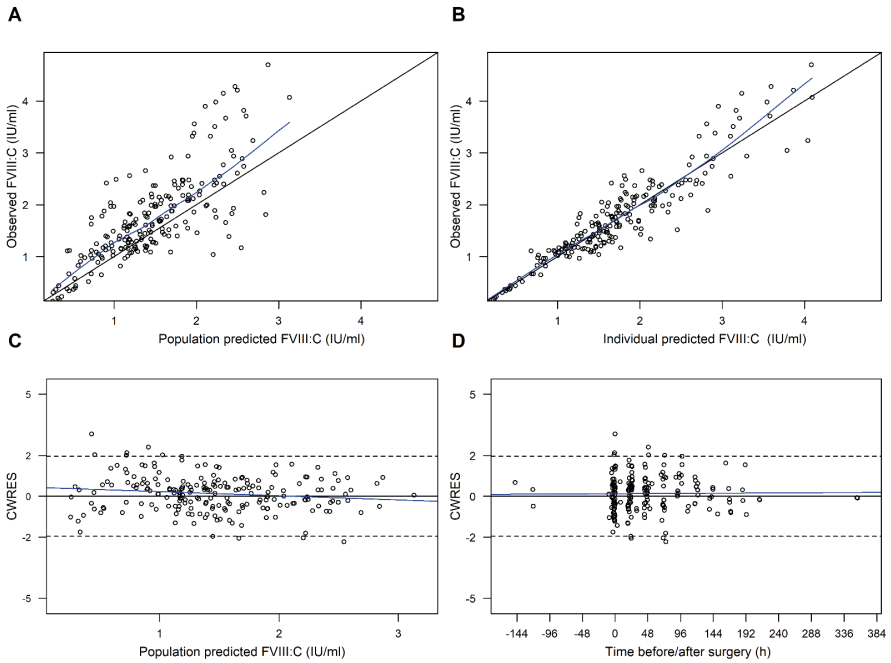

Figure 1. The goodness of fit plots of the validation of the intermediate FVIII population pharmacokinetic model for a specific VWF/FVIII concentrate (Haemate P®/Humate P®) with 20 independent patients. (**A**) Population predicted and (**B**) individual predicted FVIII levels are compared to observed FVIII levels. Conditional weighted residuals (CWRES) representing the difference between the observed and predicted FVIII levels are compared to the (**C**) population predicted levels and (**D**) time before/after surgery. The individual data (black circles) are visualised as a trend line (blue solid line) that approximate the line of identity (black solid line). The blue line should be close to the line of identity, indicating that no bias is present in the pharmacokinetic model.


8

Chapter 8

Population pharmacokinetics of the von Willebrand factor - factor VIII interaction in patients with von Willebrand disease

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ABSTRACT

Recent studies have reported that patients with von Willebrand Disease treated perioperatively with a von Willebrand Factor (VWF)/factor VIII (FVIII) concentrate with a ratio of 2.4: 1 (Humate P® / Haemate P®) often present with VWF and/or FVIII levels outside pre-specified target levels to prevent bleeding. Pharmacokinetic (PK)-guided dosing may resolve this problem. As clinical guidelines increasingly recommend aiming for certain target levels of both VWF and FVIII, application of an integrated population PK model describing both VWF activity (VWF: Act) and FVIII levels may improve dosing and quality of care. In total, 695 VWF: Act and 894 FVIII levels measurements from 118 patients (174 surgeries), who were treated perioperatively with the VWF/FVIII concentrate, were used to develop this population PK model using nonlinear mixed-effects modeling (NONMEM). VWF: Act and FVIII levels were analyzed simultaneously using a turnover model. The protective effect of VWF: Act on FVIII clearance was described with an inhibitory maximum effect function. An average perioperative VWF: Act level of 1.23 IU/mL decreased FVIII clearance from 460 mL/h to 264 mL/h, and increased FVIII half-life from 6.6 to 11.4 hours. Clearly, in presence of VWF, FVIII clearance decreased with a concomitant increase of FVIII half-life, clarifying the higher FVIII levels observed after repetitive dosing with this concentrate. VWF: Act and FVIII levels during perioperative treatment were described adequately by this newly developed integrated population PK model. Clinical application of this model may facilitate more accurate targeting of VWF: Act and FVIII levels during perioperative treatment with this specific VWF/FVIII concentrate (Humate P® / Haemate P®).

INTRODUCTION

Von Willebrand disease (VWD) is an autosomally inherited bleeding disorder, with an estimated prevalence between 0.6 and 1.3% (1). Patients with VWD suffer from bleeding caused by von Willebrand factor (VWF) deficiency or dysfunction, leading to defects in the primary hemostasis as VWF promotes platelet adhesion and aggregation (2). VWF also plays a role in the secondary hemostasis as it acts as chaperone protein for factor VIII (FVIII), protecting it from degradation and clearance in the circulation. Therefore, VWD patients often also present with reduced FVIII levels. VWD is categorized into three types: type 1 patients are characterized by a partial quantitative VWF deficiency, type 2 patients by functional VWF defects and type 3 patients by a complete quantitative deficiency (2).

Treatment of VWD is usually on demand and focuses on normalization of VWF and FVIII levels in critical situations such as surgery, child delivery, acute bleeding and/or trauma (3). A therapeutic increase of VWF and FVIII levels can be achieved by administration of desmopressin, which stimulates the endogenous release of VWF and subsequently increases circulating FVIII, or by intravenous infusion of a VWF-containing concentrate when desmopressin is contraindicated or desmopressin response is insufficient (4, 5). Most plasma derived VWF-containing concentrates also contain FVIII, as acute situations necessitate readily available FVIII for adequate hemostasis (6, 7). However, during prolonged treatment with these concentrates, FVIII accumulates as FVIII production and secretion are not affected in VWD, thereby inducing a hypothetical risk of thrombosis (8, 9). Factor concentrates with varying VWF: Activity (VWF: Act)/FVIII ratios are available, and several studies have indicated that repeated dosing with VWF/FVIII concentrates with a ratio of more than 1 results in less FVIII accumulation if VWF concentrate dosing is based only on VWF levels (7, 10, 11). A commonly used plasma derived VWF/FVIII concentrate is Humate P® or Haemate P® (CSL Behring, Marburg, Germany), which has a VWF: Act/FVIII ratio of 2.4: 1 (12, 13). Nonetheless, also with this specific concentrate, FVIII accumulation is observed after perioperative treatment (14, 15).

Hazendonk et al. have reported that respectively 65% and 91% of trough VWF: Act and FVIII levels in type 1 VWD patients treated with Humate P® during surgery were ≥0.20 IU/ml higher than predetermined target levels as prescribed in clinical guidelines (14). This results in higher treatment costs than necessary and an increased risk of adverse events (14). On the other hand, this study also observed seven VWF: Act levels and FVIII levels of five patients below the pre-specified target levels during the first 36 hours after surgery, thereby increasing bleeding risk. The wide variability in achieved

levels is due to the large inter-individual variability in the pharmacokinetics (PK) of both exogenous and endogenous VWF and FVIII (12, 14, 16). A possible solution for this large variability in achieved VWF and FVIII levels is PK-guided dosing, which uses maximum *a posteriori* Bayesian estimation to determine individual PK parameters that can be used to calculate an adequate dose to achieve a target level. The application of this approach for perioperative dosing with this VWF/FVIII concentrate has been examined in two earlier studies (17, 18). The first prospective multi-center study showed that it is feasible to determine the loading dose of a VWF/FVIII concentrate based on individual PK of VWF (17). Contrastingly, in the study by Di Paolo et al., the *in vivo recovery* (IVR) of the individual PK profile performed before surgery did not match the IVR observed in the perioperative period, indicating that PK-guided dosing is less beneficial (18). However, data in this study was analyzed using a standard two compartment model without taking prior population knowledge or the influence of covariates into account. Development of a population PK model, which is based on data from a population and describes the typical PK parameters with corresponding inter- and intra-individual variability, could possibly improve the PK-guided dosing approach for VWD patients treated with this VWF/FVIII concentrate perioperatively. We have recently developed a population PK model describing FVIII PK after VWF/FVIII concentrate (ratio 2.4: 1) administration, enabling perioperative PK-guided dosing based on FVIII target levels (19). However, as several clinical guidelines advise target levels for both VWF and FVIII to ensure adequate hemostasis, application of an integrated population PK model to predict VWF: Act as well as FVIII levels may allow for more accurate perioperative dosing and therefore improve quality of care (1, 20, 21). In addition, this model may also give insight into the mechanisms of FVIII accumulation observed in these patients. Therefore, the aim of our study was to develop the first population PK model for perioperative VWF/FVIII concentrate (ratio 2.4: 1) dosing, that describes the interaction between VWF and FVIII in patients with VWD.

METHODS

Data collection

We used data from a retrospective multicenter study to develop this integrated population PK model (14). The dataset included VWD patients whom underwent surgery in one of five academic hemophilia treatment centers in the Netherlands between 2000 and 2018. All patients received multiple perioperative doses of a plasma derived VWF-containing concentrate with a VWF/FVIII ratio of 2.4: 1 (Humate P® or Haemate P®, CSL Behring, Marburg, Germany) and were included in the dataset

if at least two perioperative VWF: Act and FVIII level measurements were available. Patients were excluded if other hemostatic disorders were present or if desmopressin was concomitantly used. Dose adjustments were generally based on FVIII levels, as FVIII results were usually more rapidly available. All FVIII levels were measured by one-stage assay, whereas different centers performed different VWF: Act assays: four centers used a VWF: RCo assay, whereas one center used different assays over time (VWF: RCo assay from 2000-2005, monoclonal antibody (VWF: Ab) assay from 2005-2012), and a VWF glycoprotein 1b binding (VWF: GP1bM) assay from 2012 onwards. More detailed specifications of these assays are added to the supplementary methods. Additional information, such as patient characteristics and surgical characteristics were collected from electronic patient files. All data was collected following Good Clinical Practice and Dutch regulations. Informed consent was not obtained, as anonymized, retrospective data was used as reported in an earlier publication.[14]

Population PK modeling

A population PK model describing VWF: Act and FVIII PK after VWF/FVIII concentrate administration was constructed using nonlinear mixed-effect modeling software (NONMEM version 7.4.2, ICON Development Solution). A population PK model considers data from a whole population simultaneously instead of analyzing patients separately, enabling simultaneous analyses of patients where PK differences are expected, such as patients with different types of VWD. This technique can handle sparse data with random sampling times, as was the case in our retrospective clinical data set.

We used turnover models to describe the change of endogenous and exogenous VWF: Act and FVIII levels over time. This method enables handling endogenous baseline concentrations in PK modeling, as it is able to correct for analytic assay variability of the measured endogenous baseline level, while this cannot be done with the frequently used baseline subtraction method (22, 23). Firstly, separate PK models for VWF: Act and FVIII were developed. These models were then combined and the interaction between VWF: Act and FVIII was added. An inhibitory maximal effect (Imax) function relating VWF: Act levels and FVIII clearance was incorporated to describe the inhibitory effect of the VWF: Act levels on FVIII elimination.

During model development, the number of compartments, inclusion of inter-individual variability (IIV), inter-occasion variability (IOV) and residual error structure were evaluated. The incorporation of a separate residual error for VWF levels measured by VWF: RCo and VWF levels measured by other assays (VWF: GP1bM or VWF: Ab) was tested to correct for the use of different analysis methods. As both children and

adults were included in the dataset and a wide range of weights was present, allometric scaling to bodyweight was applied.

Covariate analysis

Patient characteristics or surgical characteristics may potentially explain part of the IIV observed in PK parameters. To identify these characteristics, a covariate analysis was performed using forward inclusion and backwards elimination. The following patient characteristics were tested: age, sex, VWD type as diagnosed by the local center, blood group and physical status as determined by the American Society of Anesthesiologists (ASA) classification (24). The influence of liver and kidney parameters was evaluated by the following covariates: alanine aminotransferase, aspartate aminotransferase, gamma glutamyl transferase, alkaline phosphatase, lactate dehydrogenase, albumin, creatinine and urea levels. Surgical characteristics included duration and severity of the procedure (25). Administration of co-medication such as tranexamic acid, nonsteroidal anti-inflammatory drugs or heparin was evaluated in the covariate analysis. Firstly, covariates were included univariately to statistically select potential covariates. Thereafter, we performed a forward inclusion and backwards elimination procedure. Finally, inclusion of IOV was evaluated and the final population PK model was constructed. More in-depth details of the modeling process can be found in the supplementary method section.

Model evaluation

To evaluate the adequacy of the population PK models to predict the VWF: Act and FVIII levels, goodness-of-fit plots were inspected. The final model was internally validated using a visual predictive check (VPC). One thousand datasets were simulated with the final model and the simulated levels were compared to the observed levels. Additionally, a bootstrap was performed to test the robustness of the model. During the bootstrap analysis 1000 new datasets were randomly created from the original dataset and the model was re-estimated using the newly created datasets.

RESULTS

The data set consisted of 118 patients with different types of VWD, aged 1 to 82 years, whom underwent 174 surgeries (table 1). Eight children (<18 years) were included with a median age of 14 years (range: 1 – 17 years) and median weight of 53.5 kg (range: 8.8 – 107 kg). Patients received a median of five doses of the VWF/FVIII concentrate per perioperative period and a total of 695 VWF: Act and 894 FVIII levels were available. None of the FVIII levels were below quantification limit (BQL<0.01 IU/mL), but three

VWF: Act levels were below the quantification limit of 0.20 IU/mL. These VWF: Act levels were excluded from analysis as the percentage of BQL samples was only 0.4%. A median of four VWF: Act levels and five FVIII levels was collected per perioperative period. These samples were taken between 171 h before start of surgery until 524 h after surgery; but 96% of the samples were collected within 168 h after surgery. After the first perioperative VWF/FVIII concentrate dose, median FVIII level was 1.30 IU/ml [range 0.41 - 3.64 IU/ml], which accumulated to a median FVIII level of 1.80 IU/ml [range: 0.59-4.21 IU/ml] on day five. The VPC, which is explained later in the results section, also illustrates the (prediction-corrected) observed VWF: Act and FVIII levels over time (Figure 1).

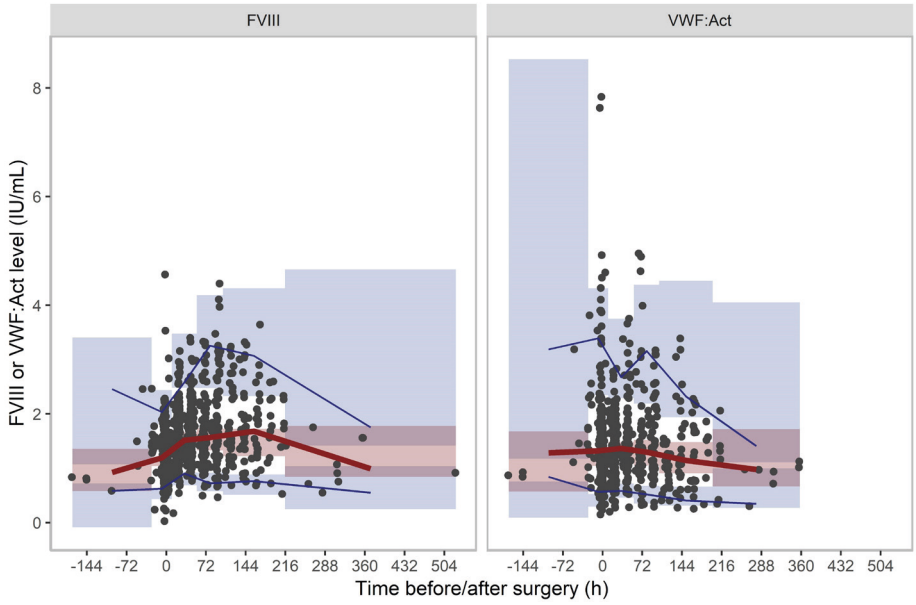

Figure 1. Prediction corrected visual predictive check of the final integrated VWF/FVIII population pharmacokinetic model. The median and 2.5th and 97.5th percentile interval of the observed levels (black dots) at each bin are presented as the red line and the blue lines, respectively. The red and blue shaded boxes represent the median and 2.5th and 97.5th percentile of the 1000 simulated prediction corrected observations at each bin, respectively. A) FVIII; B) VWF:Act

Table 1. Patient characteristics

Demographics	Median (range) or number (%)	Number of patients data available (n)
Number of patients	118	-
Sex (male)	40 (34%)	118
Age (years)	49 (0.5 – 82)	118
Body weight (kg)	77.0 (8.8 – 118.0)	118
Height (cm)	172.0 (69.0 – 194.0)	113
Historical baseline FVIII level (IU/mL)	0.41 (0.01 – 0.97)	118
Historical baseline VWF: Act level (IU/mL)	0.15 (0.00 – 0.58)	118
Historical baseline VWF: Ag level (IU/mL)	0.28 (0.00 – 0.93)	118
Von Willebrand disease type		118
Type 1	57 (48%)	
Type 2A	32 (27%)	
Type 2B	9 (8%)	
Type 2M	11 (9%)	
Type 2N	3 (3%)	
Type 3	6 (5%)	
Blood group 0	59 (52%)	113
Surgery information		
Number of surgeries (occasions)	174	-
Duration of surgery (min)	67.5 (7.0 – 470.0)	174
Severity of surgery	-	174
Minor	50 (29%)	
Major	124 (71%)	
ASA classification per surgery	-	154
II	127 (82%)	
III	26 (17%)	
IV	1 (1%)	
Treatment information		
Bolus dose in FVIII (IU/kg) (n=1036)	20.8 (5.5 – 66.1)	
Continuous infusion dose in FVIII (IU/h/kg) (n=51)	1.06 (0.19 – 4.17)	
Number of doses per surgery	5 (1 – 30)	
Tranexamic acid during surgery	68 (39%)	
NSAID use during surgery	9 (5%)	
Heparin use during surgery	71 (41%)	

Population PK model

Time profiles of both VWF: Act and FVIII were described using turnover models. In these models, the change in endogenous VWF and FVIII levels over time was described with a zero-order production rate k*in* and a first order elimination rate k*out*. Upon administration of the factor concentrate, VWF and FVIII were injected in the respective central compartments. The interaction between VWF and FVIII was described by an inhibitory effect of VWF: Act on FVIII clearance. An Imax relation was chosen to describe this relationship, following equation 1.

$$Inhibition = 1 - \frac{Imax * C_{VWF}}{IC_{50} + C_{VWF}} \quad (1)$$

in which C_{VWF} represents the VWF level, Imax the maximal inhibitory effect on FVIII clearance and IC_{50} the VWF level at which 50% FVIII clearance inhibition was established. A visual representation of the model can be found in figure 2.

In 77 surgeries, VWF: Act level before the first VWF/FVIII concentrate infusion (pre-administration level) was higher (0.26 IU/ml [range: 0.01-3.74]) than the historical baseline (lowest level ever measured; 0.15 IU/ml [range: 0.00-0.58]). The pre-administration FVIII level (0.68 IU/mL [range: 0.01-3.11]) was also higher than the historical baseline level (0.41 IU/mL [range: 0.01-0.97]) in 98 surgeries. In the turnover models, the pre-administration VWF: Act and FVIII levels were considered as baseline values instead of the historical baseline levels, assuming that the increase in endogenous VWF: Act and FVIII levels was permanent and levels would return to the pre-administration level after the perioperative period.

The structural model consisted of one-compartment turnover models for both VWF: Act and FVIII (Figure 2). Typical values for VWF 1) pre-administration baseline, 2) clearance and 3) volume of distribution with corresponding inter-individual variability values (IIV %) were 1) 0.42 IU/mL (126.4%), 2) 262 ml/h (55.3%) and 3) 4990 ml (25.2%) for a patient of 70 kg (Table 2). Using the integrated turnover model, typical values for FVIII 1) pre-administration baseline, 2) clearance and 3) volume of distribution were 1) 0.77 IU/mL (32.2%), 2) 460 ml/h (81.5%) and 3) 4350 ml. These values reflect the theoretical situation in which VWF is absent. VWF inhibited FVIII clearance with an IC50 value of 1.65 IU/mL. An average perioperative VWF: Act level of 1.23 IU/mL decreased FVIII clearance from 460 ml/h to 264 ml/h and increased FVIII elimination half-life from 6.6 to 11.4 hour.

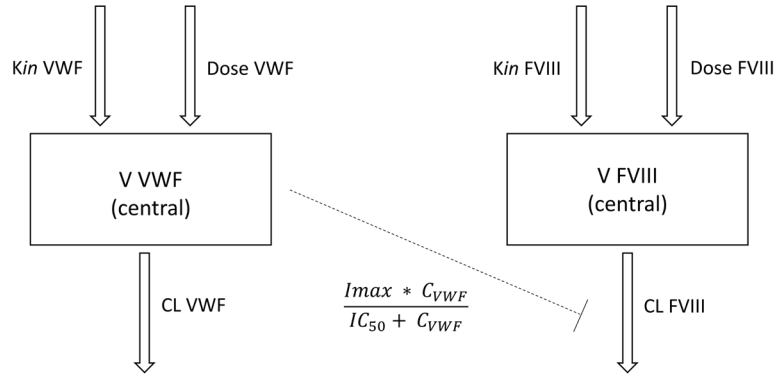

Figure 2. Schematic overview of the integrated population pharmacokinetic model consisting of one compartment models described by central volume of distribution (V) and clearance (CL). The von Willebrand Factor (VWF) activity inhibits the clearance of factor VIII (FVIII) and is described by an maximal inhibitory effect (Imax) relationship, where IC_{50} describes the VWF concentration resulting in 50% of the maximal inhibitory effect. The change in endogenous VWF and FVIII amounts over time was described with a turnover model with a zero-order production rate k*in*.

Table 2. Pharmacokinetic parameters for the structural model, final model and bootstrap analysis

	Structural model		**Final model**		**Bootstrap**	
Parameter	**Estimate**	**RSE (%) [Shr.]**	**Estimate**	**RSE (%) [Shr.]**	**Estimate**	**95% CI**
CL FVIII (mL/70kg/h)	460	39.1	1170	37.1	1188	546 - 5801
V FVIII (mL/70 kg)	4350	7.3	4440	6.5	4414	3940 - 5178
Baseline FVIII (IU/mL)	0.77	15.0	0.64	6.9	0.64	0.50 – 0.80
CL VWF (mL/70kg/h)	262	7.5	252	6.3	255	228 - 291
V VWF(mL/70 kg)	4990	4.1	5060	3.9	5058	4654 - 5570
Baseline VWF (IU/mL)	0.42	11.3	0.68	14.1	0.68	0.52 – 0.89
IC50 (IU/mL)	1.65	39.7	1.10	15.5	1.08	0.67 – 1.84
Imax	1 (fixed)	-	1 (fixed)	-	1	1 – 1
Inter-individual variability (CV%)						
IIV on CL VWF	55.3	11.3 [27.5]	49.9	12.6 [25.7]	48.2	32.0 – 63.5
IIV on V VWF	25.2	21.1 [35.4]	27.6	11.3 [31.4]	26.5	17.5 – 34.1
IIV on CL FVIII	81.5	25.6 [26.6]	85.4	13.8 [32.4]	79.8	46.7 – 314.0
IIV on Base FVIII	32.2	15.6 [11.4]	27.8	12.0 [14.3]	27.4	20.5 -35.7
IIV on Base VWF	126.4	9.8 [10.5]	85.2	11.1 [15.1]	83.4	60.9 – 111.2

Table 2. Continued

	Structural model		Final model		Bootstrap	
Parameter	**Estimate**	**RSE (%) [Shr.]**	**Estimate**	**RSE (%) [Shr.]**	**Estimate**	**95% CI**
Correlation IIV Base FVIII and Base VWF	55.4	0.0767	32.4	0.032	32.2	-2.9 – 60.3
Residual variability						
Proportional error FVIII (%)	19.4	8.0	18.7	7.4	18.5	15.3 – 21.1
Additive error FVIII (IU/mL)	0.13	25.6	0.13	22.7	0.13	0.06 – 0.18
Proportional error VWF: RCo (%)	26.7	7.6	27.0	7.0	26.9	22.9 – 30.6
Proportional error VWF: Ab and VWF: Gp1bM(%)	23.0	6.6	22.6	6.3	22.7	19.6 – 25.4
Covariate relations						
Duration of surgery on CL VWF	-	-	-0.29	30.6	-0.29	-0.56 – -0.18
VWD type 2 on baseline VWF	-	-	0.39	18.8	0.40	0.27 – 0.57
VWD type 3 on baseline VWF	-	-	0.18	65.4	0.19	0.04 – 1.01
ASA score III/IV on baseline VWF	-	-	1.53	15.4	1.48	1.15 – 2.29
VWD type 2 on CL FVIII	-	-	0.44	26.4	0.44	0.17 – 0.72
VWD type 3 on CL FVIII	-	-	0.34	52.8	0.33	0.08 – 1.20

FVIII: Factor VIII, VWF: von Willebrand factor, V: volume of distribution, CL: clearance, Imax: the maximal inhibitory effect, IC50: the VWF level where 50% of the inhibitory effect is reached, CV: Coefficient of variation calculated as √(exp(ω2)-1) * 100, RSE: relative standard error, Shr: shrinkage. Of the 1000 data subsets used for bootstrap analysis, 145 runs were terminated.

Formulas PK parameters final model:

$$CL\ VWF = \theta_{CL} * \left(\frac{Weight_i}{70}\right)^{0.75} * \left(\frac{Surgery\ duration_i}{68}\right)^{-0.29} * e^{\eta_{CL}}.$$

$$V\ VWF = \theta_V * \left(\frac{Weight_i}{70}\right)^{1} * e^{\eta_V}.$$

$$BASE\ VWF = \theta_{BASE} * 1.53_{ASA\ class\ 3,4} * 0.39_{VWD\ type\ 2} * 0.18_{VWD\ type\ 3} * e^{\eta_{BASE}}.$$

$$CL\ FVIII = \theta_{CL} * \left(\frac{Weight_i}{70}\right)^{0.75} * 0.44_{VWD\ type\ 2} * 0.34_{VWD\ type\ 3} * e^{\eta_{CL}}.$$

$$V\ FVIII = \theta_V * \left(\frac{Weight_i}{70}\right)^{1}.$$

$$BASE\ FVIII = \theta_{BASE} * e^{\eta_{BASE}}.$$

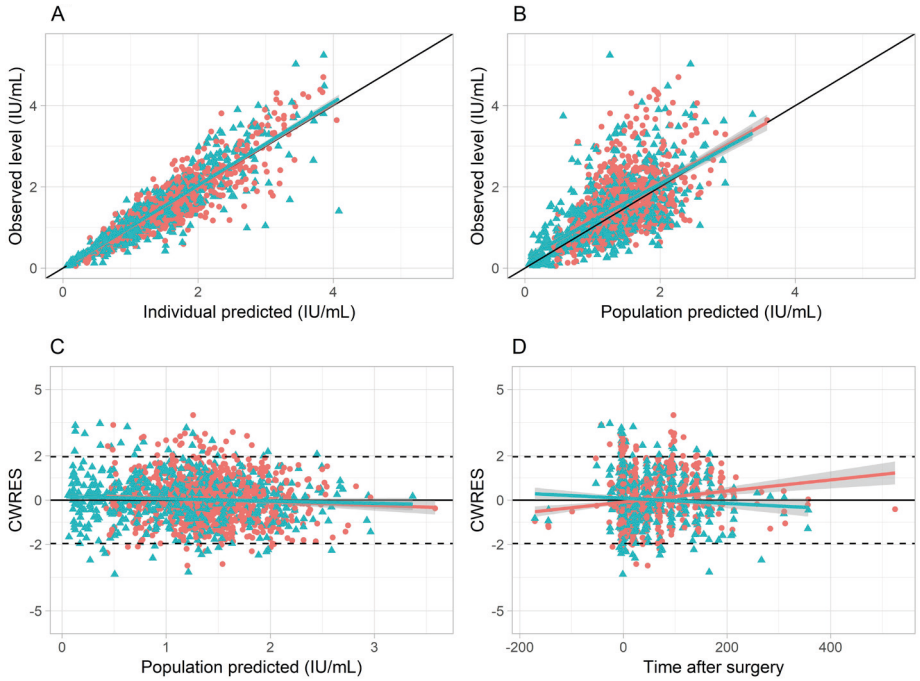

Figure 3. Goodness-of-fit plots of the final population pharmacokinetic model. Red circles (•) represent FVIII levels, while blue triangles (▲) represent VWF: Act levels. The observed level is compared to the individual predicted (A) and population predicted levels (B). The conditional weighted residuals (CWRES) are plotted to the population prediction (C) and the time before/after surgery (D).

Covariate analysis

During univariate selection, the following associations were statistically significant ($p<0.05$): surgery duration on VWF clearance, sex on VWF volume of distribution, VWD type, ASA score and age on VWF baseline, VWD type on FVIII clearance, and ASA score, age and blood group O on baseline FVIII. After forward inclusion and backward elimination, only duration of surgery on VWF clearance, VWD type and ASA score on VWF pre-administration baseline and VWD type on FVIII clearance were retained in the model ($p<0.01$). Increase in surgery duration was associated with a decrease of VWF clearance. Specifically, when the duration of surgery increased from 45 to 110 min (interquartile range), VWF clearance decreased from 284 to 219 mL/h. The VWF pre-administration baseline of VWD type 2 and type 3 patients was 61.0% and 81.8% lower than the VWF baseline of type 1 patients. For patients with an ASA score of III or IV, a 53% higher VWF: Act pre-administration baseline was observed than for patients with ASA score II. All patients had at least ASA score II, as VWD is a mild systemic disease and only completely healthy patients classify as ASA I. Patients with VWD type 2 and type 3 had a 56.4 and 65.7% lower FVIII clearance, respectively, compared to type 1.

Model evaluation

The goodness-of-fit plots of the final model demonstrate that the model describes VWF: Act and FVIII levels adequately (Figure 3). The VPC shows similar adequate model performance (Figure 1). Finally, the estimates and 95% confidence intervals of the bootstrap confirm robustness of the model (table 2).

Clinical application of the novel population PK model

To demonstrate the clinical application of the newly developed model, a 33 year old male (69 kg) with type 3 VWD who underwent ankle surgery while being treated with the VWF/FVIII concentrate (ratio 2.4: 1), was fitted with the newly developed integrated VWF/FVIII model retrospectively. The patient was not included in the original dataset and informed consent of the patient was obtained. An initial dose of ~50 IU/kg, followed by doses of ~25 IU/kg every 12 hours, following clinical guidelines pursuing prespecified VWF and FVIII target levels, were administrated to the patient (20). Figure 4A confirms that the measured FVIII and VWF levels of this patient were adequately described by the newly developed integrated VWF/FVIII population PK model, including the observed accumulating FVIII levels. Only the initial pre-administration FVIII level was estimated higher than observed, probably caused by the fact that only few type 3 patients with a low endogenous FVIII baseline were included in the model. Interestingly, during the first 36 hours after start of surgery the VWF target level of >0.80 IU/mL, as prespecified in the clinical guidelines to prevent bleeding, was not achieved after administration of the dosing scheme as described above, although no bleeding or adverse events occurred (20).

Thereafter, the individual dosing scheme was calculated that would have been necessary to reach the prespecified VWF and FVIII target levels. This advised dosing scheme was composed based on individual PK parameters retrieved from the available preoperative PK profile in which VWF and FVIII levels were measured before and at three time points after infusion of 25 IU/kg of the VWF/FVIII concentrate. When dosing was based on the individual PK parameters (PK-guided dosing), higher doses would have been necessary for this unique patient to reach the specified target levels (figure 4B).

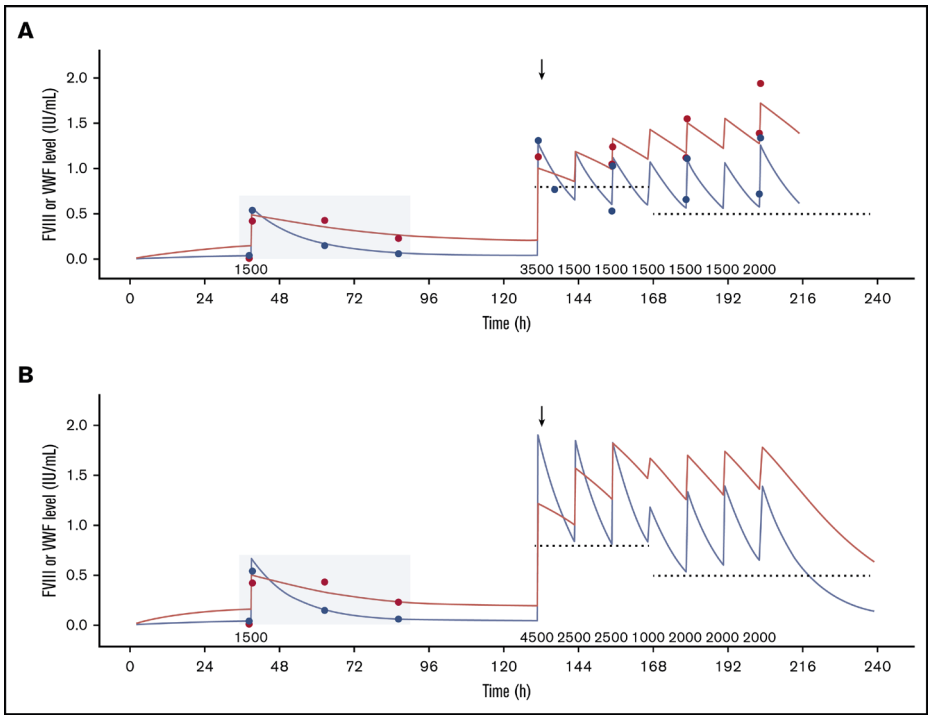

Figure 4. Clinical example of the fit using the developed integrated VWF-FVIII population pharmacokinetic (PK) model. This 33 year old patient of 69 kg is treated with VWF/FVIII concentrate (ratio 2.4:1) during ankle surgery. The VWF: Act levels (blue lines) and FVIII levels (red lines) are estimated using the new integrated VWF-FVIII population PK model. The observed VWF: Act levels are shown by the blue dots and the observed FVIII levels are shown by the red dots. The dotted lines represent the VWF and FVIII target levels according to clinical guidelines, the shaded are the PK-profiling period and the arrow the start of surgery. The numbers in the graph display the given dose of the VWF/FVIII concentrate according to FVIII dose. (A) The real-life situation in which the patient was treated according to the clinical guidelines, with an initial dose of ~50 IU/kg followed by ~25 IU/kg every 12 hours. (B) The hypothetical situation in which the doses needed to achieve the target levels are calculated based on the individual PK parameters of the patient derived from the levels measured during an individual PK-profile before surgery.

DISCUSSION

A novel population PK model was successfully developed which describes VWF: Act and FVIII levels simultaneously, illustrating their physiological interaction, after perioperative dosing with a VWF/FVIII concentrate (ratio 2.4: 1) in patients with VWD. In literature, the protective effect of VWF on FVIII metabolism and clearance has not yet been quantified in a population PK model. Moreover, the model demonstrates that the presence of VWF increased the half-life of FVIII, thereby clarifying FVIII accumulation as is generally observed after perioperative treatment with this VWF/ FVIII concentrate.

In this integrated population PK model, the observed VWF: Act and FVIII levels over time were both described by one compartment turnover models. The interaction between both coagulation proteins was captured by an Imax relation function connecting VWF: Act to FVIII clearance. The PK parameters obtained in this integrated population PK model are consistent with the values described in literature. The developed population PK model predicts a FVIII half-life of 11.4 hours in the presence of 1.23 IU/mL VWF: Act, which is similar to the average FVIII half-life of 12 h as described in literature (26, 27). In type 3 and type 2N VWD patients, the FVIII half-life without VWF presence or VWF binding can be assessed. Generally, a FVIII half-life of 2-3 hours is observed in these patients, which approaches the FVIII half-life of 6.6 hours without VWF presence as observed in the present model (28-30). In literature, VWF half-life is found to be between 12-15h (28, 30). Similarly, Lethagen et al. have described VWF half-life to be 15.6 hours in a VWD population receiving this VWF/ FVIII concentrate preoperatively. This is almost equivalent to the 13.9 h we observed in our analyses (17). In our previously published population PK model, that only describes FVIII levels after perioperative treatment with this specific concentrate, we reported a FVIII volume of distribution of 3.28 L/70 kg and clearance of 0.038 L/70kg/h (19). With these PK parameters, a typical patient of 70 kg will have a FVIII half-life of 60 hours, which does not comply with FVIII half-life of 12 hours as described in literature. As this newly developed population PK model presents PK parameters that approach values reported in literature, we assume to have captured the PK of FVIII after perioperative treatment with this VWF/FVIII concentrate more realistically.

During covariate analysis, several patient characteristics and surgical characteristics were identified that were able to explain parts of the inter-individual variability in the PK parameters. The observed negative association between surgery duration and VWF clearance may indicate that more VWF is produced and/or released when a surgical intervention takes longer to perform. Higher VWF baseline was associated with more comorbidities as defined by an ASA classification of III or IV (24). Atiq

et al. also observed the association between more comorbidities and increased VWF levels in VWD patients. This report indicated that this association was most likely explained by increasing age in especially type 1 VWD patients (31). In our study, type of VWD was found to be associated with the baseline of VWF, which is consistent with the classification system of VWD types (2). The high relative standard error value of the association between type 3 patients and the VWF baseline, is probably caused by the small number of type 3 patients in this dataset. We decided to maintain this covariate in the final model, as it displays the clinical difference between the types of VWD patients properly. Finally, an association between FVIII clearance and VWD type was observed, indicating that type 2 and type 3 patients show a decreased FVIII clearance. This observation feels contradictory, as binding of VWF to FVIII is dysfunctional in type 2N patients and endogenous VWF is normally not present in type 3 patients, causing enhanced FVIII clearance. However, exogenous FVIII and VWF may have different PK properties than endogenous FVIII and VWF. I.e., the exogenous VWF administered in type 2N patients has no dysfunctional binding to FVIII and the presence of exogenous VWF possibly lowers the enhanced FVIII clearance seen in type 3 patients not treated with factor concentrates.

During model development, we chose to model the pre-administration VWF and FVIII levels as endogenous baseline levels instead of the often lower historical (lowest ever measured) baseline level. The observed differences between the historical baseline and pre-administration VWF and FVIII levels may be caused by multiple factors, such as preoperative stress, inflammation, increasing age, comorbidities or analytical variation (15, 16). If this difference is only a temporary increase caused by e.g. preoperative stress, factor levels will return to the historical baseline in the postoperative period. However, if increasing age or comorbidity is the underlying reason, levels will approach the pre-administration level postoperatively as this reflects the current endogenous baseline. As in only one surgical procedure the FVIII and in three surgical procedures, the VWF:Act dropped below the pre-administration level and these differences were small (<0.10 IU/mL), we assumed that the baseline difference is permanent, and caused by e.g. increasing age. An endogenous baseline increase caused by increasing age is especially expected in type 1 patients with a VWF:Act baseline ≥0.10 IU/mL, while for type 1 with baseline <0.10 IU/mL, type 2 and type 3 patients the endogenous baseline is expected to remain similar over time (31). In our dataset, type 1 patients contributed most to the baseline differences (58%), but surprisingly other types of patients also showed an endogenous baseline increase compared to the historical baseline.

A limitation of this study is that type 2B (n=9), type 2M (n=11) and type 2N (n=3) and type 3 (n=6) patients were underrepresented. Although GOF-plots show adequate prediction of the

separate disease types (Supplement figure 1-3) and VWF: Act and FVIII levels of the clinical case (type 3 VWD) were adequately described, application of the population PK model in these types of patients may be less accurate. Another limitation is that we were unable to distinguish endogenous from exogenous coagulation factors in the population PK model. Endogenous and exogenous FVIII and VWF may have different PK and as a result the model may be improved by estimation of separate PK parameters for both endogenous and exogenous coagulation factors. Unfortunately, it is not yet possible to measure these coagulation factors separately and it was necessary to model the change in the cumulative sum of the endogenous and exogenous VWF: Act and FVIII levels over time. A third limitation is the high relative standard error (>30%) obtained for FVIII clearance in the final model, indicating that there is some uncertainty around the estimated value. Possibly, this can be solved by adding data with more level measurements per subject or by implementing a better sampling scheme. Finally, the population PK model was only based on data from patients receiving one particular VWF/FVIII concentrate. A study by Kessler et al. showed bioequivalent VWF PK properties for two commonly used VWF/FVIII concentrates, but PK of FVIII after administration of the concentrates is different (32). Therefore, we recommend to only use the model for patients receiving this specific concentrate. For VWF/FVIII concentrates with other ratios or multimer compositions other population PK models will have to be developed.

Clinical applicability of our newly developed model was demonstrated by the clinical case described in this manuscript. For this unique type 3 patient, higher VWF/FVIII concentrate doses would have been necessary to reach the prespecified targets, though for the majority of patients lower doses to reach the targets are expected (supplement figure 4). Undoubtedly, this single case does not confirm external validity of the model and external validation in a larger cohort is recommended. This case only demonstrates the clinical implications of PK-guided dosing using an interaction model. As clinical guidelines increasingly recommend to monitor and target both VWF and FVIII levels, this population PK model is beneficial over the previously developed population PK model based on only FVIII levels (1, 19-21). Despite the fact that this population PK model will support the targeting of sufficient VWF and FVIII levels, it is important to realize that the VWF/FVIII ratio of this concentrate is fixed and that both coagulation factors have different PK properties. Therefore, it remains challenging to achieve FVIII and VWF levels within similar ranges, and sometimes it may be unavoidable to accept higher FVIII (or possibly VWF) levels when dosing repetitively. Future prospective studies which examine the feasibility and reliability of PK-guided dosing with VWF/FVIII concentrates in perioperative VWD patients will further verify the validity of this PK-guided dosing approach and its clinical impact.

CONCLUSION

This novel integrated population PK model adequately describes VWF: Act and FVIII levels after perioperative dosing with a VWF/FVIII concentrate (ratio 2.4: 1, Humate P®/ Haemate P®). In this model, presence of VWF decreases FVIII clearance and increases FVIII half-life, thereby approaching a more physiological situation and explaining FVIII accumulation observed in this specific situation. Application of this model may facilitate PK-guided perioperative dosing with this specific concentrate based on both FVIII and VWF: Act targets, thereby potentially improving quality and cost-effectiveness of care.

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SUPPLEMENT

SUPPLEMENTARY FIGURES

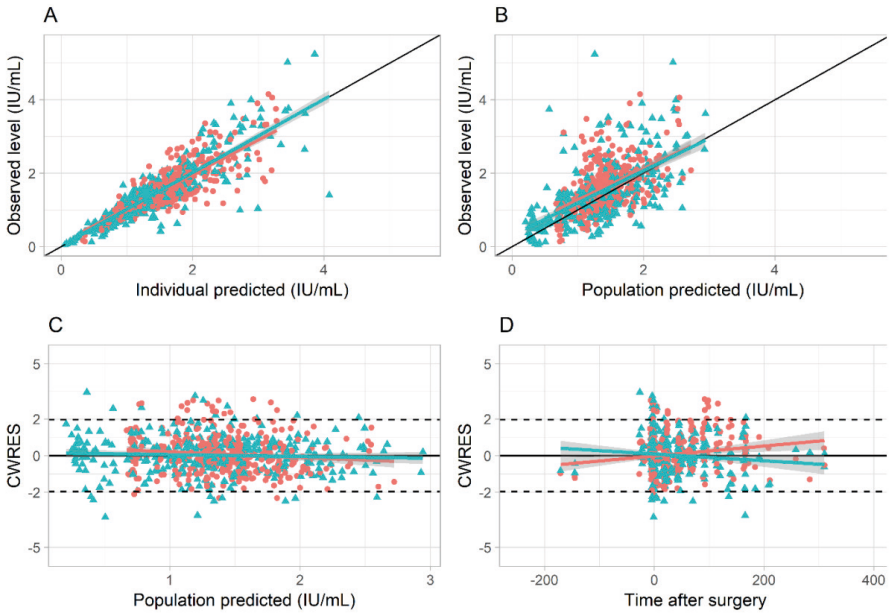

Figure 1. Goodness-of-fit plots of von Willebrand disease type 1 patients using the final population pharmacokinetic model. Red circles (•) represent FVIII levels, while blue triangles (▲) represent VWF: Act levels. The observed level is compared to the individual predicted (A) and population predicted levels (B). The conditional weighted residuals (CWRES) are plotted to the population prediction (C) and the time before/after surgery (D).

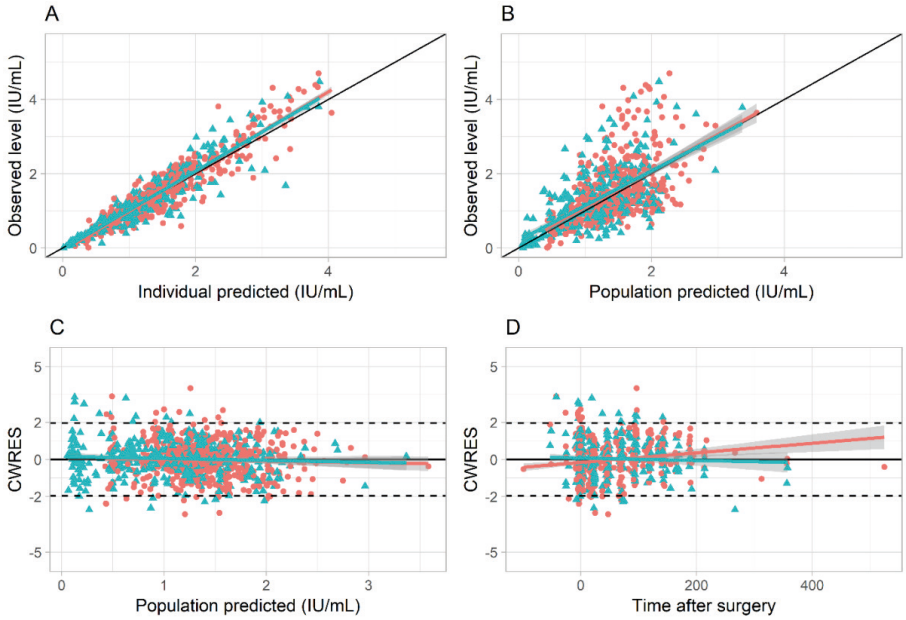

Figure 2. Goodness-of-fit plots of von Willebrand disease type 2 patients using the final population pharmacokinetic model. Red circles (•) represent FVIII levels, while blue triangles (▲) represent VWF: Act levels. The observed level is compared to the individual predicted (A) and population predicted levels (B). The conditional weighted residuals (CWRES) are plotted to the population prediction (C) and the time before/after surgery (D).

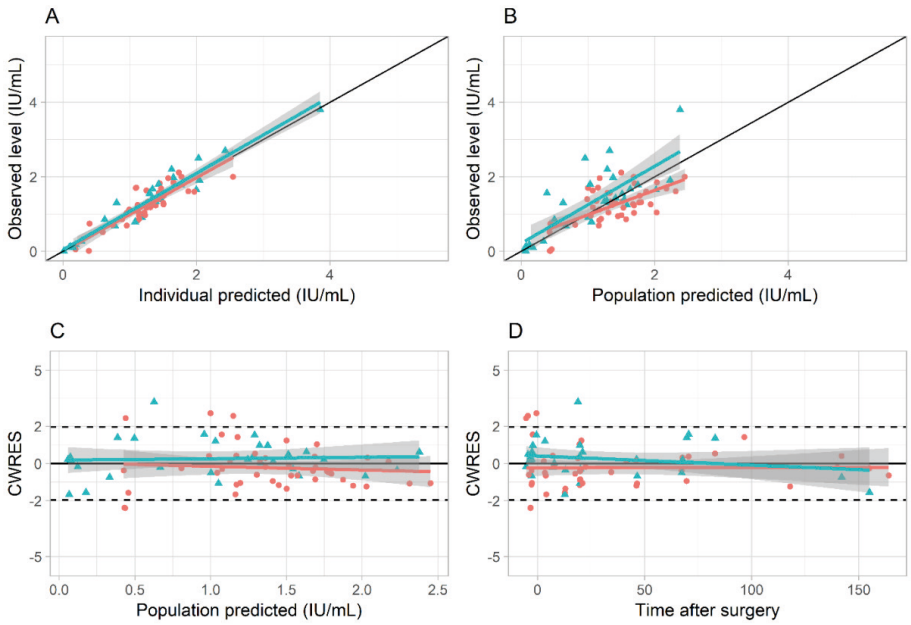

Figure 3. Goodness-of-fit plots of von Willebrand disease type 3 patients using the final population pharmacokinetic model. Red circles (•) represent FVIII levels, while blue triangles (▲) represent VWF:Act levels. The observed level is compared to the individual predicted (A) and population predicted levels (B). The conditional weighted residuals (CWRES) are plotted to the population prediction (C) and the time before/after surgery (D).

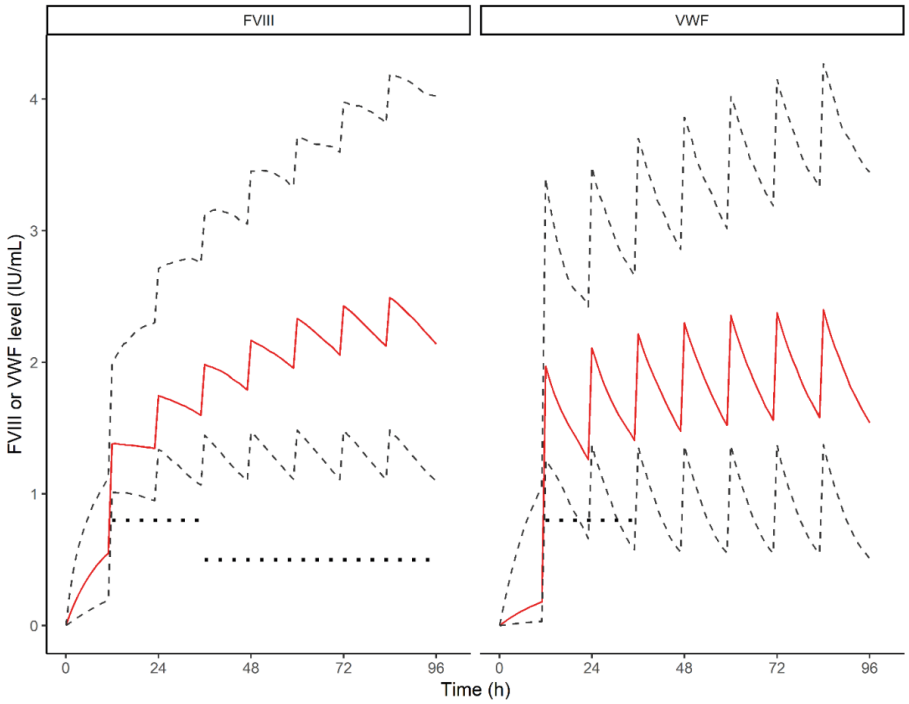

Figure 4. Predicted FVIII (left) and VWF: Act (right) levels over time for patients of 70 kg undergoing a major surgery when VWF/FVIII concentrate (ratio 2.4: 1) dosing is performed according to the Dutch clinical guidelines (loading 50 IU/kg, thereafter every 12 hours 25 IU/kg) (1). The figure is based on simulation of 1.000 virtual patients of 70 kg using the developed structural population PK model. The red line presents the median FVIII or VWF level and the dashed lines the 5th and 95th percentile. The dotted lines demonstrate the target levels for a major surgery as described in the Dutch clinical guidelines (1).

SUPPLEMENTARY METHODS SECTION

Specification of the assays used to measure VWF: Activity levels

Center 1: VWF: RCo - Chronolog aggregometer, reagens Siemens.

Center 2: VWF: RCo - measured by light aggregometry using fixed human platelets on a Chrono-Log aggregometer.

Center 3: VWF: RCo - measured by light aggregometry using fixed human platelets on a Chrono-Log aggregometer.

Center 4: VWF: RCo - ACL Top 700 (IL), VWF with ristocetine activity (IL).

Center 5: VWF: RCo between 2000- May 2005 - agglutination of fixed thrombocytes was measured using ristocetin as a cofactor on the PAP-4 or Chrono-Log aggregometer.

VWF: Ab between May 2005-2012: latex immune assay on automated coagulometer with monoclonal antibodies against the GP1ba binding site of VWF (Hemosil VWF activity; IL).

VWF: GP1bM from 2012 - was measured with the INNOVANCE VWF Ac reagent (Siemens) on a Sysmex CS-5100 analyzer using the manufacturer's protocol.

Population PK modeling

The first-order conditional estimation with interaction (FOCE+I) was chosen for estimation. Data management, evaluation and visualization were performed with R (v3.5.2) and PsN (v4.8.1).

First separate structural models for VWF: Act and FVIII were developed. The typical population estimates (θ), random variability (η) and residual error(ε) were described by equation 1 and 2. Different residual error structures (additive, proportional or combined) were examined.

$$\theta_i = \theta_{TV} * e^{\eta_i} \quad \text{(equation 1)}$$

$$C_{ij,obs} = C_{ij,pred} * \left(1 + \varepsilon_{ij,prop}\right) + \varepsilon_{ij,add} \quad \text{(equation 2)}$$

Where, θ_i is the estimated individual PK parameter of the i^{th} individual, θ_{TV} the typical value for the PKparameter and $\eta_{\neg_i}$ the inter-individual variability of the i^{th} individual.

$C_{ij,obs}$ is the observed FVIII or VWF: Act level for the i^{th} observation of the j^{th} individual, $C_{ij,pred}$ the predicted level, $\varepsilon_{ij,prop}$ the proportional residual error and $\varepsilon_{ij,add}$ the additive residual error.

PK parameters were a priori scaled to bodyweight (allometric scaling), as both children and adults were included in the dataset and a wide range of weights was present. The allometric exponents (θ_p) were fixed to 1 for volume parameters and to 0.75 for clearance parameters (equation 3) (2).

$$\theta_i = \theta_{TV} * \left(\frac{Weight_i}{70}\right)^{\theta_p} * e^{\eta_i} \quad \text{(equation 3)}$$

Turnover models were used to describe the change of endogenous and exogenous VWF: Act and FVIII levels over time (equation 4).

$$\frac{da}{dt} = Base * CL - k10 * A \quad \text{(equation 4)}$$

Where, da/dt describes the change in FVIII or VWF: Act amount over time, base the endogenous baseline of FVIII or VWF: Act, CL the clearance of FVIII or VWF: Act, k10 the elimination rate constant of FVIII or VWF: Act and A the amount of FVIII or VWF: Act.

An inhibitory Emax (Imax) function relating VWF: Act levels and FVIII clearance was incorporated to describe the inhibitory effect of the VWF: Act levels on FVIII elimination (equation 5 and 6).

$$Inhibition = 1 - \frac{Imax * C_{VWF}}{IC_{50} + C_{VWF}} \quad \text{(equation 5)}$$

$$\frac{da}{dt} = Base\ FVIII * CL\ FVIII - k10\ FVIII * A\ FVIII * inhibition \quad \text{(equation 6)}$$

Where, C_{VWF} represents the VWF level, Imax the maximal inhibitory effect and IC_{50} the VWF level with 50% inhibition.

A nested model was regarded superior if the objective function value (OFV) decreased with 3.84 points ($p<0.05$ and 1 degree of freedom).

Covariate analysis

To examine which patient or surgical characteristics explain part of the inter-individual variability observed in the PK parameters, a covariate analysis was performed. As in the structural model some shrinkage values were above 20%, empirical Bayesian

estimate diagnostics were not trusted, and covariate selection was performed based on statistical analysis (3). Continuous covariates were described by equation 7, in which describes the value of the covariate for the ith individual, Cov_{med} the median value of the covariate in the population and θ_{cov} the exponent describing the effect of the covariate. Categorical covariates were described by equation 8, in which θ_{Cov_n} is the fractional change of the parameter for category n.

$$\theta_j = \theta_{TV} * \left(\frac{Cov_i}{Cov_{med}}\right)^{\theta_{cov}} \quad \text{(equation 7)}$$

$$\theta_j = \theta_{TV} * \theta_{Cov_n} \quad \text{(equation 8)}$$

When covariate data was missing, the median value was imputed for continuous covariates or the category was set to the comparator category in categorical covariates. Addition of the covariate was regarded significant if the objective function (OFV) dropped at least 3.84 points ($p<0.05$, 1 degree of freedom) and random variability, as captured in the IIV of the associated PK parameters, decreased. During backward elimination an OFV increase of 6.64 ($p<0.01$, 1 degree of freedom) was considered significant.

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Part III

Innovative approaches to individualize treatment


9

Chapter 9

Is pharmacokinetic-guided dosing of desmopressin and von Willebrand factor-containing concentrates in individuals with von Willebrand disease or low von Willebrand factor reliable and feasible? A protocol for a multicenter, non-randomized, open label cohort trial, the 'OPTI-CLOT: To WiN' study

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ABSTRACT

Introduction

Von Willebrand disease (VWD) is a bleeding disorder, caused by a deficiency or defect of von Willebrand factor (VWF). In case of medical procedures or bleeding, patients are treated with desmopressin and/or VWF-containing concentrates to increase plasma VWF and factor VIII (FVIII). However, in many cases these factor levels are outside the targeted range. Therefore, population pharmacokinetic (PK) models have been developed, which aim to quantify and explain intra-individual and inter-individual differences in treatment response. These models enable calculation of individual PK parameters by Bayesian analysis, based on an individual desmopressin test or PK profile with a VWF-containing concentrate. Subsequently, the dose necessary for an individual to achieve coagulation factor target levels can be calculated.

Methods and analysis

Primary aim of this study is to assess the predictive performance (the difference between predicted and measured VWF activity and FVIII levels) of Bayesian forecasting using the developed population PK models in four different situations: A) desmopressin testing (n ≥30); B) medical procedures (n = 70; 30 receiving desmopressin, 30 receiving VWF-containing concentrate and 10 receiving a combination of both); C) bleeding episodes (n = 20; 10 receiving desmopressin and 10 receiving VWF-containing concentrate); and D) prophylaxis with a VWF-containing concentrate (n = 3 to 5). Individuals with all types of VWD and individuals with low VWF (VWF 0.30-0.60 IU/mL) will be included. Reliability and feasibility of PK-guided dosing will be tested by assessing predictive performance, treatment duration, hemostasis, patient satisfaction and physician satisfaction.

Ethics and dissemination

The OPTI-CLOT:To WiN study was approved by the medical ethics committee of the Erasmus MC, University Medical Center Rotterdam, the Netherlands. Results of the study will be communicated through publication in international scientific journals and presentation at (inter)national conferences.

Trial registration number

NL7212 (NTR7411); Pre-results.

INTRODUCTION

Von Willebrand disease (VWD) is the most common inherited bleeding disorder (1). It is caused by low or absent von Willebrand factor (VWF), or by a functional defect of VWF. VWF is essential for primary hemostasis as it facilitates platelet plug formation at sites of vascular injury. It also plays a role in secondary hemostasis, as it protects factor VIII (FVIII) from being cleared from the circulation. Symptoms of VWD include bleeding after trauma or surgery and (spontaneous) mucocutaneous bleeding. VWD is classified into three main types: type 1 and type 3 are respectively; a partial (VWF <0.30 IU/mL) and a complete (VWF <0.05 IU/mL) absence of VWF, whereas type 2 comprises several functional defects of VWF (2). In type 2A, binding of VWF to platelets is decreased, while in type 2B, affinity of VWF for platelets is increased. In both type 2A and 2B, there is an absence of high molecular weight VWF multimers (HMWM). In type 2M, platelet binding is decreased, but this is not caused by the absence of HMWM. In type 2N, often VWF levels are normal, however affinity of VWF for FVIII is decreased, leading to decreased FVIII levels. Individuals with low VWF have a bleeding tendency associated with VWF levels between 0.30-0.60 IU/mL (3).

Individuals with VWD are treated with desmopressin or -in more severe cases or when prophylactic therapy is needed- VWF-containing concentrates. The main reasons for treatment are acute bleeding and prevention of bleeding during medical procedures, (e.g. dental procedures, surgery or in-hospital childbirth). Prophylactic treatment to prevent spontaneous bleeding is seldom necessary and mainly applied in type 3 and severely affected type 1 and 2 patients. The aim of treatment is to accomplish sufficient hemostasis by achieving physiologically normal plasma coagulation factor levels. However, it has been previously reported in a study on perioperative treatment of VWD patients with Haemate P, that a majority of patients (65% in type 1, 53% in type 2 and 57% in type 3 VWD) achieve higher VWF activity (VWF:Act, or VWF function) levels than aimed for, and a minority (16% in type 1, 38% in type 2 and 29% in type 3 VWD respectively) does not reach sufficient levels for adequate hemostasis (4). This may lead to an increased risk of either thrombosis or bleeding. Moreover, costs of treatment are high as VWF-containing factor concentrates are expensive and frequent laboratory monitoring of plasma VWF and FVIII is required. As rising health care costs are an increasing concern, it is important to investigate alternative dosing strategies that facilitate more precise dosing, to improve quality of care with potential reduction of costs.

Currently, desmopressin dosage and dosing frequency are solely based on body weight and estimated degree of tachyphylaxis. Dosing of VWF-containing concentrates is also based on body weight, and dose calculations are made according to target VWF and

FVIII values based on the severity of the bleed or the type of medical procedure (5). However, pharmacokinetics (PK) of desmopressin and VWF-containing concentrates differ within and between patients (i.e. intra-individual and inter-individual differences), and large inter-individual differences in response to desmopressin are observed (6-8). Population PK models that describe plasma VWF:Act and FVIII after administration of desmopressin or VWF-containing concentrates have been constructed by our group (however not all models have been published yet) (9, 10). These models are based on retrospective DDAVP-testing data and VWF-containing concentrate treatment data from multiple hemophilia treatment centers in the Netherlands and in the United Kingdom. In a population PK model, the typical PK parameters and their corresponding variability are estimated. Subsequently, covariate relationships (e.g. patient characteristics and procedure characteristics), can be used to (partially) explain the estimated variability (11).

With these population PK models, we are able to perform Bayesian forecasting: all information and sources of uncertainty are combined into a predictive distribution for the future values, after which point forecasts (the predicted future values) and interval forecasts (the uncertainty level surrounding these predicted future values) can be obtained (12). In our models, individual VWF:Act and FVIII PK parameters are calculated. These PK-parameters are based on patient characteristics, combined with VWF:Act and FVIII measurements obtained after an individual test dose of desmopressin or VWF-containing concentrate, or measurements obtained during a bleeding episode or medical procedure. Based on the estimated individual PK parameters, we are able to design a personalized dosing strategy for each patient. We hypothesize that PK-guided dosing of desmopressin and VWF-containing concentrates may improve safety and efficacy of therapy, and lower treatment costs. It is essential to first evaluate the predictive performance of PK-guided dosing and the feasibility of this approach prospectively, in order to prove its effectiveness and safety.

OBJECTIVE

To prospectively investigate the reliability and feasibility of PK-guided dosing of desmopressin and VWF-containing concentrates in individuals with VWD and low VWF.

METHODS

Trial design

The OPTI-CLOT: To WiN trial is a multicenter, non-randomized, open label cohort study. The study was approved by the Medical Ethics Committee of the Erasmus MC, University Medical Center Rotterdam, the Netherlands, and was registered in the Netherlands Trial Register with trial registration number NL7212 and to EudraCT with number 2018-001631-46. The first patient was included on April 8th, 2019. The planned end date of the study is October 1st, 2023.

Study population

After obtaining informed consent, individuals with congenital VWD or low VWF will be enrolled it they will, for medical reasons, have to undergo a desmopressin test, require hemostatic treatment with monitoring of VWF:Act and FVIII during a medical procedure or during a bleeding episode, or receive prophylaxis with a VWF-containing concentrate. Patients will be recruited from Hemophilia Treatment Centers in the Netherlands.

Inclusion criteria

- Individuals of all ages with any type of VWD or low VWF with historically lowest VWF antigen (VWF:Ag), VWF:Act and/or VWF collagen binding (VWF:CB) level <0.60 IU/mL, or historically lowest FVIII level <0.40 IU/mL (only in case of type 2N VWD), who;
- Provide informed patient consent (if patient is ≥12 years), or parental informed consent (if patient is <12 years), or both (if patient is between 12 and 16 years); who;
- Are scheduled to undergo a desmopressin test, or;
- Are scheduled to undergo an elective medical procedure (e.g. dental procedure, surgery, diagnostic procedure or in-hospital child delivery), requiring treatment with desmopressin and/or a VWF-containing concentrate (Haemate P, Wilate, Wilfactin or Veyvondi) with monitoring of VWF and FVIII levels, or;
- Have a bleeding episode requiring treatment with desmopressin and/or a VWF-containing concentrate with monitoring of VWF and FVIII levels, or;
- Require prophylaxis with a VWF-containing concentrate due to frequent bleeding episodes

Exclusion criteria

- Any other known hemostatic abnormalities;
- Acquired VWD;
- Presence of VWF antibodies (>0.2 BU)

Intervention

Predictive performance will be tested in all study arms, and feasibility of PK-guided dosing will be tested in arm B, C and D:
Arm A: patients who will undergo a desmopressin test.
Arm B: patients who will undergo an elective medical procedure.
Arm C: patients with a bleeding episode.
Arm D: patients receiving or requiring prophylaxis.

Desmopressin testing (arm A)

In standard VWD care, most patients (except most type 2B VWD patients and all type 3 VWD patients) undergo a desmopressin test to determine their individual response to desmopressin. Desmopressin testing comprises measuring VWF:Act and FVIII before desmopressin administration and at 1 hour and 3-4 hours after desmopressin administration (0.3 μg/kg intravenously or subcutaneously or 300 μg (or 150 μg if body weight is <50 kg) intranasally), to assess the effect of desmopressin in the individual patient. In individuals who will undergo a desmopressin test, VWF:Act and FVIII response will be predicted a priori based on the constructed population PK-model and individual patient characteristics.

On demand treatment (arm B+C)

During elective medical procedures and during bleeding episodes, we will aim for VWF:Act and FVIII target plasma levels as defined in the national guidelines (table 1) (13). However, the treating physician will be able to set specific VWF:Act and FVIII target levels if needed, as is standard practice. These patient-specific target levels will be recorded prior to treatment and will be communicated to the clinical pharmacologist performing PK modelling. The pharmacologist will then provide a dosing strategy based on the patients' characteristics and individual desmopressin test and/or VWF-containing concentrate PK profile (performed prior to the procedure with the specific concentrate that will be used during the procedure), combined with the specific population PK model. When, at any time during the treatment period, target VWF:Act and FVIII plasma levels are not reached, additional desmopressin and/or VWF-containing concentrate can be administered by the treating physician to secure hemostasis. Therefore, bleeding risk for patients participating in the study will not be higher than in patients treated according to standard protocol.

Prophylaxis (D

In individuals receiving or requiring prophylaxis with a VWF-containing concentrate due to frequent bleeding episodes, patients will first undergo PK-profiling. This will be done in order to determine the optimal dosage of VWF-containing concentrate on

basis of VWF:Act or FVIII target trough and peak values as set by the treating physician and patients' individual PK parameters (as derived by Bayesian analysis). Patients will initially receive PK-guided treatment for 12 weeks. During this period, plasma VWF:Act and FVIII will be measured and will be compared to predicted VWF:Act and FVIII to validate the advised dosing regimen. Information on bleeding episodes will be obtained from medical records. Participants will be followed up for a period of 24 weeks in which additional data will be collected in order to assess the association between plasma VWF:Act and FVIII concentrations and bleeding events.

Table 1. Guidelines for substitution with VWF-containing concentrate in VWD according to Dutch national guidelines

Indication	Target levels
Dental extraction	FVIII:C and VWF:Act >0.50 IU/mL
Surgery	Prior to surgery and 36 hours postoperatively FVIII:C and VWF:Act >0.80 IU/mL
Major surgery	FVIII:C >0.50 IU/mL during 7-10 days
Minor surgery	FVIII:C >0.50 IU/mL during 3 days and >0.30 IU/mL during 4-7 days

FVIII:C = Factor VIII activity; VWF:Act = von Willebrand factor activity

Individual pharmacokinetic profiling

For every patient in arm B, C and D, an individualized dosing strategy will be provided based on actual body weight, type and severity of the procedure or bleeding, target VWF:Act and FVIII, baseline VWF:Act and FVIII and, if possible, an individual PK profile. Patients who will undergo a procedure requiring VWF-containing concentrate and patients who will receive prophylaxis, will undergo PK profiling with the VWF-containing concentrate of choice. Blood sampling for VWF and FVIII will be performed directly before bolus infusion and at approximately 10 minutes, 2 to 6 hours, 24 hours and 48 hours after infusion. Measuring VWF and FVIII at these time points will enable the construction of a concentration-time curve.

Population PK models

Population PK models for desmopressin and different VWF-containing concentrates have been constructed using NONMEM® software (however not all our models have been published yet) (9, 10). These models are able to predict average PK parameters for VWF:Act and FVIII (as well as the inter-individual variability of these PK parameters, and intra-individual variability of some of the PK parameters), in a population of individuals with VWD and low VWF.

In these PK models, the relationship between different patient factors and treatment factors (e.g. age, sex, weight, baseline VWF and FVIII, blood group type and VWF levels and PK parameters) are described. This allows prediction of the PK of VWF:Act and/or FVIII after desmopressin and VWF-containing concentrate administration. Combining an individual PK profile with the population PK model will allow for better prediction of the required doses and dosing frequency, -as well as better prediction of plasma coagulation factor levels- than prediction based on the population PK model alone.

Primary endpoints

Arm A (desmopressin testing): predictive performance of the desmopressin population PK model: reliability of predicted VWF:Act and FVIII levels, defined as the difference between predicted and actual VWF:Act and FVIII levels.

Arm B (elective medical procedures requiring treatment with desmopressin and/or VWF-containing concentrate): predictive performance of the Bayesian adaptive approach using the population PK model for desmopressin and/or VWF-containing concentrate, (i.e. reliability of the predicted VWF:Act and FVIII levels, defined as the difference between predicted and actual VWF:Act and FVIII levels achieved after dosing).

Arm C (bleeding episode requiring treatment with desmopressin or VWF-containing concentrate): predictive performance of the respective population PK models, (i.e. reliability of the predicted VWF:Act and FVIII levels, defined as the difference between predicted and actual VWF:Act and FVIII levels achieved after dosing).

Arm D (prophylactic treatment with a VWF-containing concentrate): predictive performance of the VWF-containing concentrate population PK models, (i.e. reliability of the predicted VWF:Act and FVIII levels, defined as the difference between predicted and actual VWF:Act and FVIII levels achieved after dosing).

Secondary endpoints

(Only in arm B, C and D): number and timing of desmopressin administrations (desmopressin dose will be standardized at 0.3 μg/kg) and/or timing and dosing of VWF-containing concentrate infusions.

(Only in arm B, C and D): hemostasis quantified by: hemoglobin levels, blood loss (ml), incidence of bleeds, incidence of thrombosis, and need for blood transfusion and/or re-operation because of bleeding.

(Only in arm B and C): duration of hospitalization (days), number of clinical visits.

(Only in arm B, C and D): Feasibility of the procedure with regard to patient and physician satisfaction and economic impact.

(Only in case of desmopressin testing or desmopressin treatment (in arm A, B and C)): desmopressin plasma concentrations.

Sample size

In this prospective study, we will explore the predictive performance of the constructed population PK models for desmopressin and VWF-containing concentrates. In bleeding and surgery, we will aim for VWF:Act and FVIII target trough levels (defined as 100-125% of VWF:Act and FVIII target trough level as stated by the treating physician and according to the national guidelines).

It is not common practice to calculate a sample size for prognostic models, and to our knowledge it is not possible to calculate a sample size for the determination of predictive performance, our primary outcome. However, as characteristics such as age, sex and disease type are not part of the inclusion criteria or exclusion criteria, the study population will be a reflection of the heterogeneous 'real life' VWD and low VWF population. Consequently, this will increase the 'effective sample size' of our study population.

To be able to provide an estimation of the sample size needed, we have calculated sample sizes for outcomes that may be seen as surrogates for the primary outcome. Based on a random sample (n=100) of our retrospective cohort of patients whom underwent a desmopressin test, we have constructed an average VWF:Act-after-desmopressin curve with 25% percentiles. In 81% of individual desmopressin tests, one or more time points fall outside of the 50% confidence interval of this average curve. Data from our retrospective cohort study on perioperative treatment with a VWF-containing concentrate (Haemate P®) show that in the total study population, 81% of FVIII trough levels in the first 36 hours was >0.20 IU/mL higher than targeted (4). Using adaptive Bayesian dosing, we estimate that we can decrease the percentage in both groups from 81% to <50%. To determine this with an alpha of 0.02 and a power of 90%, we will have to include at least 25 patients in the desmopressin test group and at least 25 patients in the perioperative VWF-containing concentrate group. To allow for dropouts, at least 30 patients will be included in the desmopressin test group, and 30 patients will be included in the perioperative VWF-containing concentrate group. For desmopressin treatment during medical procedures, scarce data is available on factor

levels during the periprocedural period. However, as we will also use the desmopressin test PK model in this setting, we assume similarity to the desmopressin test group and will also include 30 patients.

To explore the applicability of the currently available population PK models in other settings, predictive performance of the population PK models and PK-guided dose adjustments in groups for which no retrospective data is available, will be tested. Only small numbers of patients are currently treated with desmopressin in combination with VWF-containing concentrate, and it is expected that inclusion of patients with acute bleeding will be logistically challenging. Therefore, we aim to include 10 patients who will receive a combination of desmopressin and VWF-containing concentrate during a medical procedure, 10 patients with a bleeding episode receiving treatment with desmopressin and 10 patients with a bleeding episode receiving treatment with VWF-containing concentrate. As very few patients in the Netherlands receive prophylaxis, we aim to include 3-5 patients in arm D. In these settings, the population PK models for treatment with desmopressin and VWF-containing concentrate will be combined, and we will extrapolate the perioperative PK models to bleeding and prophylaxis. Due to the low sample sizes in arm C and D, predictive performance of the models (the primary endpoint) in these arms can only be assessed on an individual level, giving a rough idea of the accuracy of the models in these settings.

Data analysis plan

Primary study parameters

Predictive performance of the population PK models (defined as difference between predicted and actual FVIII and VWF:Act levels achieved after dosing) will be analyzed using Bland Altman analysis (14). Mean relative error (MRE) will be calculated to determine accuracy, and root mean squared error (RMSE) will be calculated to determine precision.

Secondary study parameters

1. In case of perioperative treatment with VWF-containing concentrate (n = 30): concentrate consumption (IU/kg) from 24 hours before surgery until stop of VWF-containing concentrate infusions will be compared to consumption in the retrospective treatment cohort, of which the data have already been published (4). If patients underwent >1 surgical procedure, only the first one will be used for analysis. The distribution of outcomes for the prospectively studied group will be tested for normality using the Shapiro-Wilk test. In case of a non-significant ($p > 0.05$) result of this test, the t-test will be used for the comparison of the primary

endpoint. In case the resulting p-value for the Shapiro-Wilk test is equal or less than 0.05 , the Wilcoxon-rank sum test will be used. The level for significance for this analysis will be set at two-sided p <0.05. Number and timing of desmopressin infusions will be defined quantitatively.

2. In the perioperative group, hemostasis will be quantified by amount of blood loss (mL). Bleeding complications or thrombotic complications will be defined quantitatively.
3. In the perioperative group, duration of hospitalization (days) will be defined quantitatively.
4. Feasibility of the procedure: patient and physician satisfaction during PK-guided treatment during surgery and bleeding will be measured using a 10-point VAS (visual-analogue scale) questionnaire and will be defined quantitatively. Economic evaluation will be performed from a health care perspective taking all health care costs (i.a. costs of medication, hospitalization costs) into account.
5. To test the correlation between desmopressin concentrations and relative increase in FVIII and VWF levels during desmopressin tests, and during desmopressin treatment during surgery or bleeding, the Pearson correlation coefficient will be calculated.

Patient and public involvement

During development of all OPTI-CLOT studies, we work closely together with The Netherlands Hemophilia Patient Society (NVHP). A member of the NVHP is also a member of the OPTI-CLOT study group and plays an advisory role in developing the studies within the consortium. The final results of the study will be communicated through international scientific journals and at international conferences. In addition, a layman summary of the results of this study will be published in the NVHP magazine. Lastly, the results of the study will be implemented in treatment guidelines and patient information will be adjusted accordingly.

Ethics and dissemination

The trial protocol was approved by the Medical Ethics Committee of the Erasmus MC, University Medical Center Rotterdam, the Netherlands. The study will be conducted according to good clinical practice (GCP) guidelines and the Declaration of Helsinki, and in accordance with the Dutch Medical Research Involving Humans Act (WMO). Written informed consent will be obtained from all participants by the investigator. Also, see online supplementary data for our regulations for data storage, amendments and compensation for injury. Results of the study will be communicated to the (inter) national medical and scientific community through publication in high-ranking peer-reviewed international journals and at (inter)national conferences. Results of the study

will be implemented in the Dutch Haemophilia Treatment Guidelines and may also be adopted by international Haemophilia Treatment Societies.

Data monitoring committee and serious adverse events

Safety risks for participants are minimal as the VWF-containing concentrates and desmopressin used in this study are registered therapeutics for treatment of von Willebrand disease. To guarantee safety for participants in this study, VWF and FVIII levels will be monitored closely to prevent any additional bleeding risk. Therefore, a data safety monitoring board is not needed.

Serious adverse events (SAE) will be communicated to the sponsor within 24 hours. The sponsor will register the SAE within 15 days on ToetsingOnline, the Dutch registration system for SAEs.

REGISTRATION

The trial is registered at the Netherlands Trial Register, number NL7212 (www.trialregister.nl/trial/7212) and to EudraCT with number 2018-001631-46.

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Chapter 10

Can von Willebrand factor propeptide / von Willebrand factor antigen ratio predict enhanced clearance of von Willebrand factor after desmopressin?

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Submitted


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Chapter 11

Desmopressin response depends on the presence and type of genetic variants in type 1 and type 2 von Willebrand disease patients

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ABSTRACT

Patients with type 1 and type 2 von Willebrand disease (VWD) can be treated with desmopressin. Although a previous study has shown that the location of the causative *VWF* gene variant is associated with desmopressin response in type 1 VWD, the association between variants in the *VWF* gene and desmopressin response is not yet fully understood. Our primary aim was to compare desmopressin response in type 1 VWD patients with and without a *VWF* gene variant. Secondly, we investigated whether desmopressin response depends on specific *VWF* gene variants in type 1 and type 2 VWD. We included 250 patients from the WiN study; 72 type 1 without a *VWF* gene variant, 108 type 1 with a variant, 45 type 2A, 16 type 2M and 9 type 2N patients. *VWF* gene was analyzed with ion semiconductor sequencing and MLPA. Complete response to desmopressin was observed in all type 1 VWD patients without a variant, 64.3% of type 1 patients with a variant and 31.3% of type 2 patients ($p<0.001$). Despite a large inter-individual variability in desmopressin response, patients with the same variant had comparable desmopressin responses. For instance, in six type 1 patients with exon 4-5 deletion, mean VWF activity at 1 hour after desmopressin was 0.81 IU/mL with a coefficient of variation of 22.9%. In conclusion, all type 1 VWD patients without a *VWF* gene variant respond to desmopressin. In type 1 and type 2 VWD patients with a *VWF* variant, desmopressin response highly depends on the *VWF* gene variants.

INTRODUCTION

Von Willebrand Disease (VWD) is characterized by a reduced or abnormal function of von Willebrand factor (VWF) (1). VWF is responsible for platelet adhesion and aggregation (2). Therefore, patients with VWD have a reduced clot formation and an increased bleeding phenotype (1). VWF also serves as carrier protein for coagulation factor VIII (FVIII), explaining the reduced FVIII levels observed in VWD patients (2). Clinical manifestations of VWD include mucosa-associated bleeding, such as menorrhagia, gingival bleeding and postsurgical bleeding (3). VWD can be classified in three types (1). Type 1 VWD is most prevalent and characterized by reduced levels of VWF. Type 2 VWD is characterized by an abnormal function of VWF, whereas type 3 VWD is characterized by a complete absence of VWF (1).

Treatment of VWD consists of increasing VWF and FVIII levels by infusion of exogenous VWF containing concentrates, or by administration of desmopressin, which stimulates the release of endogenous VWF from vascular endothelial cells (4-6). Treatment with desmopressin is preferred above VWF containing concentrates as it is more convenient since desmopressin can be administered intranasally and subcutaneously, and is less expensive. However, not all VWD patients have a sufficient increase in VWF and FVIII after desmopressin administration (6). The majority of patients with type 1 VWD respond well to desmopressin, whereas only a small number of type 2 VWD patients respond (7). In type 2 VWD patients, VWF antigen usually increases after desmopressin, but VWF activity remains low (8). Therefore, a desmopressin test dose is required in patients diagnosed with VWD, to assess the magnitude and duration of response to desmopressin (6). In patients with type 2B VWD desmopressin is contraindicated, since desmopressin administration may lead to thrombocytopenia (1, 4).

VWF levels in circulation are largely determined by the *VWF* gene, which is located on chromosome 12 (9). A broad spectrum of variants in the *VWF* gene are found in patients with VWD (9-12). However, approximately 30% of type 1 VWD patients do not have a variant in the *VWF* gene (10, 13-15). The European MCMDM-1VWD study has previously shown in 77 patients that the location of the causative *VWF* gene variant is associated with desmopressin response in type 1 VWD patients (16). The MCMDM-1VWD study also found that all of 16 included type 1 VWD patients without a *VWF* gene variant had a complete response to desmopressin (16). Larger studies are needed to compare the desmopressin response of type 1 VWD patients with and without a *VWF* gene variant. Moreover, it is unknown whether specific *VWF* gene variants explain the variability in desmopressin response in type 1 and type 2 VWD patients. It is also unclear whether

family members with a comparable phenotype of VWD have a similar response to desmopressin. By clarifying the association between genotype and desmopressin response, one may hypothetically be able to predict the desmopressin response of patients, and thereby patients may not need a test dose of desmopressin.

Therefore, we have investigated the association between genotype and desmopressin response in a large cohort of type 1, type 2A, 2M and 2N VWD patients. Our primary aim was to compare the desmopressin response between type 1 VWD patients with and without a *VWF* gene variant. Secondly, we aimed to investigate whether the desmopressin response depends on specific *VWF* gene variants in type 1 and type 2 VWD patients. Lastly, we aimed to compare the desmopressin response of index cases in whom the *VWF* gene was analyzed and affected family members in whom the *VWF* gene was not analyzed.

METHODS

Patients

We included all type 1, type 2A, 2M and 2N VWD patients from the Willebrand in the Netherlands (WiN) study in whom a desmopressin test was performed and in whom all exons of *VWF* gene was analyzed (3, 17). Affected family members from the WiN study in whom the *VWF* gene was not analyzed, but who had the same type of VWD as an index case, including comparable historical, and centrally measured VWF and FVIII levels, were also included. Inclusion criteria of the WiN study were historically lowest VWF antigen (VWF:Ag), VWF activity or VWF collagen binding (VWF:CB) equal or below 0.30 IU/mL or FVIII activity (FVIII:C) equal to or below 0.40 IU/mL (in case of type 2N VWD), and a positive family history of VWD or personal bleeding diathesis (3, 17).

Data assessment

During inclusion in the WiN study, blood was obtained and all patients filled in a questionnaire containing a self-administered Tosetto bleeding score (BS). VWF and FVIII levels prior and immediately after a test dose of desmopressin were obtained from the electronic patient files. Desmopressin was in most cases administered intravenously at a dosage of 0.3 microgram/kg in 50 mL sodium chloride 0.9% infused over 30 minutes, or intranasally a total dosage of 300µg. Venous blood samples were routinely obtained according to the institutional protocols. This consisted routinely of samples before, and 1 to 4-6 hours after desmopressin administration.

Laboratory measurements

VWF and FVIII levels were centrally measured at inclusion in the WiN study at the Erasmus University Medical Center as described before (3, 18). VWF propeptide (VWFpp) was centrally measured at the Leiden University Medical Center as described before.(161) The assessment methods of the WiN study have been described in detail previously (3, 17, 18).

VWF:Ag, VWF activity (VWF:Act), VWF:CB and FVIII:C before and after desmopressin response were measured at the local treatment centers and were obtained from the electronic patient files. VWF:Act measurements varied between centers and varied in each center over time. In short, VWF:Act was measured with the antibiotic ristocetin and platelets assay (VWF:RCo), ristocetin and recombinant GPIb fragments (VWF:GPIbR), recombinant GPIb fragments with two gain-of-function mutations (VWF:GPIbM) and monoclonal antibody assay (VWF:Ab). Although the laboratory assays that were used in each center may differ, all centers participated in external quality controls. As such, VWF and FVIII measurements were obtained from standardized assays which were used in routine diagnostic settings.

Genetic analysis

Data on genetic analysis were obtained from the WiN study, in which the 52 exons of *VWF* gene and ±20bp exon-intron boundaries were analyzed with Ion semiconductor sequencing (Ion-Torrent™) at the hematology laboratory of the Radboud University Medical Center in Nijmegen. All detected variants were confirmed with Sanger sequencing. In patients without *VWF* gene variants, MLPA was performed to detect large deletions or duplications. All presented variants in this manuscript are in heterozygous form unless specified as homozygous. Benign variants were not regarded as pathogenic and were therefore omitted from this manuscript.

Definitions

Complete response to desmopressin was defined according to the 2021 ASH/ISTH/NHF/WFH VWD guidelines: two times increase in VWF:Act (from baseline) at 1 hour after desmopressin, and VWF:Act and FVIII ≥0.50 IU/mL until 4 hours after desmopressin (6, 19). In patients with VWF:Act ≥0.50 IU/mL measured immediately before desmopressin administration, complete response was defined as VWF:Act above 1.00 IU/mL until 4 hours after desmopressin.

Reduced synthesis/secretion of VWF was defined as FVIII:C/VWF:Ag ratio ≥1.9, whereas increased clearance of VWF was defined as VWFpp/VWF:Ag ratio ≥2.2, as

described before (18, 20). FVIII:C/VWF:Ag ratio <1.9 and VWFpp/VWF:Ag ratio <2.2 was defined as undetermined pathophysiology of reduced VWF levels (18).

Affected family members

Affected family members were only included in the analyses comparing desmopressin response of type 1 VWD patients with and without a *VWF* gene variant and type 2 VWD patients, and in the analyses comparing the desmopressin response of index cases and affected family members.

Statistical analysis

Continuous data are described as median and interquartile range (IQR) and categorical data as number and percentage. Normality of data was visually assessed with histograms. In case of more than 30 patients per group, we compared groups with parametric tests, such as independent sample t-test or ANOVA. Categorical data were compared between groups using a Chi-square test.

Desmopressin response between type 1 with and without variants and type 2 VWD patients were compared with an ANOVA test. Proportion of responders to desmopressin were compared between groups with a Chi-square test. Desmopressin response per *VWF* gene variant are presented descriptively, without statistical tests, because of low number of patients per *VWF* gene variant. The variability in desmopressin response is presented as coefficient of variation (CV), which is expressed in percentages. Statistical analyses were performed with SPSS Statistics version 25 (IBM Corp., Armonk, NY, USA). A p-value below 0.05 was considered significant.

RESULTS

We included a total of 250 type 1, type 2A, 2M and 2N VWD patients. In 208 patients genetic analysis was performed and 42 patients were affected family members with the same type of VWD as the index case with a known genetic variant, and similar historical and centrally measured VWF and FVIII levels. The patient characteristics are presented in Table 1. Seventy-two patients had type 1 VWD without a *VWF* gene variant, 108 patients had type 1 VWD with a *VWF* gene variant, 45 patients had type 2A, 16 had type 2M and 9 had type 2N VWD. The median age at desmopressin administration was 36 years [24-46], and did not differ among type 1 patients with a variant, type 1 patients without a variant, and type 2 patients (Table 1). Older age was associated with a better desmopressin response (Supplement Figure 1). For instance, in type 1 VWD patients with a variant, a complete response to desmopressin was observed in 10/26 (38.5%) of

patients younger than 18, 25/37 (67.6%) of patients 18-40 and 27/33 (81.8%) of patients older than 40 years (p=0.002). In type 1 VWD patients without a variant, VWF and FVIII levels 1 hour after desmopressin were similar, but levels at 2 to 5-6 hours were lower in patients with blood group O compared to patients with blood group non-O, although not statistically significant (Supplemental Figure 2).

Table 1. Patient characteristics

	Type 1 without variant (n=72)	Type 1 with variant (n=108)	Type 2 VWD (n=70)
Age at desmopressin	37 [26-46]	35 [18-45]	38 [27-48]
Female, n (%)	48 (66.7%)	66 (61.1%)	39 (55.7%)
Blood group O, n (%)	54 (75.0%)*	60 (60.6%)*	38 (55.1%)*
Genetic analysis, n (%)	63 (87.5%)	86 (79.4%)	59 (84.3%)
Included AFMs, n (%)	9 (12.5%)	22 (20.4%)	11 (15.7%)
Bleeding score[1]	9 [5-15]*	7 [4-12]*	11 [6-14]*
Historically lowest VWF levels[2]			
VWF:Ag	0.39 [0.30-0.46]*	0.26 [0.15-0.36]*	0.31 [0.19-0.43]*
VWF:Act	0.24 [0.20-0.27]*	0.18 [0.10-0.25]*	0.09 [0.04-0.22]*
VWF:CB	0.25 [0.19-0.33]*	0.19 [0.10-0.26]*	0.15 [0.03-0.27]*
FVIII:C	0.50 [0.40-0.61]*	0.44 [0.27-0.60]*	0.38 [0.26-0.57]*
FVIII:C/VWF:Ag ratio[1]	1.6 [1.4-1.8]*	2.0 [1.6-2.5]*	1.5 [1.2-2.0]*
VWFpp/VWF:Ag ratio[1]	2.0 [1.7-2.3]*	2.8 [1.9-5.0]*	4.1 [3.1-5.6]*

Data are presented as median [interquartile range], unless otherwise specified. AFMs = affected family members. [1]obtained at the inclusion in the WiN study. [2]measured at the local laboratories were also VWF and FVIII levels before and after desmopressin administration were performed. *p-value between groups <0.05.

Desmopressin response in patients with and without a VWF gene variant

An overview of all *VWF* gene variants found in our cohort and their association with desmopressin response is provided in Supplemental Table 1. Overall, we found a clear difference in VWF and FVIII levels after desmopressin between type 1 VWD patients with and without a variant, and type 2 VWD (p<0.001 for all variables at all measurements, Figure 1A-C). Desmopressin response was at all measurements after desmopressin significantly higher in type 1 VWD patients without a variant compared to type 1 patients with a variant (p<0.001 at all measurements, Figure 1A-C). Even after adjustment for relevant confounders, these differences were present. For instance, in patients without a *VWF* gene variant, VWF:Act was at 1 hour after desmopressin β=0.36

IU/mL higher (95% CI 0.16-0.56, p<0.001) compared to type 1 VWD patients with a *VWF* gene variant (adjusted for historically lowest VWF:Act, VWF:Act immediately prior to desmopressin administration, FVIII:C/VWF:Ag ratio and VWFpp/VWF:Ag ratio). As expected VWF:Act was lower after desmopressin administration in type 2 VWD patients compared to type 1 patients with a variant (p<0.01 at all measurements, Figure 1B), whereas VWF:Ag was not different between both groups (p>0.3 at all measurements, Figure 1A).

Based on the most recently defined desmopressin response criteria, 100% of type 1 patients without a *VWF* gene variant had a complete response, whereas 64.3% of type 1 VWD patients with a *VWF* gene variant and 31.3% of type 2 VWD patients had a complete response after desmopressin (p<0.001, Figure 1D).

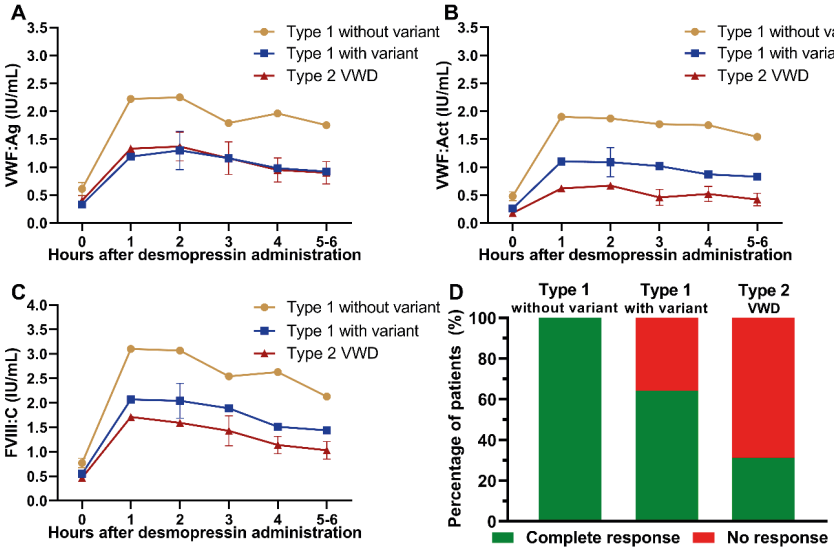

Figure 1. A large difference in desmopressin response was observed between type 1 VWD with and without a VWF gene variant and type 2 VWD. (A) VWF:Ag was higher after desmopressin in type 1 VWD patients without a variant, compared to type 1 VWD patients with a variant and type 2 VWD patients. (B-C) VWF:Act and FVIII:C after desmopressin administration was highest in type 1 patients without a variant, followed by type 1 patients with a variant and type 2 VWD patients. (D) All type 1 VWD patients without a variant had a complete response to desmopressin, whereas 66.3% of type 1 patients with a variant and 31.1% of type 2 VWD patients had a complete response. (A-C) Data are represented as mean and 95% CI.

Desmopressin response depends on VWF gene variants in type 1 VWD

In all type 1 VWD patients in whom genetic analysis was performed, the inter-individual variability in desmopressin response was large with a CV of respectively 61.9% and 48.6% at 1 hour after desmopressin and 69.7% and 69.2% at 4 hours after

desmopressin for VWF:Act and FVIII:C. However, patients with the same *VWF* gene variant had comparable desmopressin responses with small inter-individual differences (Figure 2). For instance, in six type 1 VWD patients with an exon 4-5 deletion, mean VWF:Act at 1 hour after desmopressin was 0.81 IU/mL with a CV of 22.9%, whereas at 4 hours mean VWF:Act was 0.73 IU/mL with a CV of 27.0% (Figure 2A). Similar small inter-individual CVs were observed for all other variants, except for R854Q and R924Q. One patient with R924Q had a lower desmopressin response compared to other patients with R924Q, which could be attributed to a second *VWF* gene variant (C1169W) present in this patient. One patient with R854Q had a lower desmopressin response compared to other patients with R854Q, for which no clear explanation could be found.

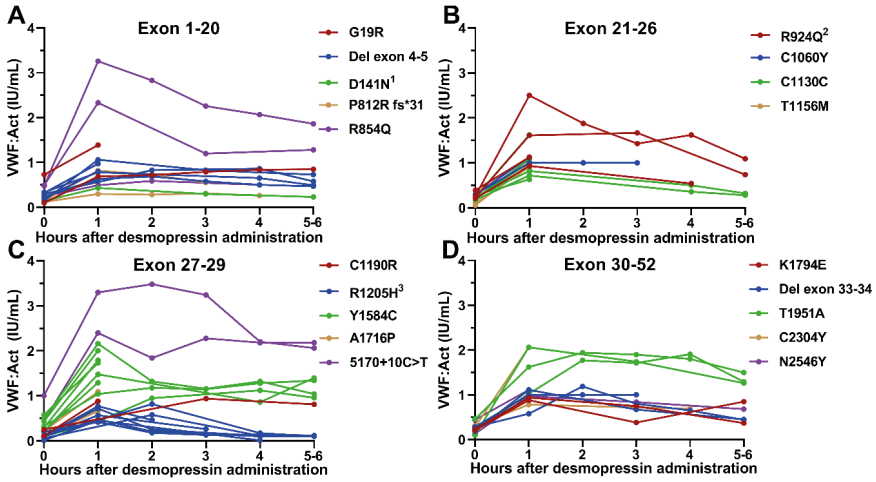

Figure 2. Type 1 VWD patients with same *VWF* gene variants have a comparable response to desmopressin. Each line represents a single patient. [1]Both patients also had R2313C. [2]The patient with lower desmopressin response also had C1169W. [3]Two patients also had 5170+10C>T.

11

Desmopressin response depends on VWF gene variants in type 2 VWD

We included 36 type 2A VWD patients with 16 different *VWF* gene variants. Overall, a large inter-individual variability was found, especially for VWF:Act with inter-individual CVs of 80.7% and 38.1% at respectively 1 hour and 4 hours after desmopressin. However, the inter-individual variability in type 2A patients with the same variant was low (Figure 3A). Patients with C1190Y had the highest VWF:Act after desmopressin followed by V1499E. In all type 2A patients, desmopressin response was very comparable between patients with the same variant.

In type 2M VWD, 14 patients were included with eight different *VWF* gene variants. Overall, also type 2M patients showed a variable response in VWF:Act at 1 hour and 4 hours after desmopressin, depending on the causative mutation (Figure 3B). However, in patients with the same variants, the interpatient variability in response was small, since two patients with R1374H both had no increase in VWF:Act after desmopressin, whereas two patients with R924Q both had a complete response to desmopressin. In five patients with F1293L, desmopressin responses were very comparable, with low inter-individual variance at measurements after desmopressin (Figure 3B).

In type 2N VWD, six patients had compound heterozygous variants, two patients had heterozygous variants and one patient had a homozygous variant. Since there was a large variety of second variants in type 2N patients, we could not assess the variability in desmopressin response between patients with exactly the same genetic background, except for R854Q+R2535* which was present in two patients with a comparable desmopressin response (Figure 3C-D). Although VWF:Act increased well after desmopressin in all type 2N patients, there was a large inter-individual variability in FVIII:C response after desmopressin (Figure 3C-3D).

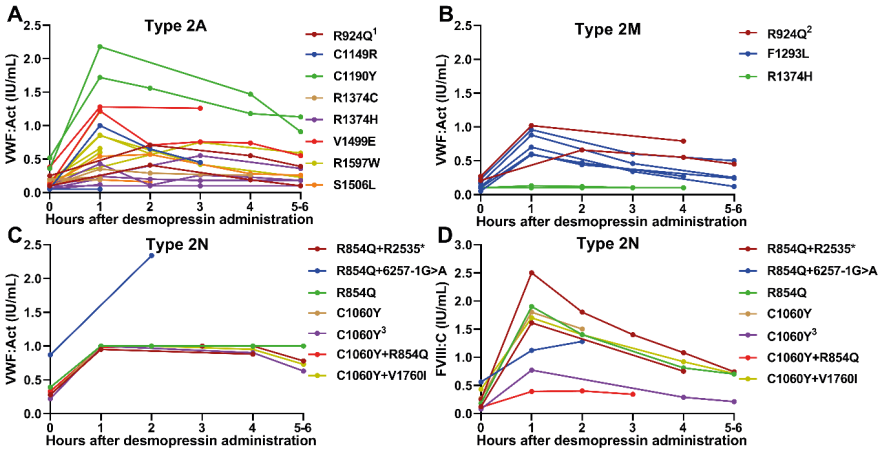

Figure 3. Type 2 VWD patients with same *VWF* gene variants have comparable responses to desmopressin. Each line represents a single patient. [1]One patient also had G1609R. [2]One patient also had R1374H. [3]Homozygous variant. VWF:Act was in most type 2N patients recorded as ≥ 1.00 IU/mL in the electronic patient files, in case of high levels, explaining the straight line from 1 to 4 hours in Figure 3C.

The association between genotype and desmopressin response is mediated via the pathophysiological defects of VWF

In type 1 VWD, all patients with reduced synthesis/secretion of VWF with normal clearance (FVIII:C/VWF:Ag ≥1.9 and VWFpp/VWF:Ag <2.2) had a complete response to desmopressin (Figure 4A). All patients with a rapid clearance of VWF with VWFpp/VWF:Ag ratio >7 had an incomplete response to desmopressin (Figure 4A). Lastly, all patients with undetermined pathophysiology of reduced VWF levels (FVIII:C/VWF:Ag < 1.9 and VWFpp/VWF:Ag <2.2) had a complete response to desmopressin (Figure 4A).

In type 2 VWD, all patients with rapid clearance of VWF with VWFpp/VWF:Ag ratio ≥6.0 had an incomplete response to desmopressin (Figure 4B). Also, all patients with a combination of both reduced synthesis/secretion and increased clearance of VWF (FVIII:C/VWF:Ag ratio ≥1.9 and VWFpp/VWF:Ag ≥2.2) had an incomplete response to desmopressin, except for one patient with a borderline response (VWF:Act of 0.56 IU/mL 4 hours after desmopressin, Figure 4B). Lastly, all four patients with undetermined pathophysiology of reduced VWF levels (FVIII:C/VWF:Ag < 1.9 and VWFpp/VWF:Ag <2.2) had a complete response to desmopressin (Figure 4B).

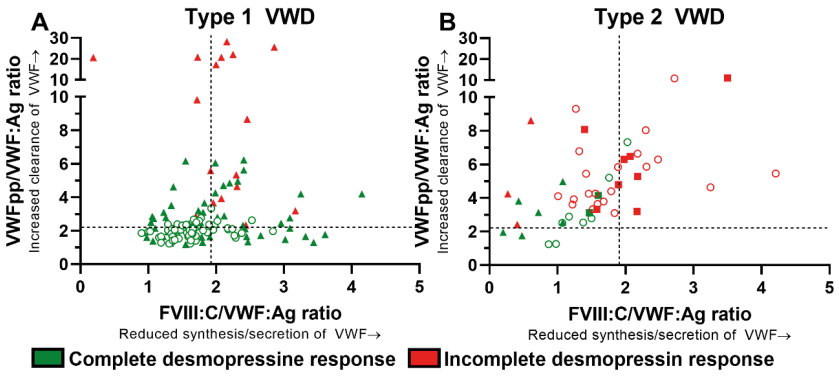

Figure 4. The association between genotype and desmopressin response is mediated via the pathophysiological defects of VWF. Each point represents one patient. Dashed lines represent the cut-off values for reduced synthesis/secretion and increase clearance of VWF. (A) Triangles represent patients with a *VWF* gene variant, whereas dots represent patients without a *VWF* gene variant. (B) Dots represent patients with type 2A, squares represent patients with type 2M and triangles represent patients with type 2N VWD.

Desmopressin response in affected family members

VWF and FVIII levels after desmopressin were similar between index cases and affected family members at all measurements after desmopressin (all p-values >0.05). In 41 type 1 VWD patients, desmopressin response was in nine families with a *VWF*

gene variant and five families without a variant, very comparable between index cases in whom the *VWF* gene was analyzed and affected family members (Figure 5A). In 19 type 2 VWD patients from seven families, desmopressin response also seemed comparable between index cases and affected family members (Figure 5B).

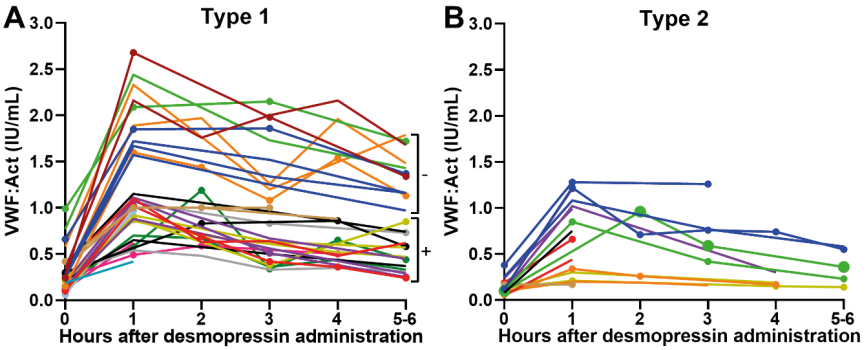

Figure 5. Index cases and affected family members have a comparable desmopressin response. Each line represents a single patient. All family members are illustrated with the same color. Index patients are indicated with dots. –Type 1 patients without a *VWF* gene variant. +Type 1 patients with a *VWF* gene variant.

DISCUSSION

In this large study in well-defined type 1 and type 2 VWD patients, we have investigated the association between genotype and desmopressin response. We found that all type 1 VWD patients without a *VWF* gene variant had a complete response to desmopressin, whereas in type 1 VWD patients with a *VWF* gene variant only 64.3% had a complete response, and in type 2 VWD only 31.3% had a complete response. Furthermore, despite a large inter-individual variation in desmopressin response in type 1 and type 2 VWD, patients with same *VWF* gene variants had very similar desmopressin responses, even in unrelated patients. Lastly, desmopressin response seemed comparable between index cases in whom the *VWF* gene was analyzed and affected family members.

The results of our study indicate that there is a clear difference in the desmopressin response of type 1 VWD patients with and without a *VWF* gene variant, confirming the results of the MCMDM-1VWD study (16). Since all type 1 VWD patients without a *VWF* gene variant had a complete response, desmopressin testing may not be needed in these patients. Similarly, it was previously found that 40/40 (100%) of patients with historically lowest VWF levels of 0.30-0.50IU/mL had a complete response to desmopressin, suggesting that desmopressin testing may not be needed in these

patients (21). Together with our previous findings that there is a clear difference in the pathophysiology of type 1 VWD patients with and without a *VWF* gene variant and that type 1 VWD patients without a mutation have higher VWF and FVIII levels, the results of our current study indicates that type 1 VWD patients with and without a *VWF* gene variant are distinct groups (22). Although until now genetic testing was not routinely performed in type 1 VWD patients, these new insights raise the question whether we should perform genetic analyses in type 1 VWD patients. Especially, since genetic analysis may have therapeutic consequences as observed that all type 1 patients without a variant respond well to desmopressin and therefore do not need a desmopressin test.

Castaman et al have previously demonstrated in 77 patients that desmopressin response is influenced by the genotype in type 1 VWD patients (16). We have confirmed their results in type 1 VWD patients with various variants, and additionally found the same association in type 2 VWD patients. Some of the variants that we describe in this manuscript have already been reported in relation to desmopressin response. As described in several previous studies, type 1 Vicenza (R1205H) is known for its poor response to desmopressin, due to fast clearance of VWF (23). We have also found that type 1 VWD patients with other variants associated with a rapid clearance of VWF based on a VWFpp/VWF:Ag ratio above 7.0 such as S2179R, do also not respond to desmopressin. Of note, despite that patients with an increased clearance of VWF may not have a complete desmopressin response, desmopressin treatment may still have a therapeutic role during minor interventions, as was previously shown for R1205H and C1130F (24). Also comparable to our study, patients with R1374H and R1374C were previously found not to respond to desmopressin (8, 16) On the other hand, Y1584C and R854Q were previously shown to be associated with a good response to desmopressin, as confirmed in the current study (8, 16). Importantly, in patients with R854Q who did not respond to desmopressin, often a second variant was identified. It was also previously shown that most patients with R1597W have a poor response to desmopressin (8, 16). In summary, these findings illustrate that desmopressin response highly depends on the specific *VWF* gene variant in type 1 and type 2 VWD patients. This suggests that if the genetic variant in a certain patient is known in literature to be associated with no response or with a very good response to desmopressin, then a desmopressin test may not be required in that patient. In type 2N VWD, only a small number of patients had the same *VWF* gene variants. Therefore, we could not reliably assess the variability in desmopressin response of patients with the same variants. In line with our study, it was previously found that type 2N VWD patients have a good response to desmopressin (16, 25). Moreover, it was also found that there is a large variability in FVIII:C increase after desmopressin in type 2N VWD patients (25).

Older age was associated with a better desmopressin response. This could mean that desmopressin response is better in older patients, which may have to do with the age-related increase of VWF level (26, 27). It could also be associated with the fact that patients that are diagnosed at a younger age, generally have a more severe bleeding phenotype, which is associated with a worse desmopressin response (28). Future studies are needed to investigate whether desmopressin response changes intra-individually with aging.

We have also observed that in index cases and affected family members with the same type of VWD and similar historical and centrally measured VWF and FVIII levels, desmopressin response was comparable between index cases and affected family members. This suggests that desmopressin test may not be needed in affected family members with the same disease phenotype as an index case. Additional prospective studies are needed to confirm these findings. However, one may choose to perform a test dose of desmopressin to assess the side-effects of desmopressin in a certain patient.

Furthermore, the pathophysiology of reduced VWF levels was strongly associated with desmopressin response, especially in type 1 VWD patients. We have previously shown that each *VWF* gene variant leads to specific synthesis/secretion and/or clearance defects of VWF, and is associated with historically lowest and centrally measured VWF levels (22). Together these findings lead to the working hypothesis that a specific *VWF* gene variant leads to specific defects in VWF synthesis/secretion and/or clearance, which determines VWF levels, and subsequently determines whether a patient responds to desmopressin or not.

The main strength of this study is that extensive VWF and FVIII measurements were performed before and after desmopressin administration and all exons of the *VWF* gene were analyzed including MLPA in a large cohort of well-defined type 1 and type 2 VWD patients. Therefore, we were able to investigate many *VWF* gene variants, and to compare the desmopressin response between several patients with the same *VWF* gene variants. Also, we had data on the pathophysiology of reduced VWF levels, and were therefore able to investigate the association between genetic variants, pathophysiological defects in VWF and desmopressin response. A potential limitation is that VWF and FVIII levels after desmopressin were measured with different assays in different laboratories. However, we have demonstrated for the four most used VWF:Act assays that overall the assays are very comparable (29). Moreover, we found a similar response to desmopressin in patients with the same variants in whom VWF and FVIII levels were measured in different laboratories. If VWF and FVIII levels

would have been measured in the same laboratory, desmopressin responses would have probably been even more comparable. Another potential limitation is that some *VWF* gene variants may lead to a laboratory phenotype of type 1 VWD in some patients and type 2 VWD in other patients. Therefore, some variants are listed in this manuscript as causing both type 1 VWD and type 2 VWD. Lastly, it should be noted that a patient who is classified as non-responder according to the desmopressin response criteria, still may benefit from desmopressin during for instance small bleeding such as epistaxis.

In conclusion, this study shows that type 1 VWD patients without a *VWF* gene variant always respond to desmopressin, and desmopressin response strongly depends on specific *VWF* gene variants in patients with type 1 and type 2 VWD. These results indicate that genetic analysis may have an important additional value for optimizing the therapeutic management of VWD patients.

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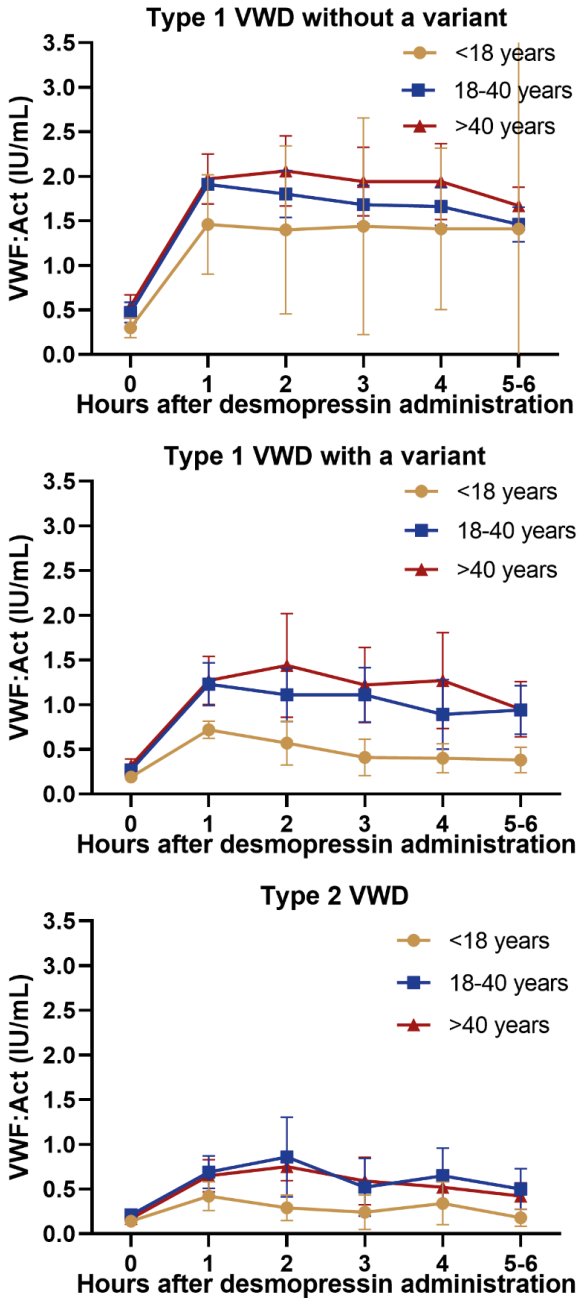
SUPPLEMENT

Supplemental Table 1. Overview of all variants found in our cohort and their association with desmopressin response

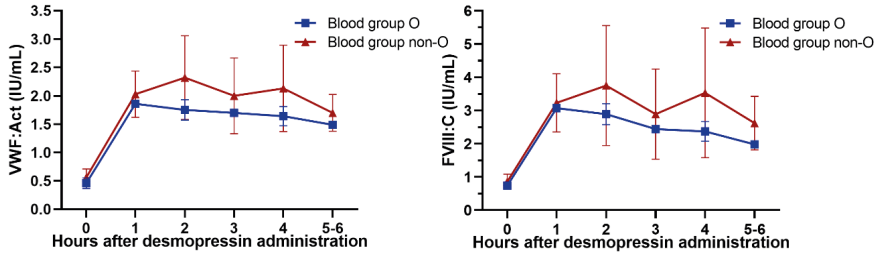
Proportion of patients with complete response to desmopressin					
	0%	**0-40%**	**40-60%**	**60-100%**	**100%**
Type 1 VWD	I482M[2] (0/1) P812R fs*31 (0/2) C1149R (0/1) R1205H[8] (0/8) R1374H (0/1) R1379C (0/1) S2179F (0/1)		D141N[1] (1/2) A1716P (1/2)	Del exon 4-5 (5/6) R854Q (2/3) C1130C (4/6) Y1584C[10] (7/8)	G19R (2/2) R236C (1/1) C440A *fs17 (1/1) C652F (1/1) 2442+4A>G (1/1) R924Q[5] (4/4) C1060Y (2/2) T1156M (2/2) P1162L (1/1) C1190R (1/1) P1266L[9] (1/1) F1369I (1/1) Dup exon 28 (1/1) K1794E (2/2) Del exon 33-34 (3/3) V1934G (1/1) T1951A (3/3) P2063S (1/1) C2304Y (3/3) C2360* (1/1) A2498D (1/1) N2546Y (2/2)
Type 2 VWD	C1130C (0/1) C1149R (0/2) L1282P (0/1) S1285P (0/1) L1288R (0/1) L1307P (0/1) R1374C (0/3) R1374H (0/8) R1374L (0/1) K1408del (0/1) N1421K (0/1) S1506L (0/2) Y1542C (0/1) I1628W (0/1) G1631D (0/1)	F1293L (1/5) R1597W (2/6)	C1060Y[7] (2/4)	R854Q[4] (3/4) R924Q[6] (3/4)	W791M[3] (1/1) C1190Y (2/2) V1499E (2/2) G1573S (1/1)

Of note, some variants, which are known in literature to cause both type 1 and type 2 VWD, are in this table listed as both type 1 and type 2 VWD, depending on the phenotype patents. Between () indicates number of patients responded/total number of patients. [1]both patients also had R2313C. [2]also had R960W and 3108+2T>G. [3]Also had P812R fs*31. [4]The patient who did not respond also had 6257-1G>A, whereas two patients who responded also had R2535*. [5]One patient also had C1169W. [6]One patient who responded also had G1609R, whereas another patient who respond had R1374H. [7]In the two patients who did not respond, one had a homozygous form and the other one also had R854Q. One patient who responded also had V1760I. [8]Two patients also had 5170+10C>T. [9]Also had V1279I. [10]The patient who did not respond had a homozygous form.

SUPPLEMENTAL FIGURES



Supplemental Figure 1. Desmopressin response is associated with age.
Data are represented as mean and 95% CI.



Supplemental Figure 2. In type 1 VWD without a variant, patients with blood group O seem to have a less sustained desmopressin response, although not statistically significant.

Data are represented as mean and 95% CI. Due to the large 95% confidence interval, none of the differences between blood group O and non-O at 1 to 5-6 hours after desmopressin were statistically significant.

Part IV

General discussion and summary


12

Chapter 12

General discussion

GENERAL DISCUSSION

STUDY OBJECTIVES AND MAIN FINDINGS

Over the last decades, limited improvements have been made in the treatment of VWD; treatment still mainly consists of administering desmopressin to release endogenous VWF from the vascular endothelium into the circulation, or infusing exogenous VWF with one of the available VWF-containing concentrates. Dosing of both treatments is still mainly based on baseline VWF and FVIII levels and the body weight of the patient, not on how treatment can be refined for the individual patient. In contrast to developments in hemophilia, there are only a limited number of novel therapeutic approaches for VWD currently under investigation, including allele-specific synthetic small interfering RNAs (siRNAs), and a novel FVIII/VWF-D'D3-fusion variant (efanesotocog alpha), that circulates independently of endogenous VWD and could therefore be a treatment option for type 2N VWD (1, 2). Increasing knowledge on individualization of current treatment with desmopressin and VWF-containing concentrates by application of population PK modelling and other modelling techniques can aid in individualizing upcoming and future therapeutic approaches.

In this thesis, outcomes of current treatments for VWD were investigated, including desmopressin and VWF-containing concentrates. Based on this analysis, easy tools for predicting desmopressin response were developed. In addition, this retrospective analysis showed that current perioperative treatment with VWF-containing concentrates resulted in significantly higher VWF and FVIII levels than needed. Adverse effects of the current approach are high treatment costs and possibly an increased risk of thrombosis. Therefore, population PK-guided dosing according to the individual patients' characteristics is interesting. We developed several population PK models for treatment of VWD with either desmopressin or a VWF-containing concentrate, which will be further evaluated in clinical practice in a prospective multicenter study.

The novel approaches described in this thesis are meaningful for individualizing treatment in VWD, as well as for improving our knowledge on the pathophysiology of VWD. We believe that recent innovations for treating patients with inherited bleeding disorders should not bypass VWD patients, and we hope to contribute to improving treatment in this frequently diagnosed bleeding disorder.

CURRENT TREATMENT IN VWD

As mentioned above, there are few treatment options to choose from for individuals with VWD and their treating physicians, as the mainstay of treatment still are desmopressin and VWF-containing concentrates. Given the high variability in response to treatment, personalizing treatment is important. Currently, dosing is only based on body weight and adapted according to the obtained FVIII-VWF peak and trough levels. In order to improve treatment we have performed several studies, mainly focusing on PK-guided dosing of current treatment modalities.

Desmopressin and VWF-containing concentrates

We evaluated the response to desmopressin during desmopressin testing and concluded that performing a desmopressin test in patients potentially eligible for treatment with desmopressin is not necessary in many cases. In patients who need a desmopressin test, only a limited number of blood samples have to be taken during testing (3). We have therefore developed and validated an easy-to-implement flow chart for selecting patients who need testing and proposed a less invasive testing regimen, which will not only reduce the burden for patients and the treatment team, but will also reduce costs for testing significantly.

In our retrospective study in VWD patients who underwent surgery while being treated with a specific plasma derived VWF/FVIII-containing concentrate (Haemate® P), we found that the majority of VWF:Act and FVIII trough levels and steady-state levels are ≥0.20 IU/mL above predefined target levels (VWF:Act: 53% in type 2 VWD to 65% in type 1 VWD, and FVIII: 72% in type 2 VWD to 91% in type 1 VWD). Some patients do not reach the predefined target levels after the initial dose and need additional dosing (4). Over-exposure may lead to an increased risk of thrombosis and leads to unnecessarily high treatment costs (5). Underdosing may lead to bleeding. So far, only a limited number of studies have been performed on the clinical implications of achieving coagulation factor levels higher or lower than aimed for, especially in VWD. In our retrospective VWF/FVIII concentrate (Haemate® P) study, none of the 103 patients developed thrombo-embolic complications and in only 3.4% of surgeries a clinically relevant bleeding complication occurred (4). In addition, we found no correlation between lower VWF:Act or FVIII levels than aimed for, and bleeding. However, more evidence for lack of this correlation needs to be established in a larger cohort.

National and international guidelines on the treatment of VWD recommend different VWF:Act and FVIII target trough levels and peak levels during the perioperative period. For instance, the American National Heart Lung and Blood Institute (NHLBI) guidelines

recommend targeting of VWF:Act peak levels >1.00 IU/mL perioperatively, without mentioning a target for FVIII peak levels, whereas the Dutch guidelines recommend targeting of both VWF:Act and FVIII trough levels >0.80 IU/mL directly preoperatively until 36 hours after surgery (6, 7). Both guidelines do recommend maintaining FVIII >0.50 IU/mL for several days after the surgical procedure, however the duration of treatment differs (7-14 days vs 7-10 days), with the NHLBI recommending to also keep VWF:Act >0.50 IU/mL. The most recent international American Society of Hematology, International Society on Thrombosis and Hemostasis, National Hemophilia Foundation and World Federation of Hemophilia (ASH ISTH NFH WFH) guideline recommends maintaining both VWF:Act and FVIII trough levels ≥0.50 IU/mL for at least three days after surgery over solely targeting FVIII, as was previously recommended (8). It should be noted that all the above-mentioned recommendations are based on low to very low-certainty evidence (9). However, studying which minimum levels and minimum treatment duration are needed to avoid bleeding is difficult, as this is considered risky.

Notably, in most studies reporting on outcomes of perioperative hemostatic treatment, hemostatic efficacy is excellent in almost all cases. More specifically, bleeding complications are rare, and thrombotic events are virtually inexistent (10, 11). With the current treatment strategy, VWF-containing concentrate is often dosed higher than strictly needed to accomplish hemostatic target levels, while factor concentrates are expensive. It will therefore be interesting to explore if the recommended dose can be lowered while still reaching target levels and accomplishing hemostasis and targeted VWF and FVIII levels. For patients who do not reach the desired target levels with the current dosing strategy, PK-guided dosing will probably be most beneficial, as it can be predicted beforehand that they need a higher dose, leading to a decreased need for additional infusion of VWF-containing concentrate and probably a lower risk of bleeding.

Within the 'OPTI-CLOT' study group, we have identified this unmet need and therefore aim to individualize dosing and treatment of patients with bleeding disorders by PK-guided dosing of desmopressin, factor concentrates and other hemostatic products.

INDIVIDUALIZING TREATMENT BY POPULATION PHARMACOKINETIC MODELLING

VWD is a complex disease due to its heterogeneous pathophysiology: VWF function can be reduced due to a quantitative defect or one of several functional defects.

Thereby, VWF also functions as a chaperone protein for FVIII, protecting it from degradation and clearance from the circulation. Both VWF and FVIII levels are additionally influenced by many known and unknown intrinsic and extrinsic factors, as described in the introduction. Although others have studied the use of PK-guided dosing in VWD (12), the 'OPTI-CLOT' study group is the first to construct and describe population PK models for treatment of VWD patients (13-15). We have created models for desmopressin and for the most widely available and most used VWF-containing concentrate (Haemate® P), in the Netherlands. These models are described in this thesis.

General challenges with regard to population PK modeling in bleeding disorders and especially VWD are the following: firstly, it is well known that the PK of VWF and FVIII differ under varying circumstances. As explained, for example during surgery, more VWF and FVIII is consumed, while endogenous VWF -and thereby FVIII- will increase due to an acute phase reaction or stress (20). Therefore, it is essential to collect data from many different situations, such as bleeding episodes, surgical procedures and prophylactic treatment, and to incorporate these data into existing population PK models and into future models to refine predictions. Secondly, it is vital that data from a substantial number of patients with a wide variety of characteristics are included. This means that individuals who were often excluded from pre-marketing trials, e.g. children <12 years and patients with comorbidities, must be included in order to attain a real-world disease population and to serve the total population, as is currently a requirement in clinical studies (16). Moreover, population PK-guided dosing may prove to be most beneficial in patients in whom blood sampling is often difficult or burdensome – for instance in young children and elderly patients -, as the classical PK approach, in which many blood samples must be taken, is often not desirable due to the number of samples and amount of blood that is needed. Also, this approach asks for substantial time investments of the health care workers obtaining the samples.

In order to check the reliability of each model, it is good practice to internally validate population PK models after construction by performing a Monte Carlo simulation (17). During such simulations, a large number of virtual patients -generally thousand or more- is created, for whom individual PK parameter values are generated. These simulated individual PK parameter values are then used to create concentration-versus-time curves after administering a virtual drug dose. This enables calculation of the concentration at every desired time point from a hypothetical large population of which the variation coincides with the available data. Mostly, an identical data set is used for construction and internal validation of the population PK model, making it of the utmost importance that the population included in the data set resembles the

real-life population as closely as possible (21). We have therefore chosen to only exclude patients with additional hemostatic disorders and patients with antibodies against VWF (acquired VWD).

Another complicating factor is that different laboratories use varying assays for measuring VWF and FVIII activity. These assay variations cause differences in the estimated population parameters and may therefore influence the recommended doses during PK-guided dosing. VWF:Act can, amongst others, be measured by VWF ristocetin cofactor (VWF:RCo) assay, monoclonal antibody (VWF:Ab) assay, or VWF glycoprotein 1b binding (VWF:GP1bM) assay (11). FVIII activity can be measured by one-stage assay or chromogenic assay. Although we did not find significant differences in measurements by the different assays, in the population PK models described in this thesis, these potential differences must be taken into account (22). Ideally, all data incorporated in a population PK model should be measured with the same assay, while also using the same assay when performing PK-guided dosing based on that model.

Unfortunately, we are not yet able to distinguish endogenous VWF and FVIII from exogenously administered VWF and FVIII. As endogenous and exogenous coagulation factors are expected to have different PK -especially when they are functionally impaired-, it would be valuable to be able to estimate PK parameters for both entities separately. Currently, it is being investigated if endogenous VWF and FVIII can be distinguished from exogenous VWF and FVIII. Proteomic research using mass spectrometry may overcome these barriers in the near future (18).

Specific challenges in pharmacokinetic modelling for desmopressin and VWF-containing concentrates

As explained earlier, when desmopressin is administered, endogenous VWF is released from the vascular endothelium, concomitantly producing an increase in circulating endogenous FVIII. It is well known that inter-individual variability in achieved VWF and FVIII levels after desmopressin administration is large (19). It is therefore common practice to perform a desmopressin test in every VWD patient potentially eligible for treatment, to analyze the patients' VWF and FVIII response. Often, mobilization of VWF and FVIII through desmopressin testing is seen as a model for intrinsic reactivity of VWF and FVIII in VWD and non-severe hemophilia A, possibly associated with the patients' bleeding phenotype (20). Therefore, modelling of this response may also be valuable to predict bleeding in these individuals (21).

As no actual VWF or FVIII is administered during desmopressin testing, we developed a population PK model to predict VWF:Act response after desmopressin

administration, using a descriptive or empirical approach. Only actual measured plasma VWF:Act levels were used for its construction, as the amount of endogenously released VWF is unknown (14). As a consequence, the estimated *CL* and *V* are *apparent* parameters. Nevertheless, by applying these parameters, we were able to describe the inter-individual variability of VWF:Act levels after administration of desmopressin. In addition, we were able to explain some of the observed inter-individual variability by several of the included covariates (weight, age, VWD type and sex). However, the final PK parameters of the residual inter-individual variability on bioavailability (*F*) (60.5%), *CL* (76.5%) and *V* (26.9%) remained significant. Ultimately, we will strive to establish population PK-pharmacodynamic (PD) models, in order to gain insight into effects on the hemostatic system. The PK of desmopressin itself and the subsequent PD response embodied by VWF:Act can be studied using a mechanism-based approach. Theoretically, this will lead to a better description of the continuous release of VWF:Act as provoked by desmopressin. We described an integrated population PK-PD model for VWF:Act release after desmopressin. In this mechanism-based model, we observed a much lower residual inter-individual variability than in the descriptive PK model.

As endogenous VWF release may be influenced by many intrinsic and extrinsic factors, including comorbidity, physical exercise and hormonal changes during the menstrual cycle, part of the inter-individual variability in circulating VWF remains unexplained as it is difficult to quantify and integrate all possible influencing factors in the model (22, 23). We therefore presume that our preliminary explorations, followed by findings from current research into using more advanced data analyzing techniques, will help to further unravel these associations.

As Haemate® P is the most widely used VWF-containing concentrate in the Netherlands and many other countries, we have first focused on collecting data and developing population PK-models for this specific concentrate. However, as different plasma-derived concentrates contain different ratios of VWF and FVIII (Haemate® P has a VWF:FVIII ratio of 2.4:1, while Wilate® has a VWF:FVIII ratio of 1:1 and Wilfactin® contains virtually only VWF), they also have different PK properties and hemostatic characteristics due to varying multimer properties (24). Also, the recombinant pure VWF concentrate Veyvondi®, which came to the market a few years ago, has different PK properties than plasma derived VWF (25). Hence, it is essential to also construct population PK models for these VWF-containing concentrates, as they are widely used in other countries and are increasingly prescribed in the Netherlands. Some studies have previously examined application of PK-guided dosing of Haemate® P following surgery. A study by Lethagen et al. demonstrated feasibility in selection of the loading dose prior to elective surgery based on the PK profile of the patient (12). Di Paola et al.

observed a poor correlation between the in vivo recovery (IVR) values before and after surgery, questioning the potential benefits of PK-guided dosing (26). However, this study, in which PK-guided dosing of this VWF-containing concentrate was evaluated with a standard two-compartment model, did not take the prior information of the population and possible influencing covariates into account. A covariate analysis is however important, as international guidelines recommend specific target levels depending on the type and extent of the surgical procedure (8).

STRENGTHS AND LIMITATIONS OF OUR STUDIES

Strengths

Over the last decades, VWD treatment has not changed significantly, and as research into new treatment modalities is sparse, desmopressin and VWF-containing concentrates will most probably remain the mainstay of treatment in the coming years. However, by increasing knowledge on the development and implementation of PK-PD models for VWD treatment we are contributing to further personalization of treatment.

For our studies, we were able to include data from a large and varying group of VWD patients, including significant numbers of patients from all VWD disease types. A significant part of these patients participate in the multicenter Willebrand in the Netherlands (WiN study) and are already being followed for years (27). Patients included in our study on VWFpp had participated in a local study at the Erasmus MC (28). Because of this remarkable research on VWD in the Netherlands, we were able to collect data and stored plasma samples from a large group of well-characterized individuals.

Performing a desmopressin test in patients potentially eligible for treatment with desmopressin is unnecessary in many cases as was proven in our study, and in some cases less blood samples need to be taken during the test (3). We developed a simple tool for deciding which patients need a desmopressin test and deciding how many blood samples need to be taken if the patient needs to be tested. This will not only reduce the burden for patients and the treatment team, but will also reduce costs for testing and labor significantly.

We also performed several other studies in patients who underwent desmopressin testing. In one study, we measured VWF propeptide levels – a marker of VWF synthesis - after administration of desmopressin and found a limited correlation between baseline VWFpp/VWF:Ag ratio and VWF:Ag increase. It is also known that a baseline VWFpp/

VWF:Ag ratio >2.2 is predictive of increased clearance of VWF. In another study, we observed that the presence or absence of a mutation and the type of mutation influences desmopressin response in VWD patients. Incorporating VWFpp levels, VWFpp/VWF:Ag ratio and genetic variants into population PK models for treatment of VWD may strengthen the predictive performance of these models and may provide better insight into the pathological mechanisms in individual patients.

Lastly, we have an excellent and unique cooperation between clinicians, pharmacologists and laboratory specialists for our clinical PK studies, which is vital for providing the right dosing advice. Working together also enables us to learn about specific topics from other disciplines, leading to better understanding between the different specialists and often provoking thoughts for new research questions

Limitations

Study limitations concern applicability and reliability of the developed population PK models due accessibility, varying assays and precision of data, lack of knowledge of minimal VWF and FVIII levels to prevent bleeding in different circumstances, as well as a historical lack of a clear definition of bleeding and therefore of bleeding phenotype.

Generally, population PK models are internally validated by performing Monte Carlo simulations (29). However, as the same data set is used for constructing the model as well as validating the model, results may be biased when using this approach. For the Haemate® P FVIII population PK model described, we were able to collect data from 20 additional patients after the initial data set was collected. This enabled us to perform external validation to overcome this limitation. External validation showed a small bias and acceptable inaccuracy of the predictive performance of the constructed population PK model (13).

PK-guided dosing is increasingly applied in clinical practice. However, PK modelling tools are not yet readily accessible for clinicians and patients and require time investment and specific knowledge from a trained pharmacologist to get a reliable dosing advice. For hemophilia treatment, easy-to-use online dosing tools are already available, enabling clinicians without specific knowledge on MAP Bayesian techniques and without specific software, to perform PK-guided dosing for their patients (30). For VWD, online dosing tools are under development and not yet easily accessible, which we aim to change in the near future. When we have managed to develop these tools and have made them accessible, wider implementation of PK-guided dosing in clinical practice will be the following hurdle needed to be taken. From studies on

implementation of PK-guided dosing in clinical practice we know there are some barriers that may make some patients and clinicians reluctant to use PK-guided dosing. These barriers, among others, include the need to perform an individual PK-profile in every patient and possibly to increase the frequency of dosing, especially in individuals receiving prophylactic treatment (31, 32). The importance of performing a PK profile is especially difficult as the majority of VWD patients are treated on demand and only require treatment when they are bleeding or in order to prevent bleeding. In addition, several factors may also influence the feasibility of PK-guided dosing. First, the accuracy of the assays used to perform factor level measurements may influence the observed factor levels, and thereby influence the accuracy and precision of the predicted factor levels. Second, imprecise or inaccurate registration of the administered doses, and timing of dosing and blood sampling may influence the accuracy and precision of the estimation. This results in less accurate or precise individual PK parameter estimates and calculation of suboptimal treatment doses. Additionally, the exact amount of clotting factor activity may differ between different batches of the same VWF-containing concentrate.

As mentioned earlier, guidelines on the treatment of VWD prescribe target VWF and FVIII levels for the perioperative period. However, as these target trough levels are lower than the levels reached in healthy individuals, evidence for these specific levels is scarce, and the correlation between these low factor levels and bleeding risk is limited (4, 33). Performing perioperative studies with lower target levels to study the relationship with bleeding is considered undesirable for obvious reasons. It is therefore important to closely document the amount of blood loss and number and nature of bleeding complications in patients receiving PK-guided dosing, as we expect them to reach lower VWF and FVIII levels during treatment than the currently used body weight-dependent dosing. Additionally, it is also important that other patient characteristics concerning genotype and phenotype that are associated with higher bleeding risk are documented in these individuals. For instance, in hemophilia A, patients with blood group O have a higher chance of bleeding during perioperative treatment than those without blood group O (34).

Another limitation is that in our retrospective study on perioperative management of VWD with Haemate® P, we did not use a specifically defined measure of bleeding: we quantified hemostasis by hemoglobin levels, amount of blood loss, incidence of bleeds, incidence of thrombosis and the need for blood transfusion and/or re-operation because of bleeding. This is also the definition used in the prospective 'OPTI-CLOT: To WiN' trial (Netherlands Trial Registry number 4711; EudraCT number 2018-001631-46). In the literature, many different measures of type of bleeding and amount of blood loss

have been described. However there has been no consensus on a uniform definition of type and severity of bleeding for a long time. Recently, an expert panel has proposed new criteria for the definition of bleeding (35). The lack of uniformity in bleeding definitions used in literature however makes it difficult to compare between previous results and our own results (35, 36).

FUTURE PERSPECTIVES

In our opinion, PK-guided dosing is important to improve targeting of VWF levels peri-operatively, at the time of a bleeding event, and in the prophylactic setting, when compared to standard body weight dosing. In the 'OPTI-CLOT: To WiN' trial, we are currently testing this hypothesis in a cohort of prospectively included VWD patients, while also enriching our already developed population PK models, to optimize their predictive performance (37). Within 'OPTI-CLOT: To WiN', we additionally aim to construct population PK models for other VWF-containing concentrates than Haemate® P. We have already collected data on treatment with several of these factor concentrates from collaborating hemophilia treatment centers in the Netherlands and United Kingdom, but these international collaborations need to be further expanded in order to obtain data from large patient groups. The ultimate goal is to develop an integrated population PK model for all different VWF-containing concentrates, describing the PK of all available concentrates simultaneously.

In the studies we have performed, pregnant individuals and treatment during delivery or Caesarean section were not included as VWF and FVIII are known to increase and even normalize during pregnancy (38). However, as the risk of postpartum hemorrhage is significantly higher in individuals with VWD than in those without a bleeding disorder, further characterization of the PK of VWF and FVIII and evaluation of adequate target levels before, during and after delivery is of the utmost importance in this vulnerable population (39). In the prospective 'OPTI-CLOT: To WiN' trial, we will therefore include a separate cohort of VWD patients scheduled for an in-hospital delivery or Caesarean section. Furthermore, the Dutch national guidelines on treatment of pregnant VWD patients have been changed and now recommend treatment with a VWF-containing concentrate if VWF:Act is <0.80 IU/mL in the third trimester, and to target peak VWF:Act levels of ≥1.50 IU/mL. This strategy is based on the finding that in healthy women, VWF and FVIII levels rise to >2.00 IU/mL at the time of delivery, and the fact that achieving VWF levels around 1.00 IU/mL during delivery still results in an increased risk of bleeding in VWD patients (40, 41). This new treatment strategy will be prospectively evaluated in the 'PRIDES' study (NCT NL6770, NTR 6947) (36).

Similar treatment strategies, aiming for more physiological coagulation factor levels during delivery, are currently also being investigated by other groups (42, 43).

We described that standard dosing of a specific VWF-containing concentrate (Haemate® P) often leads to VWF levels ≥0.20 IU/mL above target level, resulting in higher treatment costs and possibly a higher thrombosis risk (4). This can be partially explained by well-known inter-individual differences in the PK of factor concentrates. In contrast to previous PK studies for Haemate® P that did not analyze the individual PK-profile after a testing dose, or did only analyze the data of the individuals (12, 26), Bayesian forecasting takes the inter-individual differences in PK parameters into account and generates the individualized dose that will produce the target activity level, thereby increasing efficacy and patient safety. Our subsequent studies confirmed that PK-guided dosing will likely improve treatment by making it more efficient, improving costs and benefits (13, 15). As this factor concentrate is readily available, rapid implementation of this strategy is feasible. Studies on the implementation of PK guided dosing are needed. Therefore, in the near future implementation of our models in clinical practice is our most important task.

Currently, performing PK-guided dosing for VWD requires specific knowledge and time investment from a trained pharmacologist. Also, performing an individual PK profile requires giving the patient an extra dose of medication and taking multiple blood samples. This leads to additional treatment costs and deployment of staff e.g. nurses, hematologists and laboratory technicians. However, as online PK-dosing tools for VWD will also become available in the near future, clinicians will be able to calculate the most optimal dosing for their patients more easily by an automated process. Although we underline that intensive communication with a clinical pharmacologist remains key to generate high-quality personalized dosing regimens. Subsequently, when data has reached a certain volume for all VWD subtypes and VWF-containing concentrates, it may be possible to lower the number of blood samples required for individual PK-profiling, reducing efforts and costs for the treatment team. Furthermore, we have found that most patients treated with a VWF-containing concentrate receive higher doses than necessary for securing hemostasis (4). As a consequence, during PK-guided dosing, most patients may require lower or less frequent doses of VWF-containing concentrate. As VWF-containing concentrates are expensive, this will reduce treatment costs. Importantly, countries with limited health care resources may therefore especially benefit most from these potential cost reductions. Therefore, future studies should be performed on the cost-effectiveness of PK-guided dosing.

Currently, online dosing tools already exist for treatment of hemophilia. These tools however lack transparency about their underlying population PK models (30). The studies we are now conducting on the development and implementation of an online dosing tool are ongoing (18). As this consortium is mainly governmentally funded, we will be able to give full transparency on the underlying population PK models -which is often lacking in commercially funded PK models underlying PK-guided dosing tools in hemophilia-, and on how these models were validated, thereby helping clinicians to better understand how a dose is calculated. Furthermore, these tools may be used to collect additional data, which can be incorporated into the existing models to further refine them. Ideally, in the future pharmaceutical companies should be obliged to perform population PK-modelling using their pre-marketing trial data, and to publish these data or make them publically available, enabling clinicians and clinical pharmacologists to generate the most optimal dosing advice for their patients.

CONCLUSION

With the innovative work presented in this thesis, important steps towards individualization of dosing in the treatment of VWD have been taken. This is urgent as refinement of treatment in VWD has long been neglected compared to hemophilia. With ongoing data collection and subsequent construction of population PK models and ultimately PK-PD models, development of easy-to-access online dosing tools, and further translational research, truly personalized dosing is finally within reach for individuals with VWD.

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Chapter 13

Summary / Samenvatting

SUMMARY

The focus of this thesis was on individualizing dosing of treatment in von Willebrand disease (VWD), the most common inherited bleeding disorder. VWD is caused by a partial or complete absence of von Willebrand factor (VWF) (type 1 and type 3 VWD respectively) or by a functional defect of VWF (type 2 VWD). Symptoms mainly include mucocutaneous bleeding such as bruising, nose bleeds and menorrhagia, and bleeding after trauma or surgery. Due to its varying pathophysiology, diagnosis and treatment of VWD is complex.

First, current treatment strategies were evaluated and reviewed to identify where improvements can be made. Secondly, several population pharmacokinetic (PK) models were developed, which can be applied for individualized PK-guided dosing of the currently available treatment. Thirdly, a study protocol on implementation of PK-guided dosing of desmopressin and VWF-containing concentrates in clinical practice was presented, as well as new covariates that can be useful for future research.

Part I. Current treatment in VWD

In **Chapter 2**, current management of VWD was reviewed. Treatment has remained essentially unchanged over the last 30 years and mainstay of treatment are desmopressin -which stimulates the release of endogenous VWF from the vascular endothelium- and intravenously administered VWF-containing concentrates.

Individuals with VWD usually undergo a desmopressin test after diagnosis, to analyze their endogenous VWF and factor VIII (FVIII) response. In **Chapter 3**, retrospective data on measurements performed during the first six hours after desmopressin administration in 377 patients with different types of VWD were analyzed, and results were compared between responders and non-responders using baseline VWF activity (VWF:Act) cut-off levels in order to categorize response. It was concluded that all individuals with type 1 VWD with baseline VWF:Act ≥0.34 IU/mL and type 2 VWD with baseline VWF:Act ≥0.28 IU/mL respond adequately to desmopressin. Therefore we have suggested that these individuals do not need testing before desmopressin treatment. The results were subsequently validated in a group of 30 VWD patients, with similar results. Implementing this protocol in clinical practice will lead to a significantly lower burden of testing for patients and health care providers, and will reduce costs.

If an individual does not respond sufficiently to desmopressin or needs a medical intervention with a relatively high bleeding risk, a VWF-containing concentrate is

administered according to current guidelines. In **Chapter 4,** current perioperative management of VWD patients with Haemate® P, the most commonly used VWF-containing concentrate in the Netherlands, was analyzed. VWD patients (n=103) with various types of VWD whom underwent surgery were included. Overall, treatment resulted in high VWF:Act and FVIII (≥0.20 IU/mL above target), and levels below VWF and FVIII target were rare. Clinically relevant bleeding occurred in five cases and was not related to low VWF:Act or FVIII, but were probably due to surgical factors. No thrombotic complications occurred, despite 18 patients reaching very high (≥2.70 IU/mL) FVIII levels due to accumulation of FVIII after repetitive dosing. We concluded that many VWD patients are 'over-treated', leading to unnecessarily high costs. Therefore, we suggest that individualized PK-guided dosing of VWF-containing concentrates may lead to lower treatment costs, without compromising therapeutic efficacy or safety.

Part II. Individualizing treatment by population pharmacokinetic modelling

In **Chapter 5**, the relationship between desmopressin dose, its plasma concentration and the VWF:Act response in 47 type 1 VWD patients was investigated, using a newly developed integrated population pharmacokinetic-pharmacodynamic (PK-PD) model. The plasma desmopressin concentration versus time profile was best described by a one-compartment PK model. In the PD turnover model, the relationship between desmopressin plasma concentration and endogenous VWF:Act release was best described with a maximum effect (Emax) model. VWF:Act typically increased with 45% with a half-maximal (EC50) plasma desmopressin concentration of 0.17 ng/mL. Monte Carlo simulations showed that the current dosing regimen of 0.3 mcg/kg desmopressin is effective, with >90% of patients with a VWF:Act baseline >0.20 IU/mL achieving VWF:Act levels >0.50 IU/mL up to four hours after desmopressin administration. In individuals weighing >100 kg, the desmopressin dose can be capped at 30 mcg safely, as the response will remain sufficient.

A population PK model for VWF:Act after desmopressin administration in VWD patients (n=207) was developed and is described in **Chapter 6**. The aim was to analyze, quantify, and explain the large interpatient variability in achieved plasma VWF:Act after administration of desmopressin. PK was best described with a one-compartment model using allometric scaling. The bioavailability *F* of VWF:Act (the amount of circulating VWF:Act) increased with age, and clearance (*Cl*) of VWF:Act depended on VWD type and sex. VWF:Act was removed from the body fastest in type 2A and type 2M patients, and was faster in males than in females. Inclusion of these covariates into the model resulted in lower variability in *F* (81.7% to 60.5%) and lower variability in

Cl (92.8% and 76.5%). This model is a starting point towards more accurate prediction of VWF:Act after desmopressin administration.

Population PK models for the VWF/FVIII-concentrate Haemate® P were also developed. **Chapter 7** provides one piece of the puzzle: here the PK of FVIII in VWD patients during the perioperative period is analyzed. Ninety-seven VWD patients - who underwent 141 surgical procedures - were included for building the model. Subsequently, the model was externally validated and re-estimated with data of an additional 31 surgeries performed in 20 patients. The model was proven to adequately describe FVIII following perioperative Haemate® P administration. The observed PK-profiles were best described using a one-compartment model. Higher VWF:Act, decreased physical status (ASA class >2), and longer surgery duration were associated with decreased *Cl* of FVIII.

The aim in **Chapter 8** was to complete the PK puzzle. A population PK model for the complex interaction between VWF and FVIII during perioperative treatment with Haemate® P was developed. One hundred and eighteen patients who underwent 174 surgeries were included and linear mixed-effects modelling was performed. VWF:Act and FVIII were analyzed simultaneously using a turnover model. This enabled the description of the protective effect of VWF on clearance of FVIII: an average VWF:Act level of 1.23 IU/mL decreased FVIII *Cl* from 460 mL/h to 264 mL/h, and almost doubled FVIII half-life (from 6.6 hours to 11.4 hours). This is in line with the finding from **Chapter 4** that FVIII accumulates after repetitive dosing of Haemate® P. The model adequately describes VWF:Act and FVIII levels during the perioperative period and may facilitate more accurate targeting of VWF:Act and FVIII levels during perioperative treatment with this concentrate in the future, when PK-guided dosing is implemented in clinical practice.

Part III. Innovative approaches to individualize VWD treatment

In **Chapter 9,** a study protocol for a prospective, non-randomized, multicenter, post-marketing cohort study is presented, in which it is currently being investigated whether PK-guided dosing of desmopressin and VWF-containing concentrates is reliable and feasible in individuals with VWD. The main objective of this study is to assess the predictive performance (the difference between predicted and measured VWF:Act and FVIII) of Bayesian forecasting using the developed population PK models. This is tested in four different situations: 1) desmopressin testing; 2) during medical procedures; 3) during bleeding episodes and 4) in the prophylactic setting. Data is also collected on treatment duration, hemostasis, patient satisfaction and physician satisfaction to assess the feasibility of implementing individualized PK-guided dosing in clinical practice.

Novel laboratory tests and methods may help to improve diagnosis and treatment of VWD in the future. VWF levels represent a balance between synthesis, secretion and clearance of VWF in an individual. VWF propeptide (VWFpp) is a marker of VWF synthesis, and an increased VWFpp/VWF:Ag ratio reflects increased VWF clearance. In **Chapter 10**, the release of VWFpp in patients with either VWD or hemophilia A after desmoressin administration was investigated. It was concluded that incorporation of VWFpp concentrations and VWFpp/VWF:Ag ratios into population PK models for treatment of VWD can aid in further refinement of these models.

Not all patients respond to desmopressin adequately. In **Chapter 11**, it was investigated whether desmopressin response is affected by genotype in type 1 and type 2 VWD patients (n=250). It was found that in type 1 VWD, all patients without a VWF gene variant respond to desmopressin, whereas a significant part of patients with a gene variant do not respond. As expected, there was large heterogeneity in desmopressin response in both type 1 and type 2 VWD patients. However, in individuals with the same VWF gene variant, everyone had a similar desmopressin response, suggesting that the response depends on these specific gene variants.

Finally, the outcomes of the studies were discussed and placed into a broader perspective in **Chapter 12**. Furthermore, suggestions for future research were given.

SAMENVATTING

In dit proefschrift werd gefocust op het individualiseren van de behandeling van von Willebrandziekte (VWD), de meest voorkomende erfelijke bloedingsziekte. VWD wordt veroorzaakt door een gedeeltelijke dan wel complete afwezigheid van von Willebrand factor (VWF) in respectievelijk type 1 en type 3 VWD, of door een afwijkende functie van VWF in type 2 VWD. De symptomen bestaan voornamelijk uit huid- en slijmvliesbloedingen, -zoals hematomen, neusbloedingen en menorragie- en bloedingen na een verwonding of chirurgische behandeling. Door de verschillende pathofysiologie van de verscheidene vormen van VWD is de diagnose en behandeling complex.

Allereerst werden de huidige behandelstrategieën geanalyseerd om zodoende te achterhalen waar verbeteringen mogelijk zijn. Vervolgens werden een aantal populatie-farmacokinetische (PK) modellen ontwikkeld en beschreven, die kunnen worden toegepast voor het individueel PK-gestuurd doseren van de huidige beschikbare behandelingen. Als laatst hebben werden toekomstperspectieven gepresenteerd: een studieprotocol voor het implementeren van PK-gestuurd doseren van desmopressine en VWF-bevattende concentraten in de klinische praktijk en nieuwe covariaten die nuttig kunnen zijn voor toekomstig onderzoek.

Deel I. Huidige behandeling van VWD

In **Hoofdstuk 2** werd de huidige behandeling van VWD beschreven, die vrijwel onveranderd is gebleven gedurende de laatste 30 jaar. De voornaamste behandelopties zijn desmopressine -dat het vrijkomen van endogeen VWF uit het vasculaire endotheel stimuleert- en intraveneus toegediende VWF-bevattende concentraten.

Individuen met VWD ondergaan, nadat de diagnose is gesteld, gewoonlijk een desmopressinetest, om hun endogene VWF- en Factor VIII (FVIII)-respons te analyseren. In **Hoofdstuk 3** werden retrospectieve VWF- en FVIII-metingen die uitgevoerd werden gedurende de eerste zes uur na toediening van desmopressine, geanalyseerd in 377 patiënten met verschillende vormen van VWD. Patiënten werden gecategoriseerd als 'respondenten en 'niet-respondenten' en vervolgens konden afkapwaardes voor de basis-VWF-activiteit (VWF:Act) worden vastgesteld, waarmee voorspeld kan worden of een individu adequaat reageert op desmopressine. Wij concludeerden dat alle individuen met type 1 VWD met een basis-VWF:Act ≥0.34 IU/ml en individuen met type 2 VWD met een basis-VWF:Act ≥0.28 IU/ml adequaat reageren op desmopressine. Daarom stelden wij dat deze individuen geen desmopressinetest hoeven te ondergaan voorafgaand aan een de behandeling.

De resultaten van dit onderzoek werden vervolgens gevalideerd in een groep van 30 individuen, met vergelijkbare resultaten. Het in de praktijk brengen van dit protocol zal leiden tot een significant lagere testbelasing voor patiënten en behandelaars en tot kostenreductie.

Als een individu niet voldoende reageert op desmopressine of wanneer er een medische ingreep met een hoog bloedingsrisico moet plaatsvinden, dan wordt volgens de huidige richtlijnen een VWF-bevattend concentraat toegediend. In **Hoofdstuk 4** werd de huidige perioperatieve behandeling van VWD-patiënten met Haemate® P, het meest gebruikte VWF-bevattende concentraat in Nederland, geanalyseerd. VWD-patiënten van verschillende typen (n=103) die een operatie hadden ondergaan, werden geïncludeerd. In het algemeen leidde de behandeling tot VWF:Act- en FVIII-concentraties ≥0.20 IU/ml hoger dan de streefwaarde, terwijl VWF:Act- en FVIII-concentraties onder de streefwaarde bijna niet voorkwamen. In vijf gevallen was er sprake van een klinisch relevante bloeding. Deze bloedingen waren echter niet gerelateerd aan lage VWF:Act- of FVIII-waarden, maar waren waarschijnlijk het gevolg van chirurgische factoren. Er werden geen trombotische complicaties geobserveerd, ondanks het feit dat 18 patiënten zeer hoge (≥2.70 IU/ml) FVIII-concentraties bereikten door stapeling van FVIII na herhaaldelijke doses Haemate® P. Er werd geconcludeerd dat veel individuen meer factorconcentraat kregen dan noodzakelijk, wat leidt tot onnodig hoge kosten. Onze hypothese is daarom dat geïndividualiseerd PK-gestuurd doseren van VWF-bevattende concentraten zal leiden tot lagere behandelkosten, zonder dat daarbij de effectiviteit of veiligheid van de behandeling in het geding komt.

Deel II. Het individualiseren van de behandeling middels populatie-farmacokinetisch modelleren

In **Hoofdstuk 5** werd de relatie tussen de dosis en plasmaconcentratie van desmopressine en de VWF:Act respons in 47 type 1 VWD-patiënten onderzocht, gebruikmakend van een nieuw ontwikkeld geïntegreerd populatie-farmacokinetisch-farmacodynamisch (PK-PD) model. Het plasma desmopressine-concentratie-versus-tijdprofiel werd het best beschreven door een ééncompartiments PK model. In het PD omzettingsmodel werd de relatie tussen de plasmaconcentratie van desmopressine en de endogene VWF:Act-secretie het best beschreven met een maximumeffect (Emax)-model. Gemiddeld steeg VWF:Act met 45% bij een half-maximale (EC50) plasmadesmopressineconcentratie van 0.17 ng/ml. Monte Carlo-simulaties toonden aan dat het huidige doseringsregime van 0.3 mcg/kg desmopressine effectief is, waarbij >90% van de patiënten met een basis-VWF:Act-concentratie >0.20 IU/mL, VWF:Act-concentraties boven 0.50 IU/ml bereiken, die aanhouden tot in ieder geval vier uur na desmopressinetoediening. Bij individuen met een gewicht >100 kg kan de

dosering veilig worden gemaximeerd op 30 mcg, omdat de respons bij deze dosering voldoende blijft.

Een populatie-PK model voor VWF:Act na toediening van desmopressine in VWD-patiënten (n=207) werd beschreven in **Hoofdstuk 6**. Het doel was om de grote interpatiëntvariabiliteit in bereikte VWF:Act-plasmaconcentraties na toediening van desmopressine te beschrijven, te kwantificeren en te verklaren. De PK werd het best beschreven middels een ééncompartiments PK model, waarbij gebruik werd gemaakt van allometrisch schalen. De biologische beschikbaarheid *F* (de hoeveelheid circulerende VWF:Act) nam toe met de leeftijd en de klaring (*Cl*) van VWF:Act was afhankelijk van het type VWD en geslacht. VWF:Act werd het snelst geklaard in patiënten met type 2A en 2M VWD en mannen klaarden VWF:Act sneller dan vrouwen. Inclusie van deze covariaten in het model resulteerde in een lagere variabiliteit in *F* (van 81,7% naar 60,5%) en lagere variabiliteit in *Cl* (van 92.8% naar 76,5%). Dit model vormt een beginpunt voor accuratere voorspellingen van VWF:Act na toediening van desmopressine.

Er werden ook populatie-PK modellen ontwikkeld voor het FVIII/VWF-concentraat Haemate® P. **Hoofdstuk 7** bevat één stukje van de puzzel: hier werd de PK van FVIII in VWD patiënten gedurende de perioperatieve periode geanalyseerd. Om het model te kunnen bouwen werden retrospectief 97 VWD-patiënten geïncludeerd, die in totaal 141 operaties hadden ondergaan. Vervolgens werd het model gevalideerd en werden nieuwe schattingen gemaakt met de data van 31 operaties, uitgevoerd in 20 patiënten. Er werd aangetoond dat het model de FVIII-concentraties na perioperatieve toediening van Haemate® P adequaat kan voorspellen. De geobserveerde PK profielen konden het best worden beschreven met een ééncompartiments PK model. Een hogere VWF:Act, verminderde fysieke gesteldheid (d.w.z. ASA klasse >2) en een langere operatieduur waren geassocieerd met een verminderde FVIII-klaring.

De PK-puzzel compleet maken: dat was het doel in **Hoofdstuk 8**. In dit hoofdstuk werd de ontwikkeling beschreven van een populatie-PK model om de complexe interactie tussen VWF en FVIII gedurende de perioperatieve behandeling met Haemate® P te modelleren. Er werden 118 patiënten geïncludeerd die gezamenlijk 174 operaties hadden ondergaan en er werd *mixed-effects modelling* uitgevoerd. VWF:Act en FVIII werden tegelijkertijd geanalyseerd, gebruikmakend van een PD omzettingsmodel. Hiermee kon het beschermende effect van VWF op de klaring van FVIII beschreven worden: een gemiddelde VWF:Act-concentratie van 1.23 IU/ml verminderde de FVIII-klaring van 460 ml/u naar 264 ml/u en zorgde ervoor dat de halfwaardetijd van FVIII bijna verdubbelde (van 6.6 uur naar 11.4 uur). Dit sluit aan bij de bevinding in **Hoofdstuk 4**

dat FVIII-stapeling optreedt na achtereenvolgende toediening van meerdere doses Haemate® P. Het model beschrijft de VWF:Act- en FVIII-concentraties gedurende de perioperatieve periode adequaat en kan daardoor in de toekomst bijdragen aan het gerichter nastreven van de VWF:Act- en FVIII-streefwaarden gedurende de perioperatieve behandeling, wanneer PK-gestuurd doseren geïmplementeerd wordt in de klinische praktijk.

Deel III. Innovatieve manieren om de behandeling van VWD te individualiseren

In **Hoofdstuk 9** werd een protocol gepresenteerd voor een prospectieve, niet-gerandomiseerde, multicenter, *post-marketing* cohortstudie waarin op dit moment onderzocht wordt of PK-gestuurd doseren van desmopressine en VWF-bevattende concentraten betrouwbaar en haalbaar is in individuen met VWD. De belangrijkste onderzoeksvraag van deze studie is hoe nauwkeurig de voorspellende waarde (het verschil tussen de voorspelde en gemeten VWF:Act- en FVIII-concentraties) van Bayesiaans voorspellen middels de ontwikkelde populatie PK-modellen is. Dit wordt getest in vier verschillende situaties: 1) desmopressinetesten; 2) gedurende medische ingrepen; 3) gedurende bloedingsepisoden; en 4) gedurende profylactische behandeling. Er worden ook data verzameld over de behandelduur, de bloedstelping en tevredenheid van patiënten en behandelaars, om te onderzoeken of geïndividualiseerd PK-gestuurd doseren praktisch haalbaar is.

Nieuwe laboratoriumtesten en -methoden kunnen helpen om de diagnose en behandeling van VWD in de toekomst te verbeteren. VWF-concentraties representeren de balans tussen synthese, secretie en klaring van VWF in een individu. VWF-propeptide (VWFpp) is een marker van VWF-synthese en een verhoogde VWFpp/VWF:Ag ratio reflecteert een verhoogde VWF-klaring. **In Hoofdstuk 10** onderzochten wij de secretie van VWFpp in patiënten met VWD en hemofilie A na toediening van desmopressine. Er werd geconcludeerd dat het toevoegen van VWFpp concentraties en VWFpp/VWF:Ag ratios aan populatie PK modellen voor de behandeling voor van Willebrandziekte kan leiden tot verdere verbetering van deze modellen.

Niet alle patiënten vertonen een adequate reactie op desmopressine. In **Hoofdstuk 11** werd onderzocht of de desmopressinerespons beïnvloed wordt door het genotype in type 1- en type 2 VWD-patiënten (n=250). De uitkomst was dat alle type 1 VWD-patiënten zonder VWF-genvariant een desmopressinerespons vertoonden, terwijl een significant deel van de patiënten met een genvariant geen respons vertoonden. Zoals verwacht was er een grote heterogeniteit in desmopressinerespons in zowel type 1- als type 2 VWD-patiënten. Individuen met dezelfde VWF-genvariant vertoonden allemaal

eenzelfde desmopressinerespons, wat suggereert dat de respons afhankelijk is van deze specifieke genvarianten.

Afsluitend werden de uitkomsten van de studies bediscussieerd in **Hoofdstuk 12**, waarbij de uitkomsten van deze studies in breder perspectief geplaatst werden. Verder werden suggesties gedaan voor toekomstig onderzoek.

Part V

List of publications
Acknowledgements
Dankwoord
About the author
PhD portfolio

LIST OF PUBLICATIONS

This thesis

Can von Willebrand factor propeptide / von Willebrand factor antigen ratio predict enhanced clearance of von Willebrand factor after desmopressin?
***Heijdra JM**, Dirven R, Kruip MJAH, Zwaan CM, Leebeek FWG, Eikenboom HCJ, Cnossen MH. Submitted*

Desmopressin testing in von Willebrand disease: lowering the burden
***Heijdra JM**, Atiq F, Al Arashi W, Kieboom Q, Wuijster E, Meijer K, Kruip MJHA, Leebeek FWG, Cnossen MH in collaboration with the "OPTI-CLOT" study group.Research and Practice in Thrombosis and Haemostasis, 2022*

Quantification of the relation between desmopressin concentration and von Willebrand factor in von Willebrand disease type 1: a pharmacodynamic study
Heijdra JM*, *Cloesmeijer ME*, de Jager NCB, Leebeek FWG, Kruip MJHA, Cnossen MH*, Mathôt RAA* for the "OPTI-CLOT" study group. *Shared first and last authorships.Haemophilia, 2022*

Desmopressin response depends on the presence and type of genetic variants in type 1 and type 2 von Willebrand disease patients
Atiq F, **Heijdra JM**, *Snijders F, Boender J, Kempers E, van Heerde, WL, Krouwel S, Schoormans SC, de Meris J, Maas DPMSM, Schols SEM, van Galen KPM, van der Bom JG, Cnossen MH, Meijer K, Fijnvandraat K, Eikenboom HCJ, Leebeek FWG fort he WiN study group. Blood Advances, 2022*

Is pharmacokinetic-guided dosing of desmopressin and von Willebrand factor-containing concentrates in individuals with von Willebrand disease or low von Willebrand factor reliable and feasible? A protocol for a multicenter, non-randomized, open label cohort trial, the 'OPTI-CLOT: To WiN' study
Heijdra JM, *Al Arashi W, de Jager NCB, Bukkems LH, Zwaan CM, Leebeek FWG, Mathôt RAA, Cnossen MHBMJ Open, 2022*

Population pharmacokinetics of the von Willebrand factor - factor VIII interaction in patients with von Willebrand disease
Bukkems LH, **Heijdra JM**, *de Jager NCB, Hazendonk HCAM, Fijnvandraat K, Meijer K, Eikenboom HCJ, Laros - van Gorkom BAP, Leebeek FWG, Cnossen MH*, Mathôt RAA* for the 'OPTI-CLOT' study group. *Shared last authorship. Blood Advances 2021.*

Population pharmacokinetic modelling of von Willebrand factor activity in von Willebrand disease patients after desmopressin administration
Heijdra JM*, De Jager NCB*, Kieboom Q, Kruip MJHA, Leebeek FWG, Cnossen MH*, Mathôt RAA* for the OPTI-CLOT group. *Shared first and last authorship.Thrombosis and Haemostasis, 2020*

One piece of the puzzle: population pharmacokinetics of FVIII during perioperative Haemate® P/Humate P® treatment in von Willebrand disease patients
De Jager NCB, Bukkems LH*, **Heijdra JM**, Hazendonk , Fijnvandraat CJ, HCAM, Meijer K, Eikenboom J, Laros-van Gorkom BAP, Leebeek FWG, Cnossen *, Mathôt RAA* for the "OPTI-CLOT" study group. *Shared first and last authorship.Journal of Thrombosis and Haemostasis, 2020*

Analysis of current perioperative management with Haemate® P / Humate P® in von Willebrand disease: Indicating the need for personalized treatment
*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. Haemophilia 2018.*

Current and emerging options for the management of von Willebrand disease
***Heijdra JM**, Cnossen MH, Leebeek FWG. Drugs 2017.*

Other

A novel quantitative method for analysis of desmopressin in human plasma using liquid chromatography tandem mass spectrometry
*De Jager NCB, **Heijdra JM**, Pistorius M, Kruip MJAH, Leebeek FWG, Cnossen MH, Mathôt RAATherapeutic Drug Monitoring, 2020*

Von Willebrand factor and factor VIII clearance in perioperative hemaophilia A patients
*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 HCJ, Mathôt RAA, Cnossen MH for the OPTI-CLOT study group.Thrombosis and Haemostasis, 2020*

A novel, enriched population pharmacokinetic model for recombinant factor VIII-FC fusion protein concentrate in haemophilia A patients
***Heijdra JM**, Bukkems LH*, Mathias M, Collins PW, Hay CRM, Tait RC, Mangles S, Myers B, Evans G, Bailiff B, Curry N, Payne J, Austin S, Goedhart MCHJ, Leebeek FWG, Meijer K,*

Fijnvandraat K, Chowdary P, Mathôt RAA, Cnossen MH* for the UK-EHL Outcomes Registry – OPTI-CLOT collaboration. *Shared first and last authorship. Thrombosis and Haemostasis 2020*

A randomized controlled trial studying the effectiveness of group medical appointments on self-efficacy and adherence in sickle cell disease (TEAM study): study protocol
*Madderom MJ, **Heijdra JM**, Utens EMWJ, Polinder S, Rijneveld AW, Cnossen MH. BMC Hematology, 2016*

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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.

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.

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"Alleen kun je sneller, maar samen kom je verder." - Afrikaans gezegde

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ABOUT THE AUTHOR

Jessica Heijdra was born on November 22nd, 1989 in Rotterdam, the Netherlands. She was raised in Vlaardingen, and after graduating from secondary school (Het College Vos, Vlaardingen), she started studying Medicine at the Erasmus University Medical Center Rotterdam. It was during her Bachelor's program that her enthusiasm in pediatrics and hematology was sparked and she connected with prof. dr. M.H. Cnossen from the Department of Pediatric Hematology at the Erasmus University Medical Center - Sophia Children's Hospital. She provided her with the opportunity to conduct a research project on sickle cell disease, which led to a fruitful collaboration. Following her internships, Jessica became involved in the "OPTI-CLOT: To WiN" study and wrote her Master's thesis on "Perioperative consumption of VWF/FVIII concentrate and desmopressin in von Willebrand disease".

After graduation, Jessica began her PhD project "Individualizing Treatment in von Willebrand Disease" at the Department of Pediatric Hematology of the Erasmus University Medical Center - Sophia Children's Hospital in January 2017, under supervision of prof. dr. M.H. Cnossen, prof. dr. C.M. Zwaan and prof. dr. F.W.G. Leebeek. During her PhD program, she received the 'Professor Heimburger Award 2018', and was selected for the PhD curriculum of 'Training Upcoming Leaders in Pediatric Science' (TULIPS). Thanks to the established international collaboration within the "OPTI-CLOT" study group, she got the opportunity to live abroad for four months while collecting data at the Royal Free Hospital (London, UK), under supervision of prof. dr. P. Chowdary.

In 2019, Jessica got married to Tim, and later that year, their son Olaf was born. In 2022, they welcomed a daughter, Freya. Jessica has worked as a pediatric resident at Reinier de Graaf Hospital, and is currently working as a youth healthcare resident at CJG Rijnmond.

In the upcoming years, the projects that Jessica has initiated during her PhD trajectory will be continued within the SYMPHONY consortium, a national collaboration of multidisciplinary research which aims to orchestrate personalized treatment for patients with bleeding disorders. The aim of this study is to continuously improve knowledge on pharmacokinetics and pharmacodynamics in treatment of von Willebrand disease, ultimately contributing positively to the lives of people impacted by the disease.

PHD PORTFOLIO

Name PhD student: J.M. Heijdra
Erasmus MC Department: Pediatric Hematology
Research School: COEUR

PhD period: January 2017 – December 2020
Promotor(s): C.M. Zwaan, F.W.G. Leebeek
Supervisor: M.H. Cnossen

1. PhD training

	Year	Workload (Hours/ECTS)
General academic skills		
Scientific Integrity	2017	0.3
BROK ('Basiscursus Regelgeving Klinisch Onderzoek')	2017	1.5
Biomedical English Writing and Communication	2020	3.0
Research skills		
CPO Course Patient Oriented Research	2017	0.3
NIHES course Biostatistical Methods I: Basic Principles	2017	5.7
Basic training Open Clinica	2018	0.3
Specific courses (e.g. Research school, Medical Training)		
NVTH PhD course on Thrombosis and Hemostasis (3x)	2017-2019	1.8
COEUR courses (3x: Congenital Cardiology, Intensive Care, Sex and Gender)	2017-2018	1.5
Regionale Nascholing Hematologie	2018	0.3
NONMEM work meeting and journal club (monthly)	2017-2020	4.0
Seminars and workshops		
Sophia Research Day (2x)	2017+2019	0.6
Promeras PhD Day	2017	0.3
COEUR PhD Day (2x)	2017+2018	0.6
TULIPS Young Investigators Day	2017	0.3
NVTH PhD Day (2x)	2018+2020	0.6
Masterclass Paul Monagle	2019	0.3
Masterclass David Lillicrap	2019	0.3
TULIPS PhD curriculum	2019-2021	2.0
Oral presentations		
Grand Round Sophia Children's Hospital	2017	0.3
SLAM session Sophia Research Day	2017	0.3
BIC - Rome	2017	0.3
CSL Behring Prof. Heimburger Award Symposium – Marburg	2018	0.3
NVTH Symposium	2019	0.3
Masterclass Paul Monagle	2019	0.3
MasterCLass David Lillicrap	2019	0.3
Poster presentations		
ISTH Congress – Berlin	2017	0.2
ISTH Congress – Melbourne (2x) – *presented by I. van Moort*	2019	0.4
NVTH Symposium	2019	0.2
BIC - Rome - *presented by L.H. Bukkems*	2019	0.2

National meetings & conferences		
NVTH Symposium (3x)	2017-2019	1.8
Nijmegen Symposium	2017	0.3
NVK Congress (1 day)	2017+2019	0.6
Van Creveld Symposium	2017	0.3
TULIPS Child Health Symposium	2018	0.6
International meetings & conferences		
ISTH congress - Berlin	2017	1.5
Bari International Conference – Rome	2017	0.9
UKHCDO Conference – London	2017	0.3
EHC Round Table Meeting – Brussels	2017	0.3
CSL Behring Prof. Heimburger Award Symposium – Marburg	2018	0.3
EAHAD congress - Prague	2019	0.9
EAHAD congress - The Hague	2020	0.9
2. Teaching		
Lecturing		
3-4x yearly Lecture "Hemostasis and Thrombosis" to nurses and midwives	2017-2020	0.9
Supervising practicals and excursions, Tutoring		
1st year students "Kennismaking BeroepsPraktijk"	2018	0.1
Supervising Master's theses		
Medical student Quincy Kieboom (1x 20 weeks)	2017	0.7
3. Other		
NVTH board member – PhD representative	2017-2020	0.9
Hematology work meetings and journal club (weekly)	2017-2020	6.0 (1.5/year)
4. Awards and grants		
CSL Behring Prof. Heimburger Award 2018 (€ 20.000,-)	2018	-
Total		**44.4**

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