PERSONALIZED MANAGEMENT OF PATIENTS WITH BLEEDING DISORDERS

Lorenzo G.R. Romano

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Colofon

PERSONALIZED MANAGEMENT OF PATIENTS WITH BLEEDING DISORDERS

De gepersonaliseerde behandeling van patiënten met bloedingsstoornissen

Proefschrift

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

Prof. dr. ir. A.J. Schuit

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

dinsdag 6 januari 2026 om 13.00 uur

door

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geboren te Maastricht.

Erasmus University Rotterdam

Promotiecommissie

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CHAPTER 1

GENERAL INTRODUCTION
AND THESIS OUTLINE

General introduction and thesis outline

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Hemostasis

Hemostasis is a tightly regulated process, that is activated after trauma in order to stop bleeding and repair damage to vasculature and surrounding tissue[1]. After endothelial damage, platelets bind to the exposed site and the subendothelium and are activated. At activation, multiple mediators such as adenosine diphosphate (ADP) and thromboxane A2 are released, leading to platelet aggregation at the site of injury, forming a hemostatic plug (primary hemostasis). Simultaneously, this endothelial damage causes tissue factor (TF) to be exposed, and bind and activate factor VII (FVII) to FVIIa, starting the initiation phase of the secondary hemostasis. The formed TF:VIIa complex activates factor X (FXa), which together with activated factor V (FVa) converts prothrombin into thrombin. Thrombin finally converts fibrinogen into fibrin, leading to the formation of multiple fibrin fibers, consolidating and stabilizing the hemostatic plug. The amplification phase of the secondary hemostasis starts by the increasing amounts of thrombin leading to the activation of factor XI (FXI) and factor VIII (FVIII), the latter amplifying the activation of FX together with activated FIX (FIXa). The final phase, the propagation phase, takes place on surfaces with phospholipids, such as activated platelets, where activated FXI (FXIa) converts FIX to FIXa. The FIXa/FVIIIa complex strongly enhances the conversion of FX to FXa, after which the FXa/FVa complex produces sufficient thrombin to greatly produce fibrin fibers (Figure 1). These fibrin fibers are further strengthened by factor XIIIa, forming covalent crosslinks between the fibrin chains.

These processes are balanced by multiple anticoagulant factors to prevent excessive clot formation, such as protein S, protein C, antithrombin and tissue factor pathway inhibitor (TFPI)[1]. Furthermore, the formed clot is slowly degraded by fibrinolysis to prevent extensive clot formation. Plasminogen activators, such as tissue plasminogen activator (tPA), convert plasminogen into plasmin, which cleaves fibrin fibers into fibrin degradation products. Disturbance in one of these processes in hemostasis can cause bleeding or thrombosis.

Hemophilia A

Hemophilia A is a rare X-linked bleeding disorder characterized by coagulation factor VIII (FVIII) deficiency[2]. Disease severity is classified based on residual FVIII levels (FVIII:C): mild (>0.05-0.40 IU/mL), moderate (0.01 - 0.05 IU/mL) or severe (<0.01 IU/mL) hemophilia. In severe patients, bleedings such as joint bleeds, muscle bleeds or hematuria, can occur spontaneously, or after trauma or a medical procedure. In non-severe patients, bleeding usually only occurs after trauma or during and after a medical procedure.
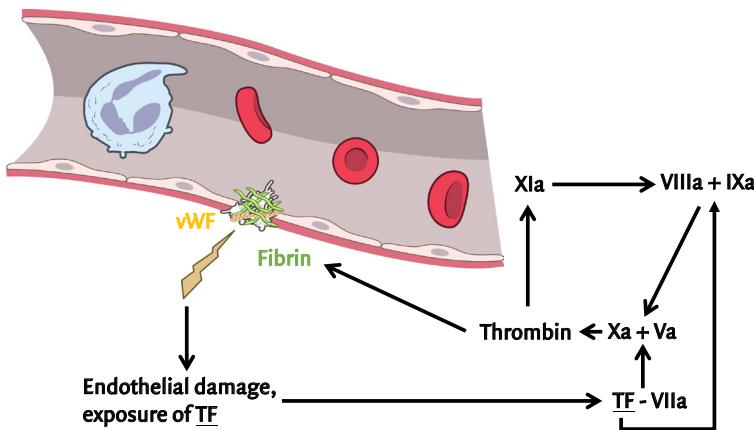
Figure 1: summarized overview of the secondary hemostasis.
After endothelial damage, tissue factor (TF) is exposed as it binds and activates factor VII (FVII) to
FVIIa. The formed TF:VIIa complex activates factor X (FXa), which together with activated factor V
(FVa) converts prothrombin into thrombin, leading to formation of fibrin by converting fibrinogen.
In the amplification phase, thrombin activates factor XI (FXI) and factor VIII (FVIII), the latter
amplifying the activation of FX together with activated FIX (FIXa). In the propagation phase, FXI
(FXIa) converts FIX to FIXa. The FIXa/FVIIIa complex strongly enhances the conversion of FX to FXa,
after which the FXa/FVa complex produces sufficient thrombin to greatly produce fibrin fibers.
The formed clot, which mainly consists of activated platelets, red blood cells and von Willebrand
factor (vWF), is stabilized by these formed fibrin fibers.

Treatment modalities in hemophilia A patients
Treatment of hemophilia A patients mainly consists of FVIII concentrate administration or
the use of desmopressin (DDAVP). Desmopressin is a synthetic derivative of antidiuretic
hormone (ADH) binding to the V2 receptor, which releases FVIII from pulmonary endothelial
cells[3-5]. Desmopressin is on the World Health Organisation (WHO) List of Essential Medicines
and widely available. The use of desmopressin is characterized by a high interindividual
variation of FVIII response. Most patients with mild hemophilia A have a good response
to desmopressin, leading to FVIII:C levels >0.50 IU/mL. However, some patients with
mild and most patients with moderate hemophilia A have a limited response (<0.30 IU/
mL) and therefore desmopressin is not used for the treatment of bleeding or before an
intervention[6]. Known determinants of this FVIII response in hemophilia A patients are *F8*
mutation, age, most recently measured FVIII:C and historically lowest FVIII:C[7-9]. Side effects
of desmopressin are usually transient and mild, related to its vasomotor and antidiuretic
effect[10]. The repeated use of desmopressin in a short period of time results in a reduced
FVIII:C response, also known as tachyphylaxis. The second FVIII:C response is reported to
be approximately 30% lower than the FVIII:C response after the first administration[11]. The
use of desmopressin in pregnancy for bleeding disorders is debated, because of possible

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safety concerns related to desmopressin's antidiuretic and vasoactive effects, which may lead to fluid overload and hyponatremia. Nonetheless, it has been used in pregnancy for patients with diabetes insipidus, with no parental nor neonatal adverse events[12]. However, in diabetes insipidus, desmopressin is administered daily, but in a lower dose than in bleeding disorders, thereby reducing the risk of the occurrence of adverse vasoactive events. The data on the efficacy of desmopressin in pregnancy is limited, despite the high morbidity and even mortality due to bleeding episodes during delivery as is the case in post-partum hemorrhage[13].

Despite its ubiquity, current use of desmopressin is suboptimal in hemophilia A patients, even in those with an adequate FVIII:C response. In a Dutch multicenter study, the desmopressin response exceeded the level targeted with FVIII concentrate in 54% of patients whose bleed was treated with one dose of FVIII concentrate[14]. For these patients, for instance those without contraindications for desmopressin, the use of desmopressin could have been preferred above the use of FVIII concentrate. In case of an inadequate response or lack of endogenous FVIII, as in severe hemophilia A, or in case of contraindications for desmopressin, desmopressin cannot be applied and only FVIII concentrate is used.

Despite the efficacy of FVIII concentrate, an important disadvantage is that it can lead to the formation of FVIII inhibiting antibodies (inhibitors) with a reported prevalence of 5% to 15% in mild hemophilia A and up to 30% in severe hemophilia A.

Inhibitor formation is associated with an increased morbidity and mortality[15-17]. Risk factors for inhibitor formation in non-severe hemophilia A patients are the extent of FVIII concentrate exposure, including high-dose FVIII concentrate treatment (>45 IU/kg) and exposure days[15,18]. Patients with inhibitors requiring hemostatic treatment are treated with bypassing agents, such as recombinant FVIIa (rFVIIa) or activated prothrombin complex concentrate (aPCC). The use of desmopressin on the other hand does not lead to the formation of inhibitors, as it induces an endogenous FVIII release. Another disadvantage of the use of FVIII concentrate are the high costs of FVIII concentrate treatment in hemophilia A. In the United States annual healthcare costs range from $59,101 (mild patients, with unspecified use of prophylaxis) up to even $301,392 (severe hemophilia patients with use of prophylaxis)[19,20]. Studies from the Netherlands and Scandinavian countries reported predicted annual costs of €51,832 without prophylaxis and costs of prophylaxis ranging from €135,476 to €224,712[21,22]. In recent years the costs of factor concentrate have decreased, but these costs remain high. A global survey by the World Federation of Hemophilia (WFH) reported that despite 24% of the worldwide hemophilia population is living in the Americas and Europe, they consume 79% of the available worldwide FVIII concentrate[23]. Novel treatment modalities or a different application of current treatment are therefore necessary.

A recent novel treatment modality is emicizumab, a bispecific monoclonal antibody mimicking the effect of FVIII, which serves as an alternative option for prophylaxis in (severe) hemophilia A patients[24]. However, its use is also costly and in inhibitor patients, the combination of emicizumab with aPCC may be associated with thrombotic microangiopathy[25]. More recently, adeno-associated virus (AAV) vector gene therapy has been introduced and phase 3 studies showed a significant FVIII:C increase in severe hemophilia A patients. However, its response is highly variable per patient, its use is costly, and long-term studies have shown a FVIII:C decrease after 2-3 years of follow-up[26,27]. Current gene therapy cannot be used for patients with FVIII inhibitors (current or in the past) and children, as they have been excluded from the phase 3 studies.

The lack of efficacy due to a limited FVIII:C response after desmopressin in combination with the high costs and limited availability of FVIII concentrate and its risk of inhibitor formation, calls for a new treatment approach, namely a combination of both treatments: first desmopressin to (slightly) increase FVIII to non-therapeutic levels, immediately followed by factor VIII concentrate administration to reach the target FVIII:C level, thereby reducing the amount of factor concentrate needed. The current World Federation of Hemophilia (WFH) guideline also states that the combination of desmopressin and FVIII concentrate could address the downsides associated with use of only either option[6]. However, studies to investigate the safety and efficacy of this combination approach are lacking.

Population pharmacokinetic modeling
In hemophilia A patients, dosing of FVIII concentrates for prophylaxis and/or treatment of bleeding is currently based on body weight[28]. However, several studies have shown that this approach is associated with a high interpatient variability in achieved FVIII:C, leading to off-target FVIII:C levels[29,30]. This could be overcome by using pharmacokinetic dosing. Traditionally, individual PK parameters are assessed by taking 10-15 serial samples after administration. This is however unpractical and a significant burden for the patient. Bayesian forecasting has the advantage that individual PK parameters can be obtained with sparse (2-4) samples. Bayesian forecasting technique uses both individual and population pharmacokinetic (PK) information. A population PK model is constructed using data from a specific patient population over a period of time after dose administration, i.e. peri-operative hemophilia A patients receiving FVIII concentrate followed by multiple FVIII:C measurements. Bayesian forecasting combines the observed activity levels of an individual patient with those comprised in an established population PK model, thereby estimating the PK parameters (clearance, volume of distribution) of the individual patient. Subsequently, these individual PK

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parameters can be used to calculate a specific FVIII concentrate dose to achieve a predetermined FVIII:C target and thereby personalizing their treatment. Multiple validated population PK models for the use of desmopressin or FVIII concentrate have been published in order to improve FVIII:C target attainment[31,32].

Aims and outline of this thesis

In this thesis, we aim to personalize management of hemophilia A by investigating the use of PK-guided dosing using Bayesian forecasting together with combination treatment of desmopressin and FVIII concentrate. Furthermore, we review the use of desmopressin in pregnancy and investigate patient perspectives on the use of desmopressin. In addition we performed *in vitro* studies with factor IX-FIAV, a possible novel treatment modality for hemophilia A patients with FVIII inhibiting antibodies. The use of aforementioned combination treatment of desmopressin and FVIII concentrate is reported as a possible solution to overcome drawbacks related to treatment with desmopressin or FVIII concentrate alone[6]. **In chapter 2,** the results of the DAVID and Little DAVID studies, both investigating the use of combination treatment of desmopressin and FVIII concentrate in the peri-operative setting, are reported. These are the first clinical trials of PK-guided combination treatment applied peri-operatively in non-severe hemophilia A patients. Repeated, daily use of desmopressin is associated with tachyphylaxis, a temporarily reduced FVIII:C response[11]. **In chapter 3,** tachyphylaxis of desmopressin is quantified and the reproducibility of the FVIII:C response will be assessed in patients included in the DAVID studies. As FVIII concentrate is costly in comparison to desmopressin, combination treatment could possibly lead to a reduction in total costs. **In chapter 4,** a cost-minimalization study will be reported which assesses the total costs of combination treatment compared to body weight based dosing of FVIII concentrate. The use of desmopressin as treatment in non-severe hemophilia A patients is reported as suboptimal, despite the fact that they may benefit from this treatment[14]. **In chapter 5,** non-severe hemophilia A patients' perspectives on the use of desmopressin will be studied in order to find ways to increase its use. The use of desmopressin in pregnancy and at the time of delivery is still debated due to safety concerns. **In chapter 6,** the safety and efficacy of desmopressin in pregnancy and during delivery will be investigated by performing a systematic review of the literature. Hematuria is usually regarded as a benign symptom in non-severe hemophilia A patients and attributed to the bleeding disorder of the patient. However, it may be the first manifestation of an underlying more severe disease. **In chapter 7,** the diagnostic evaluation and subsequent treatment of hematuria is studied retrospectively in a cohort of patients with hemophilia A in our treatment center. In hemophilia A patients with inhibitors, FVIII concentrate cannot be used to treat and prevent bleeding and other treatment options including bypassing agents have limitations. **In chapter 8,** we analyze the efficacy of FIX-FIAV, a novel FIX

variant which can be activated independently of FVIIIa and could therefore be used in hemophilia A patients, in hemophilia A patient plasma using thrombin generation assays and intrinsic coagulation activity in order to assess whether FIX-FIAV can be applied in hemophilia A patients with or without inhibitors. **In chapter 9,** we will summarize and discuss our findings and provide new focus areas for future research.

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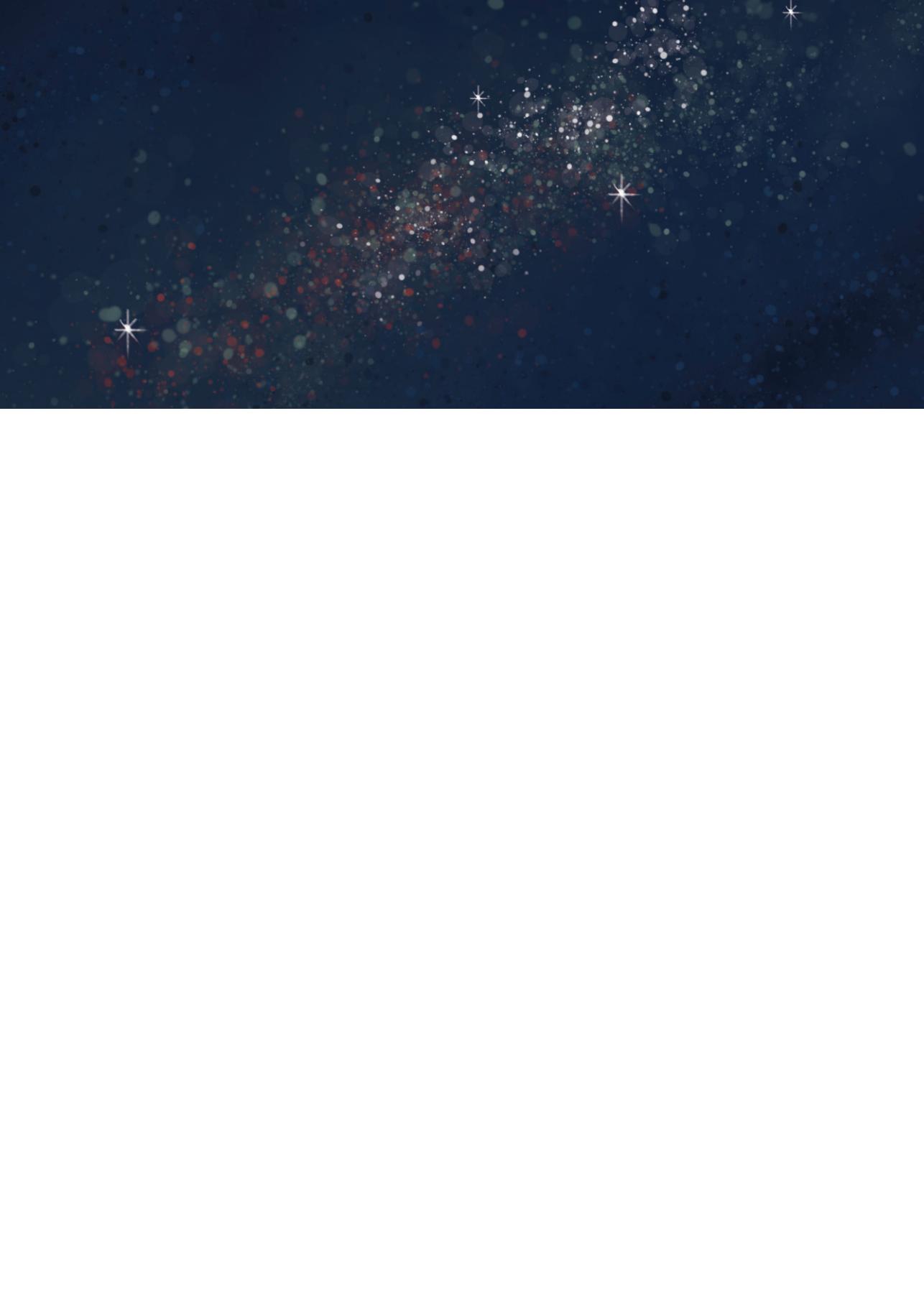
CHAPTER 2

PERI-OPERATIVE DESMOPRESSIN COMBINED WITH PHARMACOKINETIC-GUIDED FACTOR VIII CONCENTRATE IN NON-SEVERE HEMOPHILIA A PATIENTS

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Abstract

Introduction
Non-severe hemophilia A patients can be treated with desmopressin or factor VIII (FVIII) concentrate. Combining both may reduce factor consumption, but its feasibility and safety has never been investigated.

Aim
We assessed the feasibility and safety of combination treatment in non-severe hemophilia A patients.

Methods
Non-severe, desmopressin responsive, haemophilia A patients were included in one of two studies investigating peri-operative combination treatment. In the single-arm DAVID study intravenous desmopressin (0.3 μg/kg) once-a-day was, after sampling, immediately followed by PK-guided FVIII concentrate, for maximally three consecutive days. The Little DAVID study was a randomized trial in patients undergoing a minor medical procedure, whom received either PK-guided combination treatment (intervention arm) or PK-guided FVIII concentrate only (standard arm) up to two days. Dose predictions were considered accurate if the absolute difference between predicted and measured FVIII:C was ≤0.2 IU/mL.

Results
In total 32 patients (33 procedures) were included. In the DAVID study (n = 21), of the FVIII:C trough levels 73.7% (14/19) were predicted accurately on day 1 (D1), 76.5% (13/17) on D2. On D0, 61.9% (13/21) of peak FVIII:C levels predictions were accurate. In the Little DAVID study (n = 12), on D0 83.3% (5/6) FVIII:C peak levels for both study arms were predicted accurately. Combination treatment reduced pre-operative FVIII concentrate use by 47% versus FVIII monotherapy. Desmopressin side effects were mild and transient. Two bleeds occurred, both despite FVIII:C >1.00 IU/mL.

Conclusion
Peri-operative combination treatment with desmopressin and PK-guided FVIII concentrate dosing in non-severe hemophilia A is feasible, safe and reduces FVIII consumption.

Introduction

Haemophilia A is an inherited X-linked bleeding disorder characterized by a deficiency of factor VIII (FVIII)[1]. Non-severe hemophilia A patients (FVIII:C ≥ 0.01-0.40 IU/mL) mainly suffer from bleeding complications after trauma or surgery. In order to prevent bleeding peri-operatively, non-severe hemophilia A patients are treated with desmopressin or factor VIII (FVIII) concentrate. Desmopressin increases FVIII:C plasma levels by releasing von Willebrand factor (VWF) and FVIII from extrahepatic endothelial cells[2-5]. If FVIII:C response is sufficient, minor medical procedures can be performed with desmopressin only. However, the FVIII:C response to desmopressin varies strongly from patient to patient, and is often considered insufficient. In such cases, patients are treated with FVIII concentrate. Additionally, desmopressin's use is suboptimal in many patients who have an adequate FVIII:C response[6].

Both desmopressin and FVIII concentrates have certain drawbacks. In hemophilia A patients exposure to FVIII concentrate is associated with the risk of developing FVIII inhibitors, thereby increasing the risk of morbidity and mortality[7-9]. On the other hand, desmopressin is associated with vasoactive side effects. These are generally mild and transient, such as flushing. Rarely, severe side effects occur, such as hyponatremia, which is usually preventable by restriction of fluid intake[10]. Importantly, repeated administration of desmopressin over short periods of time (12 - 24 hours) leads to a reduced response (tachyphylaxis)[11].

Both desmopressin and FVIII concentrate are treatments with high interpatient variability in FVIII:C response[11,12]. Recent studies have shown that FVIII concentrate dosing based on body weight leads to postoperative FVIII:C trough levels above and below target ranges in a large proportion of patients[13,14]. This is clinically relevant as levels below targeted peak or trough level increase bleeding risk and levels above targeted peak levels might increase the risk of thrombosis[15-17]. Consequently, population pharmacokinetic (PK) models of both FVIII concentrate and desmopressin treatment have been developed to optimize dosing[12,18-20]. These models can be applied to personalize hemostatic treatment peri-operatively [20].

The 2020 World Federation of Hemophilia (WFH) guideline stated that the downsides associated with exclusive use of only desmopressin or FVIII concentrate can be overcome by combination treatment using both desmopressin and FVIII concentrate[21]. Since desmopressin is less expensive than FVIII concentrate, is available in many parts of the world, and is on the WHO's list of Essential Medicines, combination treatment may lead to considerable FVIII concentrate savings and is useful when FVIII concentrate resources are limited. However, no studies on personalized combination treatment have been performed. Therefore, we initiated two studies in non-severe hemophilia patients applying peri-operative desmopressin followed by PK-guided FVIII concentrate dosing to evaluate the feasibility, predictive performance and safety of this combination treatment.

Materials and methods

Study description and primary study endpoints
DAVID study
The DAVID study was designed as an observational multicenter single-arm study to assess the feasibility, safety and predictive performance of combination treatment peri-operatively in non-severe hemophilia A patients, focusing on major surgical procedures. The DAVID study protocol has been published before[22]. In short, combination treatment consisted of intravenous desmopressin (0.3 μg/kg body weight with no capped dose), immediately after full desmopressin administration and blood sampling followed by a PK-guided dose of FVIII concentrate pre-operatively (D0) and possibly post-operatively, with a maximum of three consecutive days combination treatment. If needed, patients were treated with FVIII monotherapy from day 3 onwards, with a possibility of combination treatment between day 6 to 8 as well. The use of peri-operative antifibrinolytics such as tranexamic acid was allowed. A general fluid restriction of 1.5 liters for 24 hours was applied after desmopressin administration. The primary study endpoint was the proportion of patients with measured FVIII:C levels within the physician's target FVIII:C trough range in the 72 hours of combination treatment, without the need for additional FVIII concentrate. To assess the effect of combination treatment on the FVIII concentrate consumption an hypothetical pre-operative dose of FVIII concentrate was calculated for each individual, assuming an increase of 0.02 IU/mL per IU of FVIII concentrate per kilogram body weight, as is used in standard care. An example of how combination treatment would be performed in the DAVID study is illustrated in Figure 1.

Little DAVID study
The Little DAVID study was designed as a randomized clinical trial to compare feasibility, predictive performance and safety of combination treatment with standard treatment in peri-operative non-severe hemophilia A patients undergoing minor medical procedures. Standard treatment with PK-guided FVIII concentrate (standard arm) was compared to combination treatment of intravenous desmopressin (0.3 ug/kg body weight with no capped dose), immediately after full desmopressin administration and blood sampling followed by a PK-guided FVIII concentrate (intervention arm). The Trans European Network for Clinical Trials Services (TENALEA), a web-based randomization system, was used to randomize patients (1:1), stratified according to center, severity of disease (mild or moderate), age (<18 years or ≥18 years) and bleeding risk of the procedure (low or medium bleeding risk, see Supplementary appendix 1). The use of peri-operative antifibrinolytics such as tranexamic acid was allowed. A general fluid restriction of 1.5 liters for 24 hours was applied after desmopressin administration. The primary endpoints were the accuracy of predicted FVIII:C (see Supplementary appendix 2) and FVIII concentrate consumption in U/kg.
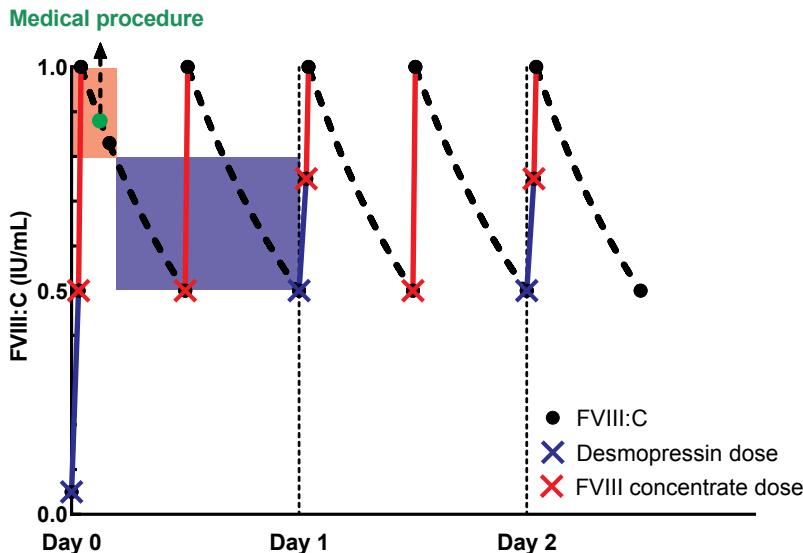
Figure 1: example of combination treatment in a DAVID study patient.

Secondary endpoints

Secondary endpoints for both studies were predictive performance of the PK-model by the Bayesian approach for measured FVIII:C (see below for definition), bleeding during D0-13 , other adverse events during D0-13, the need for off-protocol FVIII concentrate, patient reported experienced quality of care (hemophilia care and peri-operative care in general on a scale of 0 to 10), and inhibitor development.

Patient inclusion

Patients of 12 years and older with non-severe hemophilia A (FVIII:C 0.01 - 0.40 IU/mL) who were planned to undergo a (minor) medical procedure were included in either the DAVID or Little DAVID study, depending on the expected duration of treatment. All patients needing a procedure requiring ≥48 hours of FVIII concentrate administration were included in the DAVID study (major medical procedure). Patients who were expected to require <48 hours of FVIII concentrate administration were included in the Little DAVID study (minor medical procedure).

Patients were recruited from a Dutch hemophilia treatment center (Rotterdam, Groningen, Eindhoven, Nijmegen, Utrecht, Leiden, Amsterdam and Maastricht) for the DAVID study and five hemophilia treatment centers (Rotterdam, Nijmegen, Groningen, Maastricht) for the Little DAVID study.

Exclusion criteria were: not responsive to desmopressin (<0.2 IU/mL absolute FVIII:C increase one hour after desmopressin administration in the past), clinically significant FVIII inhibitors (>0.5 Bethesda units), contraindications for desmopressin or interacting co-medication (see Supplementary appendix 3 for the applied list of both), or intolerance to desmopressin (Figure 2). Both the DAVID and Little DAVID studies were approved by the local Medical Ethics Committee of the Erasmus University Medical Center Rotterdam (MEC-2015-751 and MEC-2016-726) and by the boards of all participating hospitals and were registered at the Netherlands Trial Register (www.trialregister.nl; NTR5383 and NTR6036). Patients were included from 27th February 2017 to 31st December 2020 for the DAVID study and from 27th January 2018 to 31st December 2020 for the Little DAVID study.

Study procedures and definitions
In the patients receiving combination treatment first desmopressin was administered in a dose of 0.3 µg/kg body weight intravenously followed by PK-guided FVIII concentrate administration. FVIII:C was measured before and after desmopressin and FVIII concentrate administrations, see "Sampling and assays" for more details. For predictive performance, a predicted FVIII:C was considered accurate if difference between measured and predicted FVIII:C was ≤0.2 IU/mL.

As combination treatment may be more demanding for patients, patient experiences with regard to perceived quality of care were studied using a questionnaire, rating experienced hemophilia care on a scale ranging from 0-10 (worst to best) (Supplementary appendix 4).

Side effects were studied using a previously developed questionnaire before and after combination treatment[10]. All patients were followed up for 90 days to assess the occurrence of inhibitors, bleeding or thromboembolic events according to protocol[22]. Procedures were classified in bleeding risk categories, i.e. low, intermediate and high, based on the ACCP guideline for antithrombotic therapy[23].

Pharmacokinetic-guided dosing of FVIII concentrate by Bayesian forecasting, targeting physician set FVIII:C range
Bayesian forecasting of FVIII:C after dosing desmopressin and FVIII concentrate was performed in NONMEM software (version 7.3, (ICON Development Solutions, Ellicott City, MD, United States). Population PK models were previously developed by our group and available for both desmopressin and FVIII concentrate[12,22]. For the used PK model, the PK profiles of FVIII:C after intravenous administration of desmopressin and FVIII concentrate were used in both studies. The PK model of desmopressin was used to calculate the clearance of the desmopressin induced FVIII:C response, which was taken into account for the PK-guided dose of FVIII-concentrate. If the FVIII:C response after previous FVIII concentrate

administration(s) was available, these responses were used to calculate individual PK parameters to obtain the pre-operative dose of FVIII concentrate. The individual PK parameters were iteratively updated based on measured FVIII:C and doses were adjusted accordingly. If FVIII:C response was unavailable, mean population PK parameters were used.

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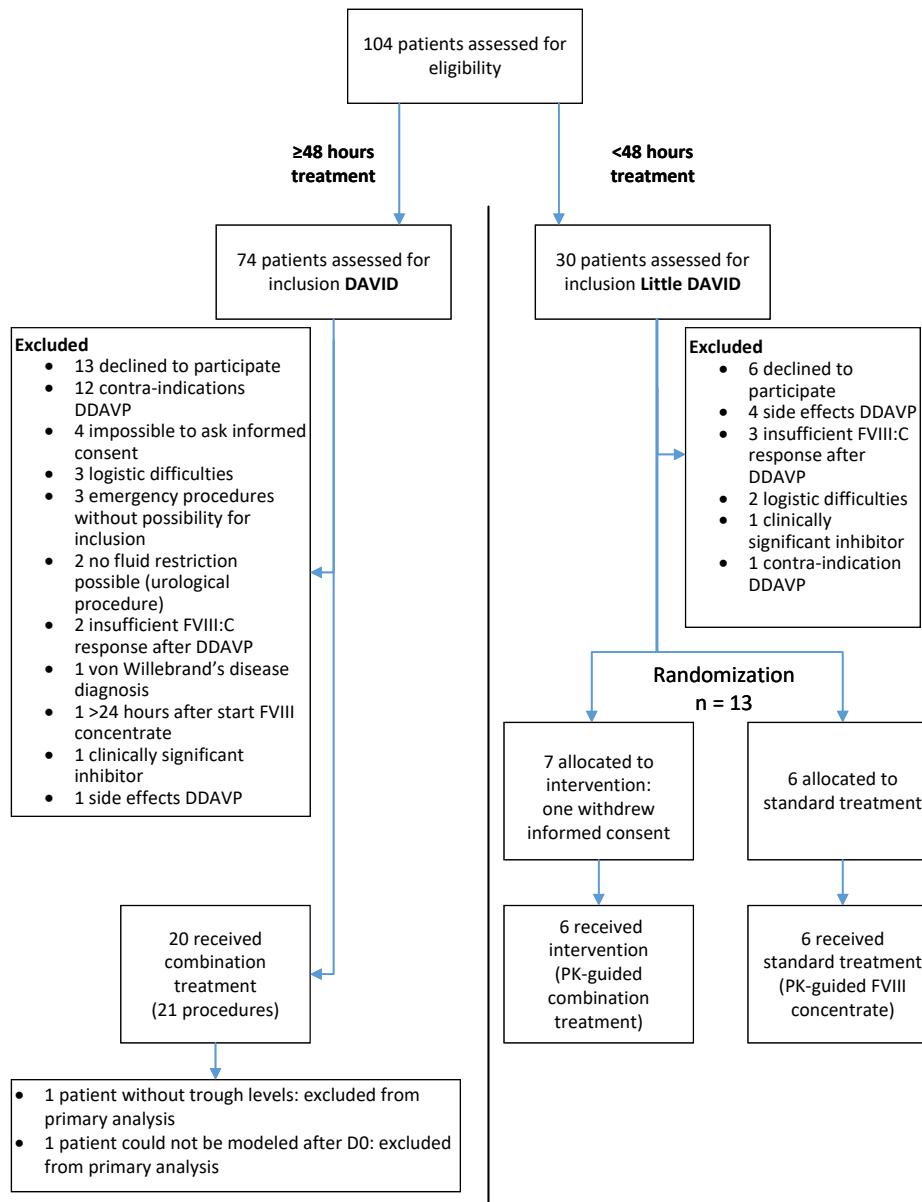
Figure 2: inclusions in DAVID and Little DAVID study.

For each included study participant, the treating physician was asked to specify the physician's desired pre-operative peak FVIII:C range or level on the day of surgery (day 0; D0), and the physician's desired postoperative target trough FVIII:C ranges or levels one day (day 1; D1) , two days (day 2; D2) and three days (day 3; D3) after surgery, if applicable. These targets were based on the national hemophilia treatment guideline, which is based on literature and the international (WFH) guideline[24]. The dose of FVIII concentrate (in IU) for D1 and D2 was calculated based on the PK model and the measured peri-operative FVIII:C on D0. With respect to anticipated desmopressin tachyphylaxis, the first five patients were modelled with 30% decrease in FVIII:C response, based on earlier studies[11]. Since the observed tachyphylaxis of these five patients was approximately 50%, an anticipated 50% decrease of FVIII:C response was used for the following patients for the second and (if applicable) third desmopressin administration.

Sampling and assays

To measure FVIII:C, blood was drawn before and fifteen minutes after every desmopressin infusion, after FVIII concentrate administration following desmopressin, and immediately after surgery. FVIII:C trough levels were also measured prior to desmopressin administration on D1 and D2 in the DAVID study. Sodium was measured before each desmopressin administration. FVIII:C were measured using a one-stage assay. FVIII inhibitor testing was performed levels according to the Nijmegen modification of the Bethesda assays.

Statistical analysis

Categorical and ordinal data are presented as frequencies and proportions. Categorical and ordinal data between multiple groups were compared using a Chi-squared (cell count >5) or Fisher exact test (cell count ≤5). Paired ordinal data (e.g. side effects before and after combination treatment) were compared using Wilcoxon signed rank test. Continuous data are presented as median and interquartile range. Continuous variables between 2 groups were compared by using Wilcoxon signed rank test with $\alpha = 0.05$ for statistical significance and Bonferroni correction for multiple testing. Continuous variables between 3 or more groups were compared by using a Friedman test. In order to assess the efficacy for the DAVID study of combination treatment in comparison to historical data[13] (proportion of 0.31 based on postoperative FVIII:C levels) with a power of 90% and alpha of 0.05, 25 procedures were needed. Non-inferiority of the accuracy of the predicted peak range between both Little DAVID study arms was assessed by studying the difference of the deviation per arm as defined and explained in Supplementary appendix 2. In order to assess non-inferiority with a power of 80% and alpha of 0.05, 68 procedures were needed. All statistical analyses were performed in IBM Statistics SPSS v25.

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Results

Patients and medical procedures
In total 32 patients underwent 33 medical procedures in the DAVID studies. Unfortunately, both studies were stopped before inclusion was complete. The main reasons for a lower inclusion rate that anticipated were that the COVID-19 pandemic resulted in a reduced number of procedures and that some patients preferred standard treatment over study participation.

DAVID study
Twenty-one procedures were performed in 20 hemophilia A patients, of whom 19 had mild and 1 moderate hemophilia A. Two patients had received desmopressin 36 to 48 hours before the procedure, which was taken into consideration with PK modeling. One patient was excluded at D1 because of logistic issues. For the analysis of the primary endpoint, two procedures were excluded due to the cessation of the study participation and additional factor concentrate treatment because of bleeding post-operatively. Four patients had received continuous factor VIII concentrate administration, of whom three also received a bolus loading dose of FVIII concentrate at D0.

Little DAVID study
Thirteen patients were included, six in the standard arm (FVIII concentrate only) and seven in the intervention arm (combination treatment). One patient (intervention arm) withdrew consent after randomization, did not receive study treatment and was not included in study analysis. One patient in the standard arm used off-protocol intranasal desmopressin the evening after the procedure and after peak FVIII:C levels were measured (D0). Inclusion for both studies is shown in Figure 2. Patient and procedure characteristics of both studies are described in Table 1.

Measured FVIII:C compared to physician's FVIII:C target range
DAVID study
Of the 19 procedures included in the primary endpoint analysis (FVIII:C levels within target in the first 72 hours), 31.5% (6/19) of all measured trough levels (D1, D2 and D3) per procedure were within or equal to the physician's target trough level. Of all measured trough levels after combination treatment, 42% (8/19), 47% (8/17) and 63% (5/8) were in target on D1, D2 and D3, respectively. Of the trough FVIII:C not in target on D1, 27% (3/11) were lower than physician's target with absolute deviations of 0.03 IU/mL, 0.05 IU/mL, 0.09 IU/mL. The other 73% (8/11) trough FVIII:C not in target on D1 were above the physician's target level but with a FVIII:C lower than 1.3 IU/mL and a maximum absolute deviation of 0.26 IU/mL. On D2 and D3 all trough FVIII:C not in target were above the physician's target level but with a FVIII:C lower than 1.15 IU/mL and a maximum absolute deviation of 0.34 IU/mL. On D0 all measured peak levels were within (12/21, 57%) or above (9/21, 43%) the physician's target FVIII:C.

Table 1: Patient and medical procedure characteristics of DAVID and Little DAVID study.

DAVID		Little DAVID	
Characteristic (n = 20)	Number (%) / median [IQR]	Number (%) / median [IQR]	
		Standard (n = 6)	Intervention (n = 6)
Hemophilia severity			
Mild	19 (95%)	5 (83.3%)	6 (100%)
Moderate	1 (5%)	1 (16.7%)	0
Lowest FVIII:C measured (IU/mL)	0.16 [0.08 - 0.21]	0.11 [0.08 – 0.14]	0.12 [0.06 – 0.19]
Age at procedure (years)	47 [38 - 59][a]	32 [23.3 – 54.8]	59.5 [46.3 – 63.5]
Weight at procedure (kg)	80 [76.35 - 93.1][a]	83 [72.5 – 95.1]	80 [70.9 – 95.0]
Time between desmopressin test and inclusion (years)	3 [0 - 11][a]	1 [0 - 10]	4.5 [1.5 -13.3]
Consecutive days of combination treatment			
One	2 (9.5%)	-	5 (83.3%)
Two	6 (28.6%)[a]	-	1 (16.7%)
Three	13 (61.9%)	-	-
Mode of FVIII concentrate administration			
Bolus	17 (81%)	6 (100%)	6 (100%)
Continuous	4 (19%)[a]	-	-
Type of medical procedure			
Orthopedic	6[a] (28.6%)	-	-
Oromaxillary/dental	6[a] (28.6%)	5 (83.3%)	2 (33.3%)
Urological	4 (19%)	-	-
Biopsy/excision	3 (14.3%)	1 (16.7%)	2 (33.3%)
Endoscopy	1 (4.8%)	-	1 (16.7%)
Lumbar puncture	-	-	1 (16.7%)
Laparoscopic colectomy	1 (4.8%)	-	-
Bleeding risk of procedure			
High	14 (66.7%)[a]	-	-
Intermediate	6 (28.6%)	2 (33.3%)	3 (50%)
Low	1 (4.8%)[a]	4 (66.7%)	3 (50%)

[a] One patient had undergone two procedures.

Little DAVID study

In the standard arm all (6/6) of the measured D0 peak levels were above the physician's target range. In the intervention arm using combination treatment, all measured D0 peak levels were within (2/6, 33%) or above (4/6, 66%) physician's target range.

The comparison between measured and physician's target FVIII:C is visualized in Figure 3 and Figure 4.
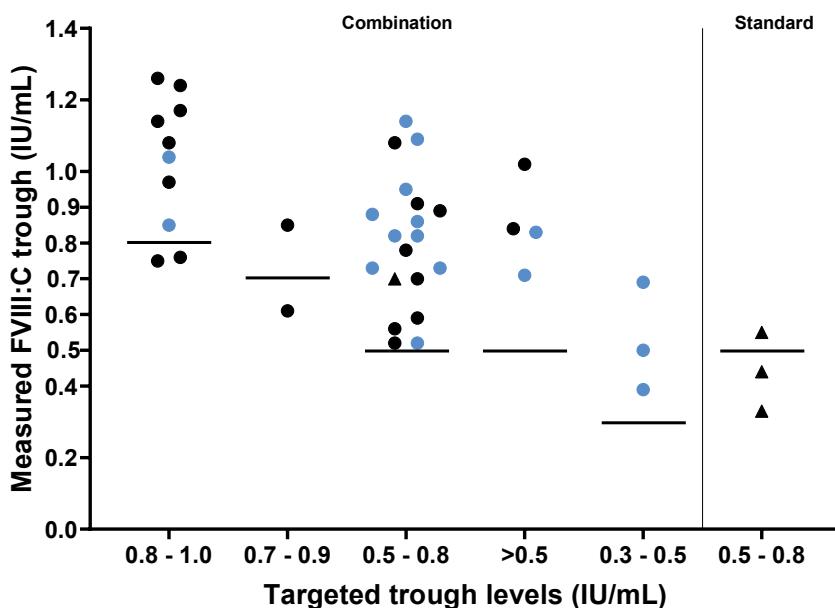
Figure 3: measured trough FVIII:C (IU/mL) in relation to physician's FVIII trough target range (IU/mL) after combination treatment (DAVID study, circles; Little DAVID study, triangles and standard treatment (Little DAVID, triangles) on D1 (black) and D2 (blue). The lower limit of FVIII target ranges is marked by a black line. For patients who received combination treatment, eight patients on D1 and two on D2 had a FVIII:C target trough range of 0.8–1.0 IU/mL, two patients on D1 a FVIII:C target trough range 0.7–0.9 IU/mL, eight patients on D1 and ten on D2 a FVIII:C target trough range of 0.5–0.8 IU/mL, two patients on D1 and two on D2 a FVIII:C target trough level > 0.5 IU/mL and three patients on D2 a FVIII:C target trough range of 0.3–0.5 IU/mL. For patients who received standard treatment, three patients on D1 had a FVIII:C target trough level between 0.5–0.8 IU/mL.

Predictive performance of the Bayesian approach of the PK-model

In addition to the aforementioned physician's target levels, we also assessed the accuracy of the PK model predictions, comparing predicted FVIII:C to measured FVIII:C.
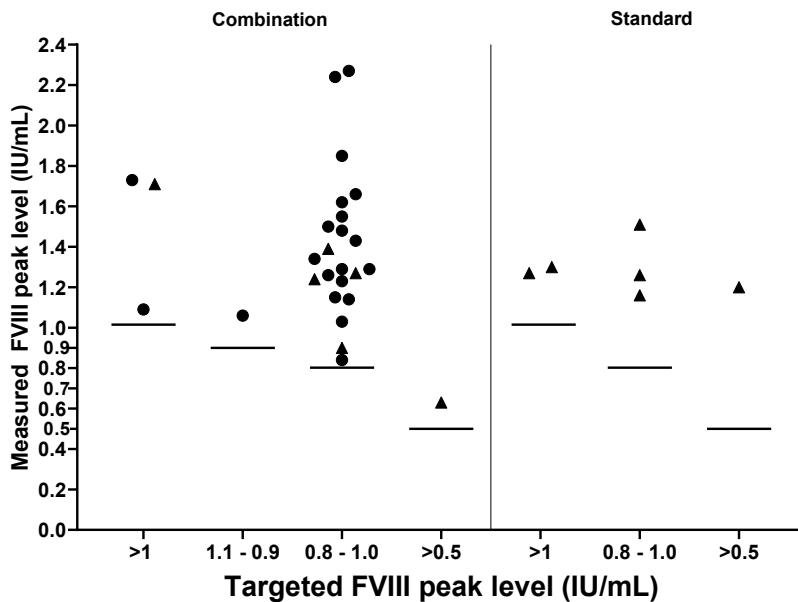
Figure 4: measured peak FVIII:C (IU/mL) in relation to the physician's target peak FVIII:C (IU/mL) at D0 of patients who received combination treatment (DAVID study (circles) and Little DAVID study (triangles)) and standard treatment (Little DAVID only, triangles). The black line signifies the lower limit of the physician's target range.

DAVID study

The Bayesian predictions were accurate for preoperative peak levels at D0 in 61.9% (13/21), accurate for trough levels at D1 in 73.7% (14/19) and at D2 in 76.5% (13/17). For ten procedures, FVIII:C levels after previous FVIII concentrate administration were available for calculation of the PK-guided FVIII concentrate dose. For these patients with previous FVIII:C pharmacokinetic data, 59.3% (16/27) levels were on target versus 76.7% (23/30) in patients without these data (n.s.).

Little DAVID study

The Bayesian predictions were accurate for preoperative peak levels at D0 in 83.3% (5/6) in the standard arm and in 83.3% (5/6) in the intervention arm. The two inaccurate predictions gave higher measured FVIII:C levels. Due to the low number of included patients, it was not possible to test for non-inferiority (Supplementary appendix 2). Figure 5 and Figure 6 show model accuracy for both studies concerning preoperative peak and postoperative trough levels after combination treatment or standard treatment with FVIII concentrate.
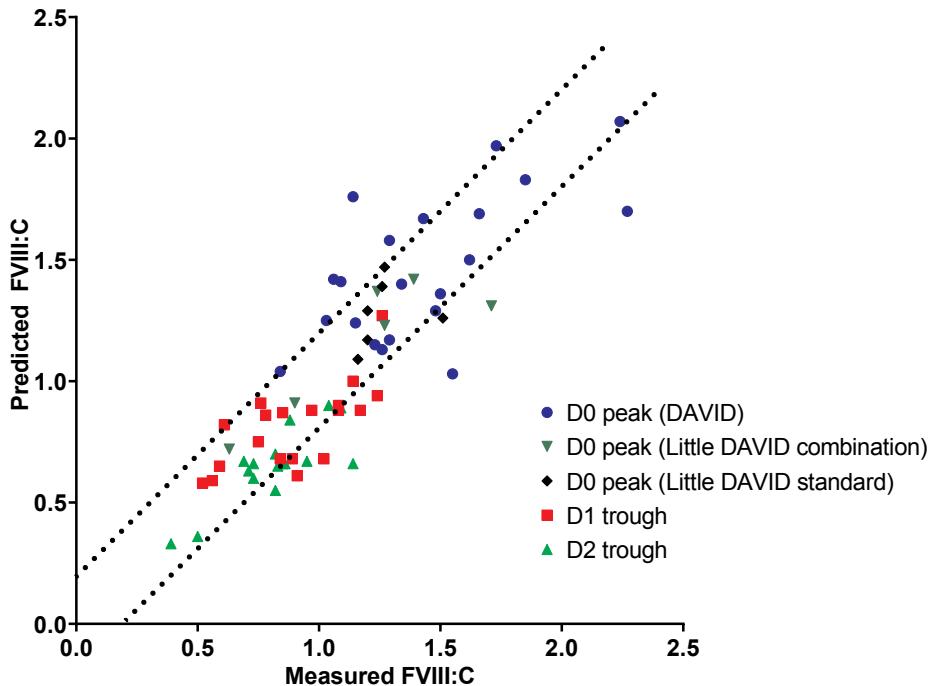
Figure 5: comparison of measured FVIII:C (IU/mL) and predicted peak and trough FVIII:C (IU/mL) in all patients (DAVID and Little DAVID study) with combination treatment or standard treatment. Dotted lines signify ±0.2 IU/mL. One patient received only desmopressin before the procedure.

Factor VIII concentrate consumption

DAVID study

After administration of desmopressin a median dose of 2000 IU FVIII concentrate (IQR 1500 – 3125 IU) was infused to reach the target FVIII:C on D0. This is significantly less (39%; p < 0.001) than the calculated median FVIII concentrate dose of 3250 IU (IQR 3250 - 4000) which would have been given as bolus infusion with FVIII concentrate based on body weight. Because of the achieved high desmopressin response, one patient only received desmopressin pre-operatively without the need of additional FVIII concentrate.

Little DAVID study

In the standard arm median PK-guided pre-operative dose of FVIII concentrate on D0 was 3750 IU (IQR 3500 – 4000 IU; 44.23 IU/kg ; IQR 37.2 - 53.3 IU/kg) versus 1750 IU (IQR 1500 – 2500 IU; 21.46 IU/kg; IQR 18.75 - 27.3 IU/kg) following desmopressin administration in the intervention arm resulting in a significant reduction of FVIII concentrate consumption (47%; p = 0.009).
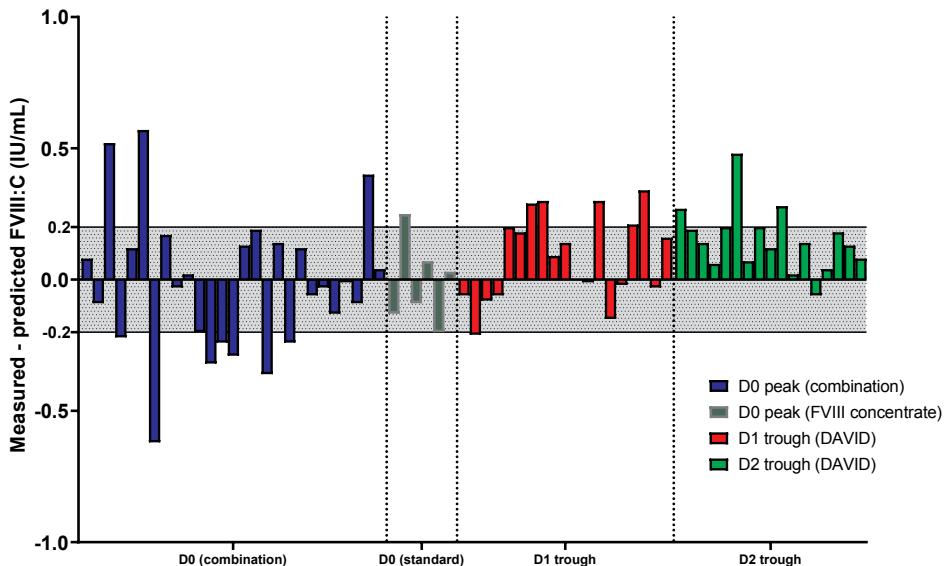
Figure 6: comparison of the absolute difference (delta) of measured FVIII:C (IU/mL) and predicted peak and trough FVIII:C (IU/mL) in patients who received combination treatment (DAVID study and Little DAVID study, *n* = 27) or standard treatment (Little DAVID study, *n* = 6). Each box represents one patient. The grey-arced background signifies ±.2 IU/mL. One patient only received desmopressin at D0.

(Serious) adverse events

In total, three bleeding events occurred in both studies. In the DAVID study, two patients suffered a bleeding event. Both patients had a high (>1.00 IU/mL) FVIII:C at the time of bleeding. In the Little DAVID study, one patient in the standard arm suffered a bleeding event six days after dental procedure. Details of these patients are given in Supplementary appendix 5.

In total, testing for inhibitor formation was performed within 3 months after treatment in 26/32 (81%) of the procedures. One patient included in the DAVID study developed an inhibitor against FVIII (6.8 Bethesda units). This changed his phenotype from mild to severe (<0.01 IU/mL FVIII:C). The *F8* mutation of this patient was c.6956C>T p. Pro2319Leu on exon 26 (C2 domain), is known to be associated with an increased risk for inhibitor formation[25]. The procedure performed was the resection of a neck cyst without any postoperative complications. In addition, in three patients inhibitor testing occurred later than three months and no inhibitor was found. No thromboembolic events were reported.

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Side effects

For patients with combination treatment, the median sodium level was 141 mmol/l (IQR 140-142) on D0 (n = 25), 139 mmol/l (IQR 137-141) on D1 (n = 19) and 140 mmol/l (IQR 136-141) on D2 (n = 16; p = 0.037). Three patients had mild asymptomatic hyponatremia on day 2, of whom two a sodium level of 133 mmol/l and one 131 mmol/l, none had symptomatic hyponatremia. Flushing was reported by 76% of patients (n = 21) after desmopressin and by none (n = 6) after treatment with FVIII concentrate only. All reported symptoms were mild and transient. No significant difference in side effects was found in the Little DAVID between the standard and intervention arm.

Experienced quality of care of combination treatment

DAVID study

14 patients rated the experienced combination treatment with a median score of 10 [IQR 8.9-10]. Six of these patients previously underwent a surgical procedure with standard FVIII concentrate treatment, of whom four preferred combination treatment above standard treatment. One patient preferred standard treatment above combination treatment because of the side effects of desmopressin and one patient had no preference.

Little DAVID study

Six patients in the standard arm and five in the combination treatment arm rated the procedure with a median score of 9.5 [IQR 8-10] in the standard arm and median score of 9 [IQR 8.3-10] in the combination treatment arm (n.s.). For the experienced care in general, six in the standard arm scored 9.4 [IQR 8-10] and five in the combination arm 9 [IQR 8.5-10] (n.s.).

Discussion

The DAVID and Little DAVID studies are the first studies on the peri-operative use of combination treatment of desmopressin immediately followed by FVIII concentrate in non-severe hemophilia A patients. Combination treatment turned out to be feasible and safe, with mild and transient side effects of desmopressin and resulted in a reduction of FVIII concentrate in comparison with PK-guided FVIII concentrate monotherapy. By using a PK-guided approach for both desmopressin administration and FVIII concentrate we were able to personalize treatment with a high predictive performance of the model.

In a previous retrospective study in non-severe hemophilia A patients treated with FVIII concentrate we have shown that only 12% of peri-operative measurements of FVIII:C levels were within the physician's target range[13]. In the DAVID study, combination treatment resulted in a higher proportion of FVIII:C levels (31.5%) in the targeted range. In both DAVID studies, almost half of the peak (D0) and trough (D1, D2, D3) FVIII:C measurements were above the targeted level (48%) and only 10% of all trough FVIII:C measurements were below the targeted level with a limited absolute deviation (<0.10 IU/mL).

In our two studies FVIII concentrate savings were evident using combination treatment due to the achieved increase in FVIII:C after desmopressin, thereby reducing the required dose of FVIII concentrate compared to monotherapy FVIII to reach target FVIII levels. Another possible FVIII concentrate saving strategy may be PK-guided dosing instead of body weight dosing. This was studied by our group in a recent randomized study (OPTI-CLOT trial) on peri-operative management of patients with moderate and severe hemophilia A. The study showed that FVIII concentrate consumption was comparable between both arms[20]. This suggests that the savings in FVIII concentrate using combination treatment with desmopressin followed by PK-guided FVIII concentrate in our studies is mainly due to the use of desmopressin, rather than PK-guidance.

Implementation of combination treatment can be facilitated for patients and clinicians by administering desmopressin subcutaneously instead of intravenously and by limiting the number of measurements to only a peak FVIII:C after the administration of combination treatment. In practice, the most savings are expected for the pre-operative FVIII dose, as illustrated by the Little DAVID study.

The Bayesian predictions for FVIII:C by the population PK model after combination treatment were accurate in the majority of cases (75%) for D1 and D2 trough levels in the DAVID study and in 83.3% of the peak levels in the Little DAVID study. We hypothesized that available PK data of previous FVIII concentrate administration in an individual

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patient could influence the accuracy of the predicted FVIII:C levels. As only 50% of included patients had prior measurements of FVIII:C levels after administrations of FVIII concentrate, statistical power was lacking to ascertain the effects of PK data on FVIII concentrate administrations on model predictions. Also, concerning PK model predictions for D0 peak levels, multiple factors influence prediction accuracy. The majority of patients was dosed using bolus administration. As a result, in case of treatment with bolus twice daily a higher than the physician's requested target peak FVIII:C level was necessary in order to achieve an adequate trough FVIII:C. This explains the difference in the accuracy of the predictive performance of this treatment strategy compared to the proportion of patients with FVIII levels within the physician's target range. Therefore, in case of body weight-dosing, a more on target peak level could have been more difficult to achieve. Moreover, previous studies on peri-operative PK-guided FVIII concentrate treatment showed that surgery and increased von Willebrand factor (VWF) levels were associated with a decreased postoperative FVIII clearance[19]. In contrast, a trend towards a small increase of FVIII clearance (p = 0.07) was found in four severe hemophilia A patients who received desmopressin followed by a bolus of FVIII concentrate[26]. In non-severe hemophilia patients, as in the present DAVID studies, the release of endogenous FVIII and VWF by surgery - a major physical stress factor – may influence levels post-surgery the most.

Side effects of combination treatment, associated with the use of desmopressin, were mild and transient. Stoof et al. reported earlier on side effects after desmopressin, where flushing was also observed after desmopressin administration[10]. Additionally, 5% (4/108) of the patients had a mild hyponatremia 24 hours after one dose, whereas in our study mild hyponatremia only occurred after multiple doses and was asymptomatic.

The experienced quality of care of combination treatment was very rated high, even up to the maximum score, despite the additional time and blood draws in our study in comparison to standard treatment. In comparison, other studies have reported a moderate to high treatment satisfaction in hemophilia A patients with FVIII concentrate prophylaxis and/or treatment[27,28].

Our studies had some limitations. The most important limitation is that we did not reach our desired number of included patients, since inclusion was hampered by patients preferring standard treatment and the COVID-19 pandemic, as less elective procedures were performed. As a result, the assessment of non-inferiority of PK-guided combination treatment versus PK-guided standard FVIII concentrate treatment in the Little DAVID study was inconclusive. In addition, as the DAVID trial was not a randomized clinical trial, we could not assess whether PK-guided dosing or combination treatment lead to more

accurate physician's target FVIII:C levels than standard treatment. Strengths of our study were the safety assessment and the savings achieved for the pre-operative FVIII dose, regardless of the procedure or base FVIII:C. Furthermore, the feasibility of our study was also shown by the accuracy of combination treatment, despite the heterogeneity of our study population, reflecting the daily practice of hemophilia care.

Conclusion

Peri-operative PK guided combination treatment of desmopressin and FVIII concentrate in non-severe hemophilia A is feasible and safe. The majority of the predicted FVIII:C trough levels for combination treatment were accurate. This novel approach may result in considerable FVIII concentrate savings in non-severe hemophilia patients undergoing medical procedures.

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Supplementary information

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Supplementary appendix 1: Risk classification of procedures for the DAVID and Little DAVID study[1].

High bleeding risk	Intermediate bleeding risk	Low bleeding risk
Thorax surgery • Lung surgery • Mediastinoscopy • Cardiac surgery	Pulmonology/cardiology • Bronchoscopy with biopsy	Gastroenterology and hepatology • Diagnostic endoscopy (gastroscopy, colonoscopy, including biopsies) • ERCP with endoprosthesis without papillotomy • Video capsul endoscopy • Stent placement (without dilatation)
	ENT/dental surgery • Oral surgery • Osteotomies • Septum correction • (Adeno) tonsillectomy • Dental extractions - 3 or more	Cardiology • Pacemaker/ICD
ENT/dental surgery • Orbita/ear surgery • Jaw reconstruction	Ophtalmology • Retinal surgery	Dental surgery • Dental extractions – less than 3
Neurosurgery • Intracranial surgery • Open vertebra surgery	Orthopedics • Knee surgery/TKP • Shoulder surgery	
Orthopedics • Open vertebra surgery • Hip surgery / THP	Surgery • Open cholecystectomy • Adrenalectomy • Mamma amputation • Onco/trauma amputation • Laparoscopic surgery	
Surgery • Pelvic surgery • Hip/femur surgery • Vascular surgery • Kidney transplantation • Neck surgery • Open resections of: esophagus/stomach/ intestines/liver/pancreas/ spleen	Plastic surgery • All major reconstructions • Vascular malformations	

2

Supplementary appendix 1: Continued

High bleeding risk	Intermediate bleeding risk	Low bleeding risk

Urology
- Open nefrectomy
- Bladder surgery
- Prostatectomy
- Percutaneous stone surgery

Internal medicine/
Gastroenterology and hepatology
- Liver and kidney biopsy
- Polypectomy
- Papillotomy (biliary or pancreas)
- Dilatation
- PEG placement
- Endo-ultrasonography
- Endoscopic coagulation
- Ablation techniques
- Rubber band ligation with esophageal varices and hemorrhoids

Neurology
- Lumbar puncture

Anaesthesia
- Epidural (guideline neuraxial anesthesia: http://richtlijnendatabase.nl/richtlijn/neuraxisblokkade_en_antistolling/samenvatting_stollingssysteem.html)

Addendum:

Several procedures are not mentioned in this table. After consultation with the concerning specialist, they are classified as followed:
- Dermatologic procedure: low bleeding risk.
- Cataract surgery: low bleeding risk.
- Treatment at the dental hygienist: intermediate bleeding risk.

Supplementary appendix 2: definition of primary outcome measurement "accuracy of treatment" and calculation of the 90% confidence interval for non-inferiority analysis in the Little DAVID study.

Accuracy of the treatment was defined as the non-inferiority of the absolute deviation of the measured pre-operative (D0) peak FVIII:C versus the predicted pre-operative (D0) peak FVIII:C by the PK-model in the intervention arm versus the standard arm. The deviation was defined as 0 (non-deviant) if the measured FVIII:C is within +/- 0.10 IU/mL of the predicted peak FVIII:C by the PK-model. If outside this range, the absolute difference between measured FVIII:C and the closest range parameter was determined and applied (e.g predicted range 0.9 - 1.1 IU/mL, measured FVIII:C 1.15 IU/mL, then the deviation would be 0.05 IU/mL). The used non-inferiority margin was set at 0.05 IU/mL. This inferiority margin is based on earlier research: unpublished, historical data had shown a mean deviation of 0.22 IU/mL with a standard deviation of 0.21 IU/mL. We expected a reduction of deviation by approximately a half by using PK-guidance, namely a mean deviation of PK-guided treatment of 0.10 IU/mL and standard deviation of 0.10 IU/mL, where we consider half of the aforementioned standard deviation as a statistically and clinically relevant margin.

The following formula was used to construct the 90% confidence interval:

$$(\bar{x}_1 - \bar{x}_2) \pm t_{0.05} \sqrt{s_p^2 \left(\frac{1}{n_1} + \frac{1}{n_2}\right)}$$

where

$$s_p^2 = \frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2}$$

and $\bar{x}_1$ and $\bar{x}_2$ equal the mean of the calculated deviation in the intervention arm and in the standard arm respectively, n_1 and n_2 equal the sample size of each arm and s_p the pooled standard deviation. This pooled standard deviation is calculated using s_1 and s_2, equal to the standard deviation of the calculated deviation of each arm. The degrees of freedom are equal to $n_1 + n_2 - 2$.

For the primary endpoint analysis in the Little DAVID study, considering the non-inferiority limit of 0.05, the constructed 90% confidence interval was [-0.07, 0.14] with a mean difference in deviation of the intervention arm minus the standard arm of 0.03

IU/mL. A mean deviation from the predicted range of 0.045 IU/mL (standard deviation 0.126 IU/mL) was found in the intervention treatment arm and a mean deviation from the predicted range of 0.083 IU/mL (standard deviation 0.082 IU/mL) was found in the standard treatment arm. As the upper limit of the 90% confidence interval is above the prespecified non-inferiority margin but the lower limit is smaller than zero, the non-inferiority analysis is inconclusive.

Supplementary appendix 3: list of contraindications and co-medications for study exclusion.

Contraindications:
- Conditions which requires diuretics
- (Predisposition to) hyponatremia
- Cardiovascular disease
- Von Willebrand disease type 2B
- Renal insufficiency with a creatinine clearance < 50 mL/min
- Thrombotic Thrombocytic Purpura
- Eye injuries or surgery
- Cerebral contusion and/or increased intracranial pressure

Interacting comedication:
- Indomethacin and other NSAIDs
- SSRIs
- Chloridpromazine
- Carbamezapine
- Tricyclic antidepressants
- Loperamide

Supplementary appendix 4: questions on experienced hemophilia care in the DAVID and Little DAVID study.

DAVID study

We would like to know how satisfied you are about the peri-operative hemophilia care. The measuring scale ranges from 0 to 100. 100 means very satisfied, 0 means very unsatisfied. Mark with a vertical line on the scale how satisfied you are and note the number on the line in the box below

Could you mark on a scale from 0 to 100 how satisfied you are about the peri-operative hemophilia care?

Example of the line and box in the DAVID study:

0	100
zeer ontevreden	zeer tevreden

Uw cijfer (0-100) [box]
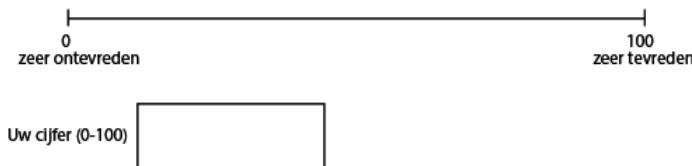
Little DAVID study
What grade do you give the hemophilia care (0-10)?

Supplementary appendix 5: details on bleeding in the DAVID study and Little DAVID study.
DAVID study
The first patient had undergone a total hip replacement, after which on D1 a local bleed was diagnosed (FVIII:C 1.12 IU/mL). Study participation was stopped and treatment with FVIII concentrate monotherapy was continued. The second patient underwent a robot-assisted laparoscopic prostatectomy, after which on D2 a postoperative bleeding occurred (FVIII:C: 0.95 IU/mL). He was already at the last day of combination treatment, and treatment with FVIII concentrate was continued. One patient underwent a partial circumcision and phrenuloplasty and a minor bleeding of the phrenulum occurred six days and ten days after the procedure. Bleeding resolved after a single administration of FVIII concentrate on both days. FVIII:C was measured on the day of the first bleed, once before and immediately after the FVIII concentrate administration on with a trough level of 0.14 IU/mL and peak level of 1.04 IU/mL.

Little DAVID study
The patient did not use the prescribed tranexamic acid after a dental extraction. Bleeding stopped after a single administration of FVIII concentrate. The measured trough FVIII:C on D1 was 0.44 IU/mL, whereas the physician's target trough FVIII:C was 0.5 IU/mL. In the intervention arm, one procedure-related event occurred. A molar extraction was complicated by a tuber fracture, for which FVIII concentrate was continued for five days. No bleeding occurred.

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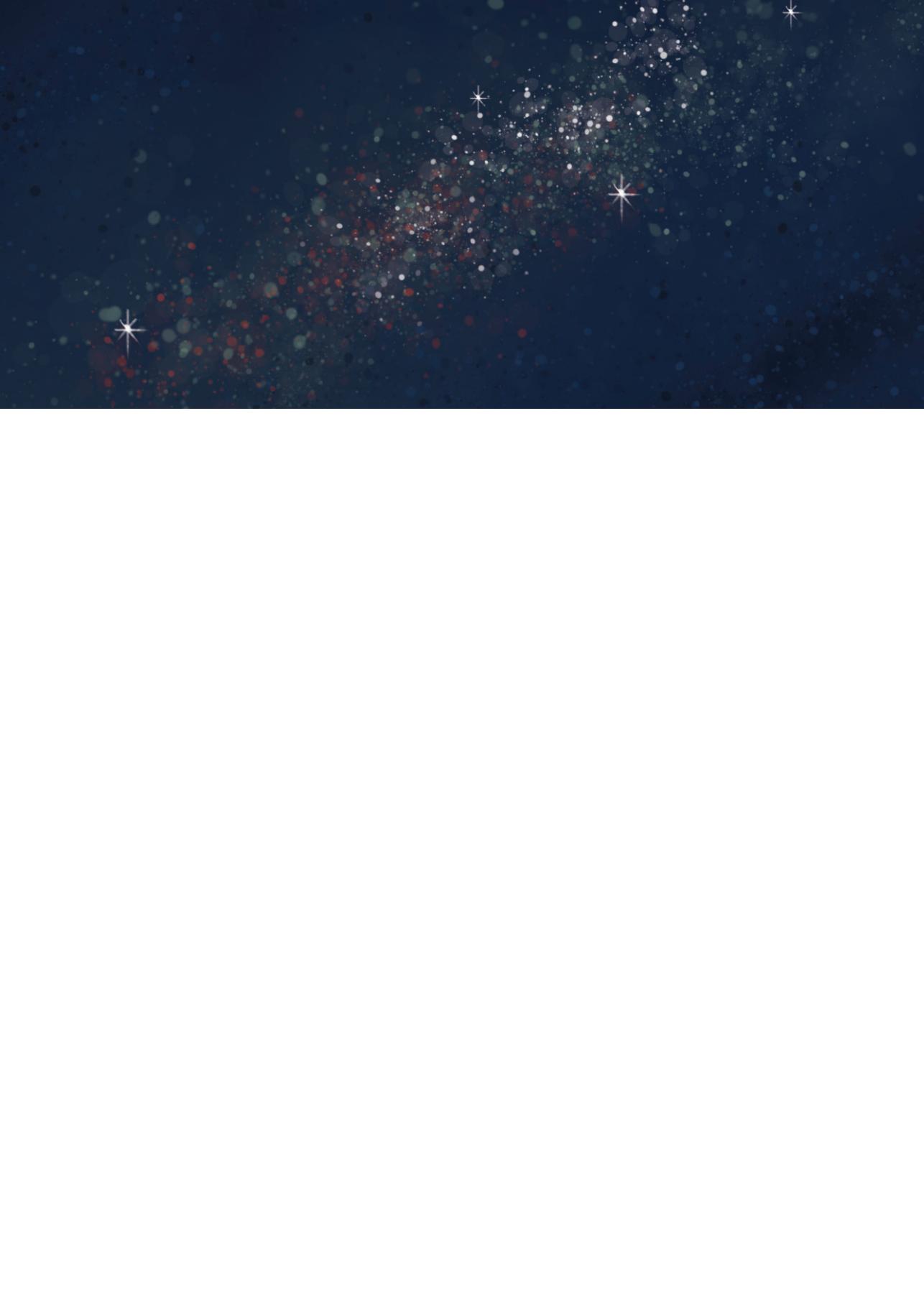
CHAPTER 3

TACHYPHYLAXIS AND REPRODUCIBILITY OF DESMOPRESSIN RESPONSE IN PERI-OPERATIVE NON-SEVERE HEMOPHILIA A PATIENTS: IMPLICATIONS FOR CLINICAL PRACTICE

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Abstract

Background
Desmopressin is frequently used peri-operatively in patients with non-severe hemophilia A. However, FVIII:C increase after desmopressin is interindividually highly variable. Tachyphylaxis has only been reported in test setting for hemophilia A patients, with a remaining response of approximately 70% after a second dose compared to a first dose.

Objectives
To study tachyphylaxis of FVIII:C response after multiple administration(s) of desmopressin in peri-operative non-severe hemophilia A patients.

Methods
We studied FVIII:C levels after desmopressin before (D0) and on day 1 (D1) and 2 (D2) after surgery in 26 patients of the DAVID and Little DAVID study. We studied tachyphylaxis by comparing the response at D1 and D2 with D0. We also assessed the reproducibility of the D0 response in comparison to an earlier performed desmopressin test.

Results
The median absolute FVIII:C increase was 0.50 IU/mL (0.35-0.74; n = 23) at D0, 0.21 IU/mL (0.14-0.28; n = 17) at D1 and 0.23 IU/mL (0.16-0.30; n = 11) at D2. The median percentage of FVIII increase after the second administration (D1) compared to the first (D0) was 42.9% (29.2%-52.5%; n = 17) and of the third (D2) compared to the first (D0) 36.4% (23.7%-46.9%; n = 11). The FVIII:C desmopressin response at D0 was comparable to the desmopressin test response in 74% of the patients.

Conclusion
Tachyphylaxis in the surgical setting was considerably more pronounced than previously reported with FVIII:C at D1 and D2 of 36-43% of the initial response. Our results may have important implications for monitoring repeated desmopressin treatment, when used peri-operatively.

Introduction

Hemophilia A is an inherited X-linked bleeding disorder characterized by a deficiency of factor VIII (FVIII)[1]. In order to prevent bleeding during and after surgery or trauma, most non-severe hemophilia A patients can be treated with desmopressin. Desmopressin increases FVIII:C plasma levels by releasing endogenous von Willebrand factor (VWF) and FVIII from extrahepatic endothelial cells[2-5]. The desmopressin response is highly variable interindividually and is partially determined by baseline FVIII:C and *F8* mutations[6,7].

Repeated administration of desmopressin over a short period of time is characterized by tachyphylaxis: a decrease of released FVIII after repeated administration. The interindividual variation and the occurrence of tachyphylaxis has led the World Federation of Hemophilia to recommend to perform a desmopressin test before clinical use of desmopressin and a treatment schedule for desmopressin up to two daily doses for a maximum of three consecutive days[8].

Tachyphylaxis was previously studied in 22 mild hemophilia A patients receiving once-a-day, intravenous desmopressin (0.3 micrograms/kg) for four consecutive days. Compared to the response on day 1, the FVIII increase after the second administration (day 2) was 70%, with a similar response for day 3 and 4[9]. In two previous studies in respectively 10 and 14 mild hemophilia A patients, the reproducibility of desmopressin FVIII:C response (intra-individual variation) was assessed[10,11]. A desmopressin response was defined as reproducible in these studies if the absolute deviation between this desmopressin response and an earlier desmopressin response was less than 20% in the first or less than 25% in the second study. The reproducibility between two desmopressin administrations for these patients, was 42% in the first and 70% in the second study[10,11].

Because of the widespread use of desmopressin, more data on the intra-patient reproducibility of the desmopressin response are needed. Furthermore, data on tachyphylaxis in non-severe hemophilia A patients undergoing a medical procedure and subsequent clinical implications have not yet been reported.

Therefore, we assessed tachyphylaxis and reproducibility of FVIII:C response after desmopressin in peri-operative non-severe hemophilia A patients who underwent a medical or surgical procedure requiring repeated daily desmopressin administrations in combination with additional pharmacokinetic-guided factor VIII concentrate (combination treatment).

Methods

This study is a substudy of the D**DAV**P treatment comb**I**ne**D** with FVIII clotting factor concentrates in patients with non-severe hemophilia A (DAVID) and Little DAVID studies, which included adult non-severe hemophilia A patients of Dutch hemophilia treatment centers who received a maximum of three consecutive days peri-operative combination treatment of both intravenous desmopressin once-a-day (0.3 micrograms/kg) followed by pharmacokinetic-guided FVIII concentrate to reach the target FVIII level (combination treatment)[12]. Patients were only included if their peak FVIII:C response was ≥0.20 IU/mL. The DAVID study was designed as an observational, multicenter single-arm study to assess feasibility, safety and predictive performance of combination treatment peri-operatively in non-severe hemophilia A patients. The Little DAVID study was a randomized clinical trial to compare feasibility, predictive performance and safety of combination treatment (intervention arm) with standard treatment of only PK-guided FVIII concentrate (standard arm). Data on hemophilia severity, consecutive days of desmopressin administration, lowest historical FVIII:C, most recent FVIII:C baseline before participation and most recent von Willebrand factor antigen (VWF:Ag) baseline were collected from patients' electronic medical records. FVIII:C was measured before (trough) and 15 minutes after (peak) each desmopressin administration on day 0 (D0, day of surgery) up to day 2 (D2). Medical ethical approval was obtained from the Medical Ethics Committee (MEC-2015-751 and MEC-2016-726) and performance of the studies was approved by the boards of all participating hospitals. Categorical and ordinal data are presented as frequencies and proportions or percentages. Continuous data are presented as median and interquartile range. Differences between two groups with continuous data were analyzed using a Wilcoxon signed-rank test. All FVIII:C levels were measured using a one-stage assay.

Definitions

Tachyphylaxis was calculated as a percentage, calculating the ratio of the absolute FVIII:C increases of two treatment days (i.e. day 1 [$FVIII:C_{after}$ - $FVIII:C_{before}$]) / day 0 [$FVIII:C_{after}$ - $FVIII:C_{before}$] * 100%).

The D0 desmopressin FVIII:C response was considered reproducible if the absolute D0 FVIII:C response deviated less than 25% from the previously performed desmopressin test absolute peak response (i.e. if 75% < day 0 [$FVIII:C_{after}$ - $FVIII:C_{before}$]) / desmopressin test [$FVIII:C_{after}$ - $FVIII:C_{before}$] *100% < 125%, day 0 response is reproducible). If this aforementioned deviation was more than or equal to 25%, the D0 desmopressin FVIII:C response was not considered reproducibile.

The similarity of the desmopressin test response related to D0 was calculated as a relative percentage by dividing the absolute desmopressin test response by the absolute D0 FVIII:C response (i.e. desmopressin test [$FVIII:C_{t=45\ min}$ - $FVIII:C_{t=0\ min}$] / day 0 [$FVIII:C_{t=45\ min}$ - $FVIII:C_{t=0\ min}$] * 100%).

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Results and discussion

In total 26 patients participated in this substudy. Twenty-one procedures in 20 patients with combination treatment were performed in the DAVID study and 6 procedures in 6 patients in the Little DAVID study. In the patients included for tachyphylaxis analyses, no bleeding complications had occurred. Two patients were excluded from all analyses (tachyphylaxis, reproducibility and similarity) because they had received a desmopressin dose <48 hours before the first pre-operative dose. Only the treatment data of the first procedure of the patient who had undergone two procedures in the DAVID study were included. Of the remaining 24 patients, seven patients hadreceived one dose of desmopressin. Therefore, 17 hemophilia A patients were included for the assessment of tachyphylaxis (one moderate and 16 mild). Of the aforementioned remaining 24 patients, one had no available post-desmopressin FVIII:C measurement. Therefore, twenty-three hemophilia A patients were included for the assessment of desmopressin response reproducibility and similarity (two moderate and 21 mild). In two of these 23 patients, a bleeding event occurred on the first day of combination treatment with a high FVIII:C >1.00 IU/mL at the time of the bleed; these two mild hemophilia A patients were withdrawn from further study participation. Of these two patients, one had an absolute FVIII:C increase of 0.39 IU/mL after desmopressin, the other 0.79 IU/mL, comparable to the other included hemophilia A patients. Patient characteristics for both groups are shown in the Table. A flowchart of the substudy inclusion is shown in Figure 1.

The FVIII:C increased from a median of 0.20 IU/mL (interquartile range 0.12-0.32) to 0.72 IU/mL (0.45-1.00; n = 23) after desmopressin administration, on D1 from 0.89 IU/mL (0.73-1.11) to 1.12 IU/mL (0.86-1.31; n = 17) and on D2 from 0.85 IU/mL (0.77-1.00) to 1.12 IU/mL (0.89-1.26; n = 11). As FVIII concentrate had been administered before baseline measurements on D1 and D2, the baseline FVIII:C levels are higher than possibly expected. The median absolute FVIII:C increase was 0.50 IU/mL (0.35-0.74; n = 23) on D0, 0.21 IU/mL (0.14-0.28; n = 17) on D1 and 0.23 IU/mL (0.16-0.30; n = 11) on D2 (Figure 2). The median percentage of FVIII increase after the second administration (D1) compared to the first (D0) was 42.9% (29.2%-52.5%; n = 17) and of the third (D2) compared to the first (D0) 36.4% (23.7%-46.9%; n = 11). The median percentage FVIII increase after desmopressin on the third day (D2) compared to the second day (D1) was 95.7% (71.4%-111.1%; n = 11).

The FVIII:C desmopressin response at D0 was reproducible to the desmopressin test FVIII:C response in 74% of the patients (Figure 3). The median similarity of the desmopressin test compared to FVIII:C response preoperative on D0 (n = 23) was 95.5% (82.1%-117.2%). In the one moderate hemophilia A patient included in the analysis of tachyphylaxis, the absolute FVIII:C increase after desmopressin on D0 was 0.35 IU/mL, on D1 0.15 IU/mL and on D2 0.16 IU/mL, comparable to the mild hemophilia A patients.
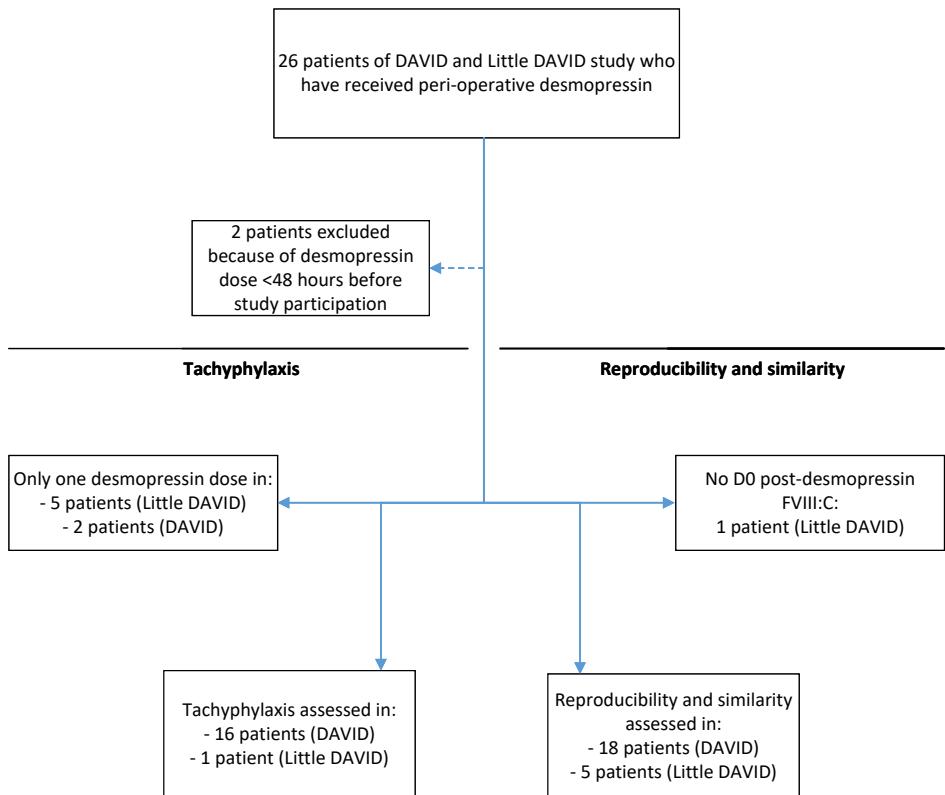
Figure 1: flowchart of the substudy inclusion for the analyses of tachyphylaxis, reproducibility and similarity in the DAVID and Little DAVID study.

Our study is the first to report on tachyphylaxis of multiple consecutive administrations of desmopressin in the peri-operative setting in non-severe hemophilia A patients. We showed that the FVIII:C increase after a second administration of desmopressin is only 43% of that obtained by the first administration and even lower (36%) after the third administration. In this substudy, patients also had received FVIII concentrate before the trough measurements on D1 and D2. As a consequence, these measured trough levels are higher than possibly expected.

Table: Patient characteristics of included patients.

Characteristics of patients in whom tachyphylaxis was analyzed (*n* = 17)	
Characteristic	***n* (%) or median [interquartile range]**
Hemophilia severity	
Moderate	1 (6%)
Mild	16 (94%)
Age (years)	47 [35 - 59]
Historically lowest FVIII:C (IU/mL)	0.15 [0.08 - 0.19]
Most recent FVIII:C at inclusion (IU/mL)	0.18 [0.11 - 0.31]
Most recent VWF:Ag (IU/mL) (n = 15)	1.24 [1.02 - 1.60]
Consecutive days desmopressin	
At least 2 days	17 (100%)
At least 3 days	11 (65%)
Type of medical procedure	
Orthopedic	4[a]
Oromaxillary/dental	6[a]
Urological	5
Biopsy/excision	1
Endoscopy	1
Laparoscopic colectomy	1

[a] One patient had an orthopedic and dental procedure combined

Characteristics of patients in whom reproducibility and similarity were analyzed (*n* = 23)	
Characteristic	***n* (%) or median [interquartile range]**
Hemophilia severity	
Moderate	2 (87%)
Mild	21 (13%)
Age (years)	49 [36 - 59]
Historically lowest FVIII:C (IU/mL)	0.15 [0.08 - 0.19]
Most recent FVIII:C at inclusion (IU/mL)	0.18 [0.11 - 0.29]
Most recent VWF:Ag (IU/mL) (n = 20)	1.28 [1.04 - 1.69]

VWF:Ag, von Willebrand antigen.
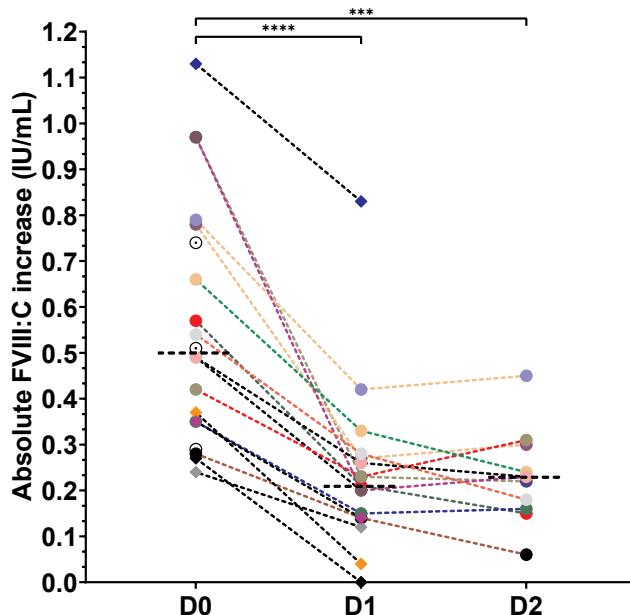
Figure 2: absolute FVIII:C increase 45 minutes after start desmopressin in non-severe hemophilia A patients on day 0 (D0; *n* = 23), day 1 (D1; *n* = 17) and day 2 (D2; *n* = 11) of a medical procedure. The median absolute FVIII:C increase of the respective day is depicted by a dotted line. Patients with only a D0 response are shown a circle with a center dot. Patients with a response on D0 and D1 are shown as a rhomboid with matching colors. Patients with a response on D0, D1 and D2 are shown as a full circle with matching colors with a connecting line.

The reduction in FVIII:C response after the second administration of desmopressin compared to the initial dose in our study was 57%. In a previous study by Mannucci et al. [9] demonstrated in a test setting with daily (every 24 hours) dosing that this reduction in FVIII:C response was only 30%. A possible explanation for this difference is that our study was performed peri-operatively in contrast to the previous study, which was performed in a test setting. In the same study also von Willebrand disease patients were included and they also showed a reduction of FVIII:C response of approximately 30% [9]. Another study in six healthy volunteers, showed a mean reduction of FVIII:C response of approximately 50% for each consecutive dose up to three doses at twelve hour dosing intervals with a high inter-individual variation[13]. We hypothesize that this difference between previous studies and our study may be caused by the additional release of stored FVIII and VWF in endothelial cells due to stress caused by the medical procedure. Also the difference in interval between dosing desmopressin (12-hour dosing versus 24-hour dosing) may contribute to the observed findings. A significant post-operative increase of FVIII:C and high-molecular-weight VWF multimers has been reported in healthy individuals undergoing a surgical procedure[14]. Similarly, this stress response may have led to a

peri-operative release of stored FVIII and VWF in our patients, eventually leading to less FVIII and VWF being available from stored endothelial pools to be mobilized by the second administration of desmopressin. The observation of our study has clinical consequences, as repeated peri-operative use of desmopressin apparently leads to a lower than previously reported response after repeated desmopressin administrations.
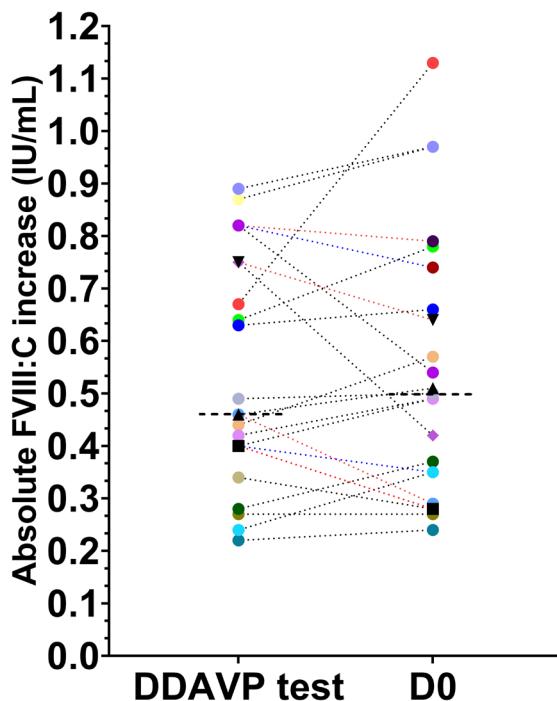
Figure 3: reproducibility and similarity of desmopressin response, comparing an earlier desmopressin test with the first pre-operative absolute desmopressin response on D0 (*n* = 23). The median absolute desmopressin (DDAVP) test FVIII:C increase (IU/mL) compared to the first pre-operative (D0) absolute desmopressin increase after administration of desmopressin (IU/mL). Matching patient data is visualized with the same shape and color with a connecting line.

The pre-operative desmopressin response had a good reproducibility of 74% compared with the previously performed desmopressin test, somewhat higher than in earlier studies (42%-70%)[10,11]. This underscores, as reported earlier, that a desmopressin test is an important and reliable tool to predict the pre-operative desmopressin response [8,10,11]. These results underline the potential of repeated desmopressin administration in peri-operative non-severe hemophilia A patients, especially in case of limited factor VIII concentrate resources.

A limitation of our study is that we did not assess the effect of certain determinants of FVIII:C levels, such as von Willebrand factor levels or the use of FVIII concentrate on tachyphylaxis. Other studies in test setting have reported certain determinants such as *F8* mutation, age, pre-desmopressin administration FVIII:C, and difference in VWF:Ag before and after desmopressin administration (at t = 1 hour). These factors could also have influenced the tachyphylaxis response in our patients[7,15,16]. FVIII concentrate has shown a minimal to no effect on FVIII and VWF clearance in peri-operative hemophilia A patients[17]. Therefore, we expect a minimal effect of FVIII concentrate on tachyphylaxis as well. Furthermore, the sample size of our cohort treated with desmopressin for three days was limited. Additionally, the comparison made with other earlier studies in test setting and our studies is indirect, with other possible factors of influence such as other formulations of desmopressin. Earlier studies were performed in a test setting and were executed many years ago.

To conclude, tachyphylaxis in peri-operative non-severe hemophilia A resulted in a FVIII:C response of only 43% after the second desmopressin administration compared to the initial response. This remaining response is lower than reported previously which may lead to lower FVIII:C levels than expected. The reproducibility of the desmopressin test response was high, which emphasizes the role of desmopressin as a low cost treatment modality with high convenience (as i.e. intranasal spray) in non-severe hemophilia A patients with an adequate. Repeated desmopressin administration should be considered as a treatment modality in the peri-operative setting in non-severe hemophilia A patients, but more pronounced tachyphylaxis should be anticipated. Our results may have important implications for monitoring repeated desmopressin treatment when used peri-operatively.

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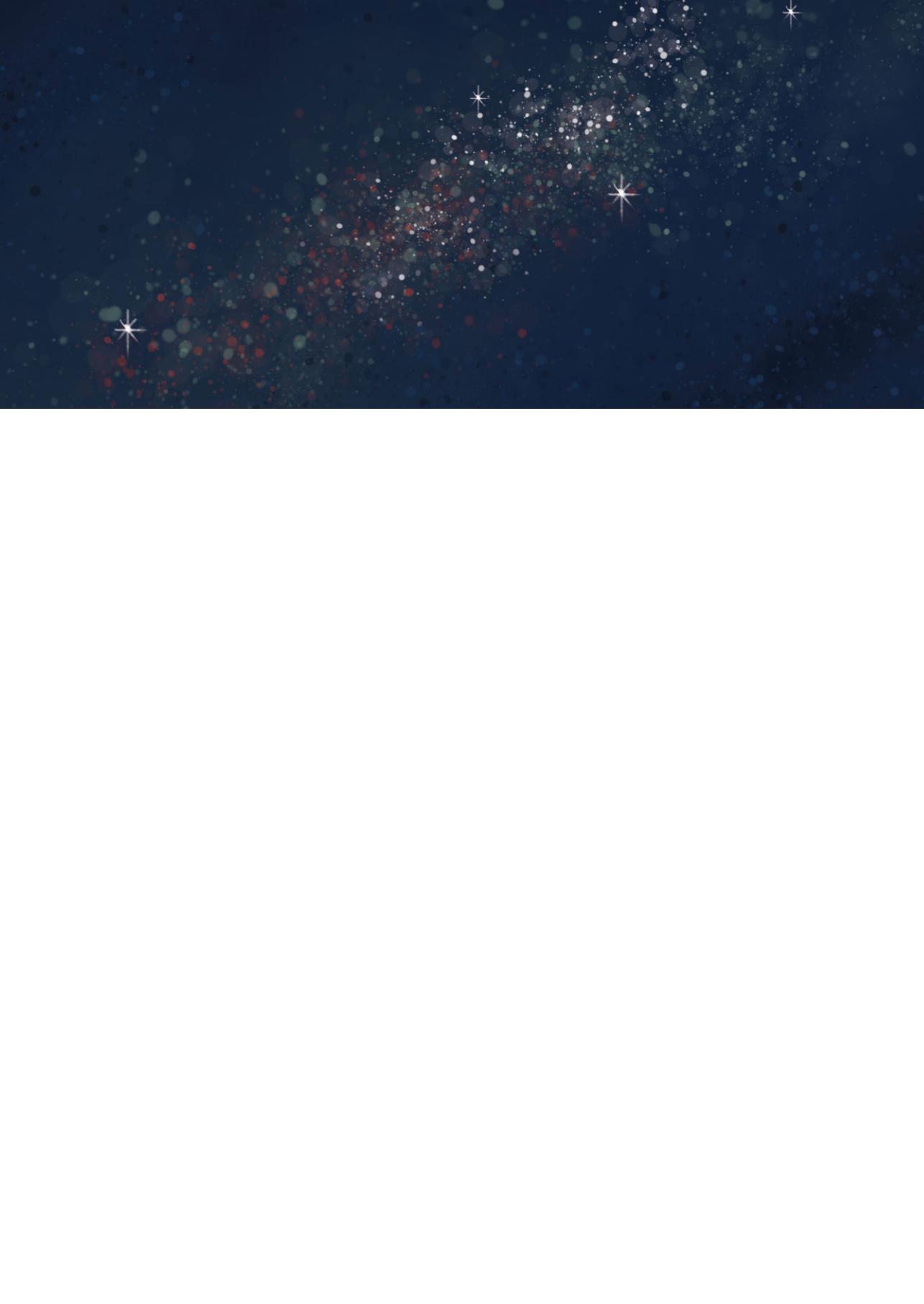
CHAPTER 4

COST STUDY OF PERI-OPERATIVE DESMOPRESSIN COMBINED WITH PHARMACOKINETIC-GUIDED FACTOR VIII CONCENTRATE DOSING IN NON-SEVERE HEMOPHILIA A PATIENTS

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Abstract

Introduction
In the DAVID study, we recently showed in non-severe hemophilia A patients that peri-operative combination treatment of desmopressin immediately followed by FVIII concentrate (based on pharmacokinetics (PK)) reduces FVIII concentrate use compared to treatment with body weight based FVIII concentrate alone (standard treatment).

Aim
We performed a substudy of the DAVID study to assess potential cost savings comparing combination treatment (PK-guided) up to three days, with standard treatment.

Methods
The cost per procedure for the average combination treatment period (maximum of three days) versus cost per procedure for the average standard treatment period (body weight based dosing in the same time period) were compared. Sensitivity analyses were performed to assess differences in costs and time for different scenarios.

Results
Combination treatment leads to a reduction of €1,127 to €1,177 (~10%) in total costs of ~€11.000 per patient per peri-operative treatment period, mainly by reducing FVIII concentrate consumption. More time is spent by nursing staff up to one hour using combination treatment in comparison to standard treatment. Subcutaneous instead of intravenous desmopressin use would save 94 to 171 minutes time spent by nursing staff, but would increase total cost with €127 to €181 in comparison to intravenous desmopressin administration. Omission of FVIII:C measurements after desmopressin would reduce 13 minutes in time spent without an evident total cost reduction.

Conclusion
Peri-operative combination treatment of desmopressin, immediately followed by FVIII concentrate, instead of FVIII concentrate monotherapy is promising as cost-saving method to reduce FVIII concentrate related costs.

Visual abstract

Cost overview in the DAVID study (PK-guided **combination treatment**)
versus body weight based dosing (**standard treatment**)

Costs expressed in €

4

PK-guided **combination treatment**

€

€

€€€

+ FVIII

Combination

€
Lab

€

Total costs
€ ± 10.000

Savings
€ ± 1000

Body weight based dosing
standard treatment

€€€€€€€

FVIII

(optional)
Lab

€

Total costs
€ ± 11.000
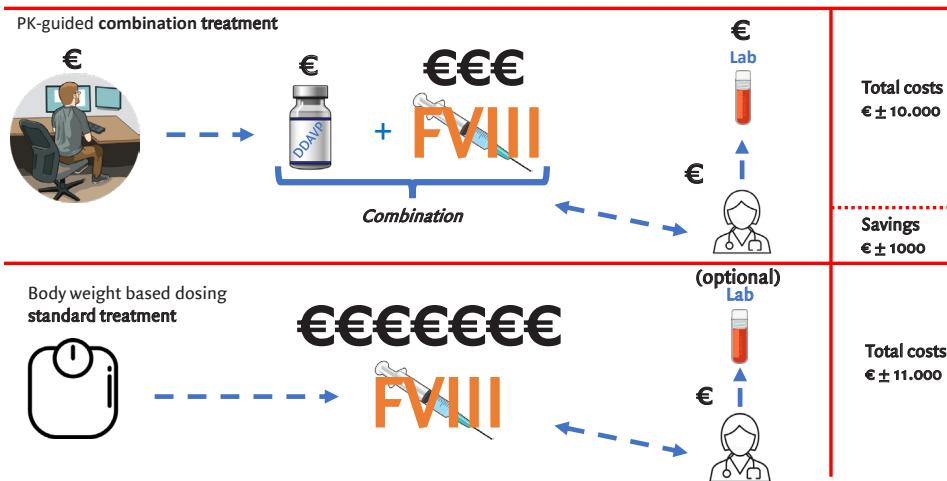
Introduction

Hemophilia A is an X-linked bleeding disorder characterized by a coagulation factor VIII (FVIII) deficiency with an estimated incidence of 1/5000[1]. Patients with hemophilia A are classified according to their plasma FVIII coagulation activity (FVIII:C): severe (FVIII:C 0.01 IU/mL), moderate, (FVIII:C 0.01-0.05 IU/mL) or mild (FVIII:C >0.05 IU/mL). Bleeding in severe patients can occur spontaneously or after trauma. In non-severe hemophilia A patients, bleeding usually occurs after trauma or during surgical or other medical procedures. In these patients, treatment in case of bleeding consists of FVIII concentrate infusion and desmopressin (DDAVP), which increases plasma FVIII and von Willebrand factor (VWF). Desmopressin can be used as treatment in non-severe hemophilia A patients depending on the FVIII:C response[2]. Desmopressin can be administered intravenously, subcutaneously or intranasally. Of these routes of administration, subcutaneous and intravenous administration lead to a similar increase in FVIII:C[3].

Unfortunately, not all non-severe hemophilia A patients reach the FVIII target level needed to safely perform a surgical intervention due to a limited response to desmopressin. Theoretically, this limited response of FVIII may be complemented by a subsequent dose of FVIII concentrate, which can be lower than the usually given FVIII dose based on body weight, as is the standard dosing protocol, in order to reach the required FVIII target level.

We recently showed in the DAVID study, performed in hemophilia treatment centers in the Netherlands, that individualized combination treatment with desmopressin immediately followed by PK-guided FVIII concentrate dosing (combination treatment) is feasible, safe and that it also lowers the consumption of FVIII concentrate in peri-operative non-severe hemophilia A patients compared to body weight based dosing of FVIII alone[2,4]. Additionally, the saved FVIII concentrate can be used for other individuals if FVIII concentrate and financial resources are scarce.

In the current cost study, we compared the direct medical costs of combination treatment (PK guided dosing of desmopressin and FVIII concentrate) with standard treatment (body weight based dosing of FVIII concentrate) for peri-operative mild and moderate hemophilia A patients.

Methods

In the DAVID study (trial number in Netherlands Trial Registry NTR5383) we included non-severe (mild and moderate, FVIII:C ≥0.01 IU/mL) hemophilia A patients who received a maximum of three days peri-operative combination treatment of intravenous desmopressin once-a-day (0.3 micrograms/kg in 30 minutes) and subsequent pharmacokinetic guided FVIII concentrate. FVIII concentrate was administered as an intermittent bolus infusion b.i.d. or as a continuous infusion, depending on the duration of treatment[4,5]. In total, 20 patients were included who underwent 21 procedures with 21 treatment periods. A treatment period is defined as the peri-operative period consisting of the day of the procedure up to the first 72 hours after the procedure. Based on a population pharmacokinetic model, a clinical pharmacologists calculated the required amount of FVIII concentrate to be administered after the administration of desmopressin to achieve prespecified desired pre- and post-operative FVIII:C.

This substudy compared the direct medical costs per procedure for the average combination treatment period (maximum of three days) in the DAVID study versus direct medical costs per procedure for the average standard treatment period. Calculation of the costs per procedure of standard treatment was a hypothetical scenario using body weight based dosing of FVIII concentrate according to routine clinical practice. For calculation of the pre-operative dose in the standard treatment arm, a 0.02 IU/mL FVIII:C increase was expected per international unit (IU) of administered FVIII concentrate per kilogram of body weight according to international treatment guidelines[2]. The pre-operative FVIII:C that was aimed for in this calculation was similar to the target pre-operative FVIII:C as pre-specified by the physician in the combination treatment. Furthermore in the standard arm half of the pre-operative loading dose was assumed to be administered b.i.d. after the procedure.

The primary effectiveness parameter in the original study was defined as the proportion of patients reaching pre-specified FVIII target levels with DDAVP and FVIII without requiring additional off-protocol FVIII concentrate. The mean amount of total used FVIII concentrate in the combination treatment group and standard treatment group was reported as mean and standard deviation, after assessment of normal distribution. In order to compare the statistical difference between the use of total FVIII concentrate, a paired t-test was performed with α = 0.05.

For each arm, the following costs were assessed: medication costs, nursing staff time (and costs), pharmacokinetic modeling costs and laboratory costs. Length of hospital stay was not dependent on the use of combination or standard treatment. Therefore, costs associated with length of stay were assumed to be equal between standard treatment and combination treatment and not added for these analyses.

Table 1: work time allocation translated into costs for each intervention (combination treatment or standard treatment) performed by a nurse.

Intervention
Procedures of intervention I: Combination treatment with intravenous desmopressin and FVIII concentrate intermittent (bolus) administration including FVIII:C measurement
Procedures of intervention II: Combination treatment with intravenous desmopressin and continuous FVIII concentrate administration including FVIII:C measurement
Procedures of intervention III: Standard treatment (only FVIII concentrate intermittent bolus administration) including FVIII:C measurement
Procedures of intervention IV: Standard treatment (only continuous infusion FVIII concentrate administration) including FVIII:C measurement
Procedures of intervention V: Combination treatment with subcutaneous desmopressin and FVIII concentrate intermittent bolus administration including FVIII:C measurement
Procedures of intervention VI: Combination treatment with subcutaneous desmopressin and continuous FVIII concentrate administration including FVIII:C measurement
FVIII:C measurement only

†Costs per minute based on collective labor agreement salary tables.

To calculate all costs associated with nursing staff's work time, a survey amongst two registered nurses at the Erasmus University Medical Center was performed regarding the time spend on the individual patients receiving treatment (Table 1).

Work time allocated to the procedures by nursing staff were collected of the following interventions: 1) combination treatment of intravenous desmopressin immediately followed by intermittent bolus of FVIII concentrate (intervention I); 2) combination treatment of intravenous desmopressin immediately followed by continuous infusion of FVIII concentrate (intervention II); 3) body weight based (standard) treatment with continuous infusion of FVIII concentrate (intervention III); 4) body weight based (standard) treatment with intermittent bolus of FVIII concentrate (intervention IV); 5) combination treatment of subcutaneous desmopressin immediately followed by intermittent bolus of FVIII concentrate (intervention V) or 6) combination treatment of subcutaneous desmopressin immediately followed by continuous administration of FVIII concentrate (intervention VI). The procedures per intervention consisted of the preparation and administration of medication, measuring of vital signs, blood sampling and record keeping of the performed tasks. As time needed for patient care can vary per patient, a minimum and maximum time (range) per intervention was specified by the two nurses. Work time allocation for pharmacokinetic modeling was assessed by surveying the clinical

Minutes (minimum)	Minutes (maximum)	Cost (€) (minimum)†	Cost (€) (maximum)†	Cost (€) per minute†
85	125	63	92	0.74
95	135	70	100	0.74
35	45	26	34	0.74
55	65	41	48	0.74
45	55	34	41	0.74
70	80	52	59	0.74
5	5	4	4	0.74

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pharmacologists themselves. Salary costs for the nursing staff and pharmacologists were based on the collective labor agreement salary tables (Table 1). The FVIII dose was rounded to the nearest available FVIII concentrate vial (to 250 IU) for calculation of the total costs.

The costs of desmopressin and FVIII concentrate used (NovoEight®, Refacto®, Kogenate® or Advate®) were based on reported costs of list price of Farmacotherapeutisch Kompas (a Dutch site published by the Dutch National Healthcare Institute). The cost of measurements of FVIII:C, sodium, hemoglobin and platelet counts were derived from the Erasmus University Medical Center. All costs were corrected for inflation up to 2020, the final year of the DAVID study, based on the Consumer Price Index of the Dutch Central Bureau of Statistics. An overview of the performed measurements in case of combination treatment is reported in Table 2.

Table 2: overview of measurements in case of combination treatment with:

A) intermittent (bolus) FVIII concentrate administration

	D0 = Day of surgery				D1			D2		
Time†	pre	post	peak	Post-proc	pre	post	peak	pre	post	peak
FVIII:C (4,5 mL)	X	X	X	X	**X**	X	X	**X**	X	X
Na	X				X			X		
Hb/Ht	X				X					
T	X				X					

X **= measurement for primary endpoint**
†pre = before DDAVP, post = after DDAVP, peak = after FVIII-concentrate

B) continuous FVIII concentrate administration

	D0 = Day of surgery				D1			D2		
Time*	pre	post	peak	post-proc	pre	post	pre-adjust	pre	post	pre-adjust
FVIII:C (4,5 mL)	X	X	X	X	**X**	X	X	**X**	X	X
Na	X				X			X		
Hb/Ht	X				X					
T	X				X					

X **= measurement for primary endpoint**
†pre = before DDAVP, post = after DDAVP, peak = after loading dose FVIII-concentrate when possible before 15.30h, pre-adjust = before second dosing adjustment of the day,

Sensitivity analyses were performed to assess the difference in costs and time spent by nursing staff for the following different scenarios: use of only subcutaneous desmopressin instead of intravenous desmopressin (intervention V or VI) , use of only continuous FVIII concentrate instead of intermittent FVIII concentrate bolus (intervention II or IV) and the omission of the FVIII:C measurement after desmopressin administration.

As no difference in clinical effectiveness was found between combination treatment and standard (body weight based) treatment[4], a cost-minimization analysis was performed.

Results

In the combination group, 8,674 ± 5,574 IU (mean ± standard deviation) was given versus 10,500 ± 2,737 IU in the standard group (*n* = 21; p = 0.10); corresponding FVIII costs (not rounded up to the nearest vial) were €9,001 (± 5797) in the combination group versus €10,751 (± 2968) in the standard group (*n* = 21; p = 0.13). Total costs were €9,727 to 9,812 in the combination group and €10,904 to 10,939 in the standard group indicating that total price is mainly dependent on the costs of the FVIII concentrate (Table 3). Application of combination treatment saves €1,127 to €1,177, approximately 10% of the total costs per treatment period, compared with standard treatment, mainly by saving FVIII concentrate (Table 3).

Other expenses were exclusively made in the combination group and were not needed using standard body weight based treatment including the clinical pharmacologist's salary costs, desmopressin costs, (more) FVIII:C measurements and the additional safety measurements (sodium, hemoglobin, hematocrit and thrombocyte measurements). In contrast, combination treatment is associated with an increase in time spent by personnel for up to one hour in comparison to standard treatment (Table 1).

Sensitivity analyses
When using only subcutaneous desmopressin instead of intravenous desmopressin, up to 94.8 to 170.65 minutes of nursing staff time per treatment period (~43–53%) was saved resulting in a relative €70 to €112 cost reduction in nursing staff costs per treatment period (~47%–57%) compared to intravenous administration. However, this subcutaneous administration leads to a €127 to €181 (~2%) increase in total costs due to the fact that desmopressin vials required for subcutaneous desmopressin are more expensive than for intravenous desmopressin (Tables 4 and 7).

Table 3: cost overview in the combination arm compared with standard treatment for 21 treatment periods (for 21 procedures).

	Cost (€, index 2020)
Procedures of intervention I[†]	63-92
Procedures of intervention II[†]	70-100
Procedures of intervention III[†]	26-33
Procedures of intervention IV[†]	41-48
Clinical pharmacologist: first dosing advice (day 0), 1.5 hours[‡]	138.75
Clinical pharmacologist: follow-up dosing advice (1 hour a day)[‡]	92.50
FVIII concentrate use rounded up to nearest vial (250 IU)[§]	NovoEight (1000 IU): 1039.86 Refacto (1000 IU): 1024.60 Kogenate (1000 IU): 1039.86 Advate (1000 IU): 1039.85
Desmopressin (medication)[¶]	5.47 (per 4 µg)
Sodium measurement (lab costs including lab personnel)[$]	1.50
FVIII:C measurement (lab costs including lab personnel)[$]	20.39
Hemoglobin measurement (lab costs including lab personnel)[$]	1.90
Hematocrit measurement (lab costs including lab personnel)[$]	1.90
Thrombocyte measurement (lab costs including lab personnel)[$]	1.90

Combination treatment (DAVID study)		Standard treatment (body weight dosing)	
Mean number	*Mean cost per treatment period (€, index 2020)*	*Mean number*	*Mean cost per treatment period (€, index 2020)*
2.1 interventions per treatment period	132.20 – 193.2	0	0
0.43 interventions per treatment period	30.10 – 43.0	0	0
1.24 interventions per treatment period	32.24 – 40.92	4.19 interventions per treatment period	108.94 – 138.27
0.29 interventions per treatment period	11.89 – 13.92	0.86 interventions per treatment period	35.26 – 41.28
1 advice per treatment period	138.75	0	0
2.81 advices per treatment period	259.88	0	0
NovoEight: 4357.1 IU per treatment period	NovoEight: 4530.82	NovoEight: 6452.4 IU per treatment period	NovoEight: 6709.57
Refacto: 1214.3 IU per treatment period	Refacto: 1244.16	Refacto: 1500.0 IU per treatment period	Refacto: 1536.9
Kogenate: 1464.3 IU per treatment period	Kogenate: 1522.67	Kogenate: 1166.7 IU per treatment period	Kogenate: 1213.21
Advate: 1642.9 IU per treatment period	Advate: 1708.37	Advate: 1250 IU per treatment period	Advate: 1299.83
62.3 micrograms per treatment period	85.2	0	0
2.67 measurements per treatment period	4.0	0	0
1.8 measurements per treatment period	17.99	0	0
1.5 measurements per treatment period	2.85	0	0
1.7 measurement per treatment period	3.26	0	0
1.6 measurements per treatment period	3.08	0	0

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Table 3: Continued

	Cost (€, index 2020)
***Total mean costs per treatment period per patient rounded to whole numbers** (€)*	-

†Cost range and time spent is based on time spent by nursing staff (see Table 1).
‡Cost range is based on time spent by modelers and their salary based on collective labor agreement.
§Based on Passantenlijst Dure Geneesmiddelen EMC 2020.

Table 4: sensitivity analysis of costs and time allocation if desmopressin is administered subcutaneously instead of intravenously in the DAVID study.

	Cost (€, index 2020)
Procedures of intervention I†	63-92
Procedures of intervention II†	70-100
Procedures of intervention V†	33-41
Procedures of intervention VI†	52-59
Desmopressin (medication)‡	5.47 (i.v.) (per 4 µg) 80.96 (s.c.) (per 15 µg)
***Subtotal mean cost per treatment period** (€)*	-
***Total time allocated per treatment period** (min)*	-

†Cost range and time spent is based on time spent by nursing staff (see Table 1).
‡Based on *Farmacotherapeutisch Kompas, desmopressine*
(URL: https://www.farmacotherapeutischkompas.nl/bladeren/preparaatteksten/d/desmopressine) and corrected for inflation (index year = 2020).

	Combination treatment (DAVID study)		Standard treatment (body weight dosing)	
	Mean number	*Mean cost per treatment period (€, index 2020)*	*Mean number*	*Mean cost per treatment period (€, index 2020)*
	9,727 to 9,812		**10,904 to 10,939**	

[1]Based on *Farmacotherapeutisch Kompas, desmopressine*
(URL: https://www.farmacotherapeutischkompas.nl/bladeren/preparaatteksten/d/
desmopressine) and corrected for inflation (index year = 2020).
[$]Based on local lab costs of the Erasmus University Medical Center.

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Intravenous (i.v.) desmopressin		Subcutaneous (s.c.) desmopressin	
Mean number (mean time spent per treatment period)	*Mean cost per treatment period (€, index 2020)*	*Mean number (mean time spent per treatment period)*	*Mean cost per treatment period (€, index 2020)*
2.1 interventions per treatment period (178.5 to 262.5 min)	132.2 – 193.2	0	0
0.43 interventions per treatment period (40.85 to 58.05 min)	30.1 - 43	0	0
0	0	2.1 interventions per treatment period (94.5 to 115.5 min)	69.3 – 86.1
0	0	0.43 interventions per treatment period (30.1 to 34.4 min)	22.4 – 25.4
62.3 micrograms per treatment period	85.2	62.3 micrograms per treatment period	336.4
-	247.5 to 321.4	-	428.1 to 447.9
219.35 to 320.55	**-**	**124.6 to 149.9**	

Table 5: sensitivity analysis of costs and time allocation of the DAVID study if no FVIII:C measurements after desmopressin administration were taken.

	Cost (€, index 2020)
Procedures of intervention I with FVIII:C measurement /without FVIII:C measurement[†]	63-92 / 59-88
Procedures of intervention II with FVIII:C measurement /without FVIII:C measurement[†]	70-100 / 66-96
Procedures of intervention III[†]	26-33
Procedures of intervention IV[†]	41-48
FVIII:C measurement[‡]	20.39
Subtotal mean cost per treatment period (€)	-
Total time allocated (min)	-

[†]Cost range and time spent is based on time spent by nursing staff (see Table 1).
[‡]Based on local lab costs of the Erasmus University Medical Center.

With the omission of FVIII:C measurement after desmopressin, 12–13 minutes of mean nursing staff time (~3-5%) is saved in combination with less lab measurement costs, with a reduction of €60 to €90 of the total costs (~0.7-1%) of combination treatment (Tables 5 and 7).

With the use of only intermittent bolus injections of FVIII concentrate instead of continuous FVIII concentrate, 10 minutes of mean nursing staff time per treatment period (~3-4%) is saved without an evident total cost reduction (Tables 6 and 7).

	DAVID study		DAVID study without FVIII:C measurements after desmopressin	
	Mean number (mean time spent per treatment period)	*Mean cost per treatment period (€, index 2020)*	*Mean number (mean time spent per treatment period)*	*Mean cost per treatment period (€, index 2020)*
	2.1 interventions per treatment period (178.5 to 262.5 min)	132.20 - 193.20	2.1 interventions per treatment period (168 to 252 min)	123.62 - 184.38
	0.43 interventions per treatment period (40.85 to 58.05 min)	30.10 - 43.00	0.43 interventions per treatment period (38.7 to 55.9 min)	28.29 - 41.14
	1.24 interventions per treatment period (43.4 to 55.8 min)	32.24 - 40.92	1.24 interventions per treatment period (43.4 to 55.8 min)	32.24 - 40.92
	0.29 interventions per treatment period (15.95 to 18.85 min)	11.89 - 13.92	0.29 interventions per treatment period (15.95 to 18.85 min)	11.89 - 13.92
	8.14 measurements per treatment period	166	5.6 measurements per treatment period	114.18
	-	**372.43 tot 457.04**	-	**310.22 to 364.54**
	278.7 to 395.2	**-**	**266.05 to 382.55**	**-**

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Table 6: sensitivity analysis of costs and time allocation if FVIII concentrate is administered as an intermittent bolus instead of continuous infusion in the DAVID study.

	Cost (€, index 2020)
Procedures of intervention I†	63-92
Procedures of intervention II†	70-100
Procedures of intervention III†	26-33
Procedures of intervention IV†	41-48
Subtotal mean cost per treatment period (€)	-
Total time allocated per treatment period (min)	-

†Cost range is based on time spent by nursing staff (see Table 1).

Table 7: overview of the mean difference in total costs per treatment period of sensitivity analyses.

	Intravenous (i.v.) desmopressin
Total mean cost per treatment period rounded to a whole number (€)	9,727 to 9,812
	Combination treatment
Total mean cost per treatment period rounded to a whole number (€)	9,912 to 9,996
	Continuous FVIII concentrate
Total mean cost per treatment period rounded to a whole number (€)	9,912 to 9,924

	Continuous FVIII concentrate		FVIII concentrate bolus	
	Mean number (mean time spent per treatment period)	*Mean cost per treatment period (€, index 2020)*	*Mean number (mean time spent per treatment period)*	*Mean number per treatment period (€, index 2020)*
	2.1 interventions per treatment period (178.5 to 262.5 min)	132.20 - 193.20	2.53 interventions per treatment period (215.05 to 316.25 min)	159.39 - 232.76
	0.43 interventions per treatment period (40.85 to 58.05 min)	30.10 - 43.00	0	0
	1.24 interventions per treatment period (43.4 to 55.8 min)	32.24 - 40.92	1.53 interventions per treatment period (53.55 to 68.85 min)	39.78 - 50.49
	0.29 interventions per treatment period (15.95 to 18.85 min)	11.89 - 13.92	0	0
	-	**206.43 to 291.04**	-	**199.17 to 283.25**
	278.7 to 395.2	-	**268.6 to 385.1**	-

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Subcutaneous (s.c.) desmopressin	Incremental costs i.v. vs. s.c. desmopressin
9,908 to 9,939	-181 to -127
Combination treatment without FVIII:C measurements after desmopressin	**Incremental costs with vs. without FVIII:C measurements**
9,850 to 9,904	62 to 92
FVIII concentrate bolus	**Incremental costs continuous vs. bolus**
9,905 to 9,989	7 to -65

Discussion

In this cost analysis of the DAVID study, we have shown that peri-operative combination treatment of desmopressin immediately followed by PK-guided FVIII concentrate (i.e. combination treatment) may lead to cost savings in comparison to standard treatment with FVIII alone. This cost reduction is mainly the result of the lower amount of FVIII used, which impact the affordability of hemophilia care. If the FVIII:C increase after desmopressin administration does not lead to the target FVIII level, only a limited amount of additional FVIII concentrate is needed for adequate hemostasis. This is obviously less factor VIII than when standard body weight based dosing of FVIII concentrate is used as monotherapy, which is current clinical practice. In the DAVID study, we measured FVIII:C at three or four time points between desmopressin administrations, but given that the response to desmopressin is known fairly well based on a previous administration, less measurements will be necessary in daily care. Less measurements may lead to a further reduction of total costs. In addition, the use of subcutaneous instead of intravenous desmopressin may also lead to a reduction in time spent on treatment, as nursing staff would be spending approximately 50% less time compared to intravenous administration. However, this is partly counterbalanced by the higher costs of desmopressin vials used for subcutaneous administration compared to the intravenous vials. The use of continuous FVIII concentrate instead of bolus injections would not lead to a clinically significant change in cost nor time spent.

A 10% reduction of the costs per treatment period using combination treatment might significantly impact the affordability of hemophilia care. Treatment of hemophilia is an economic burden on healthcare systems of countries worldwide because of the high costs of FVIII concentrate. A global survey by the World Federation of Haemophilia has reported that the Americas and Europe comprise 24% of the worldwide hemophilia A population, but these 24% of patients utilize 79% of the reported costly FVIII concentrate units, indicating a limited supply of FVIII concentrate in other countries[6]. A majority of the FVIII concentrate consumption is applied as primary prophylaxis in severe or moderate hemophilia A patients to prevent bleeds and joint disease[1]. Nonetheless, costs for non-severe hemophilia A patients are high as well in case of a bleed, after trauma or before a medical procedure to prevent a bleed. A US study on total hemophilia healthcare costs of hemophilia A reported that one mild patient annually costs $59,101 and one moderate patient annually costs $84,363, of which 54% and 74% of these total costs respectively are clotting factor-related[7]. Another US study reported an average annual hemophilia-related medical costs of $165,649 for non-prophylaxis patients, which are usually non-severe hemophilia A patients[8]. Additionally, continuous infusion of FVIII concentrate has been implied to reduce peri-operative FVIII concentrate consumption

by reducing unnecessary high peak FVIII:C[9-11]. As stated earlier, combining the use of subcutaneous desmopressin and omission of FVIII:C measurements after desmopressin could serve as a cost-neutral method to significantly reduce the time burden of nursing staff administering combination treatment. Despite literature reporting a possible cost reduction of continuous FVIII administration[9-11], we did not find any notable difference between continuous and intermittent treatment. An important factor in our calculations is the additional nursing staff time needed to perform and follow-up on the continuous infusion of FVIII concentrate versus intermittent (bolus) administration: these earlier studies reporting on continuous administration did not account for the longer time spent by nursing staff. With the current calculations in our substudy, we therefore also account for daily clinical practice.

A major advantage of desmopressin is that it is cheaper than FVIII concentrate, is widely available and is on the WHO Essential Medicines' List[2]. Based on Dutch medicine list prices in 2024, a mild hemophilia A patient weighing 80 kilograms with an adequate FVIII response after desmopressin, would require a 24 micrograms dose (0.3 micrograms/kg) of intravenous desmopressin to reach a prespecified FVIII:C level, costing €55[12]. If this patient is treated with FVIII concentrate and would need between 1,500 to 3,000 IU of clotting factor VIII, this would cost between €1,560 to €3,120. Additionally, subcutaneous administration lowers the amount of time spent by healthcare personnel on patient treatment, as patients can administer desmopressin themselves in a short period of time, whereas intravenous administration requires at least 30 minutes for full administration of desmopressin

Our study however also has limitations. First, the limited number of included patients limited the statistical power to detect a possibly significant difference in FVIII concentrate usage between combination and standard arm. Second, we used hypothetical calculations in order to assess the costs of standard treatment. FVIII concentrate dosing can also be adapted based on post-operative FVIII:C measurements instead of b.i.d. dosing of half of the pre-operative loading FVIII concentrate dose. This may have resulted in a lower FVIII dose being used clinically for subsequent dosing. Third, the costs of FVIII concentrate in the Netherlands have been decreased in the past years due to centralized purchasing, which implies that the reported costs used in our study are an overestimation of the real cost price of FVIII concentrates. However, as FVIII concentrate and desmopressin prices vary worldwide and are still high in many countries, a cost reduction may still be reached. Additionally, FVIII concentrate savings are important as FVIII concentrate is not always readily available in low resource countries. Combination therapy thus enables FVIII concentrate to be used more efficiently.

4

Conclusion

The peri-operative use of the combination of desmopressin immediately followed by PK-guided FVIIII concentrate in hemophilia A is a promising cost-saving method as it mainly reduces FVIII concentrate-related costs.

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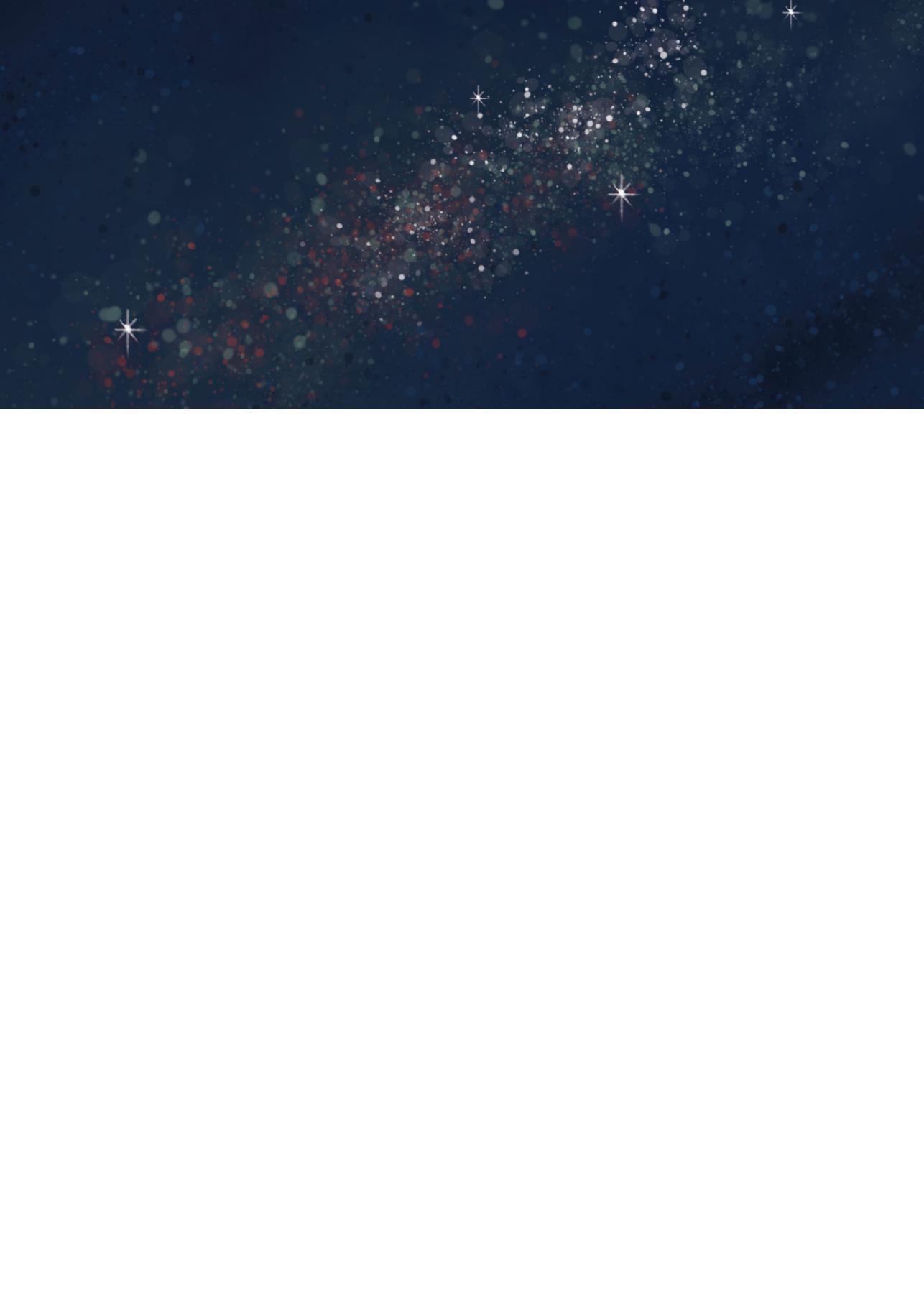
CHAPTER 5

DESMOPRESSIN IN
NON-SEVERE HEMOPHILIA A:
PATIENT PERSPECTIVES
ON USE AND EFFICACY

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Abstract

Background
Desmopressin increases plasma FVIII and von Willebrand factor (VWF) levels in non-severe hemophilia A patients. Patients' perspectives on desmopressin are relevant to increase and optimize its suboptimal use. However, patients' views on desmopressin are not reported.

Objectives
To evaluate the perspectives of non-severe hemophilia A patients on desmopressin use, barriers for its use, side effects and the knowledge of patients about desmopressin's efficacy and side effects.

Methods
Non-severe hemophilia A patients were included in a cross-sectional national multicenter study. Questionnaires were filled out by adult patients and children ≥12 years themselves. Caretakers filled out questionnaires for children <12 years.

Results
In total, 706 non-severe hemophilia A patients were included (544 mild, 162 moderate, age range 0-88 years). 234/508 (50%) patients reported historical desmopressin use. Desmopressin was considered as at least moderately effective in 171/187 (90%) patients. Intranasal administration was the modality of choice for 138/182 (76%) patients. Flushing was the most reported side effect in 54/206 (26%) adults and 7/22 (32%) children, respectively. The most frequently reported advantage and disadvantage were the convenience of intranasal, out-of-hospital administration by 56% (126/227) and side effects in 18% (41/227), respectively. Patients' self-perceived knowledge was unsatisfactory or unknown in 28% (63/225).

Conclusion
Overall, desmopressin was most often used intranasally, considered effective, with flushing as the most common side effect. The most mentioned advantage was the convenience of intranasal administration and disadvantage was side effects. More information and education on desmopressin could answer unmet needs in patients with current or future desmopressin treatment.

Introduction

Hemophilia A is an X-linked disorder characterized by a deficiency in functional coagulation factor VIII (FVIII). In non-severe hemophilia A (FVIII:C 0.01 – 0.40 IU/mL) patients mainly experience bleeding provoked by trauma or surgery. Available treatments for hemophilia A range from FVIII concentrate or desmopressin to novel therapeutics such as emizicumab and adeno-associated virus (AAV) vector gene therapy[1]. Of these treatments, desmopressin is widely available and on the World Health Organization's List of Essential Medicines[2]. Desmopressin administration increases plasma FVIII and von Willebrand factor (VWF) levels by stimulating endogenous VWF and FVIII release from endothelial cells[3-7]. VWF acts as a chaperone protein protecting exogenous and endogenous FVIII from degradation, thereby increasing its half-life[8]. Because of a high interindividual variation in FVIII response, a desmopressin test is advised to assess FVIII response[1]. If adequate, desmopressin can be used as bleeding prophylaxis before invasive procedures or treatment for minor bleeds, respectively. Advantages of desmopressin include its potential for intranasal self-administration enabling out-of-hospital management and its lower costs compared to FVIII concentrate. Furthermore, induction of VWF and FVIII release by administering desmopressin and not clotting factor concentrate may reduce the incidence of inhibitor in hemophilia A patients by reducing exposure to exogenous FVIII concentrate. The development of FVIII inhibitors in non-severe patients can cause significant mortality and morbidity with a notoriously unpredictable bleeding tendency[9]. Side effects of desmopressin such as flushing, headache and fatigue are limited and transient[10]. Contraindications for treatment are age <2 years, comorbidities that puts a patient at an increased risk of developing hyponatremia and (a high risk of) cardiovascular disease or thrombosis[1]. Recent literature has shown that its current use in non-severe hemophilia A patients with an adequate test response is suboptimal: in 54% of the bleeds treated with one dose of FVIII concentrate, the desmopressin FVIII:C response exceeded the level targeted with concentrate[11]. In other words, desmopressin could have been used for these bleeds instead of FVIII concentrate. Knowledge on patients' perspectives on desmopressin is relevant for increasing and optimizing the usage of desmopressin in these patients. Despite the worldwide use of desmopressin in patients with hemophilia and its merits, patients' views on the use of desmopressin are to our knowledge not reported in literature. Therefore, we initiated the present study to evaluate the views on treatment and use of desmopressin in non-severe hemophilia A patients. Furthermore, we evaluated to which degree non-severe hemophilia A patients have been sufficiently informed about desmopressin.

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Methods

Patient inclusion

Male hemophilia A patients (FVIII:C <0.40 IU/mL) were included in the cross-sectional national multicenter Hemophilia in the Netherlands 6 study (HiN6)[12]. For the present analysis, we included all non-severe hemophilia A patients who also participated in the survey from May 2018 and August 2019. This survey contained questions on multiple aspects of hemophilia care, such as desmopressin use, quality of care, treatment and employment. In children between 12 and 18 years old and adults, the survey was filled out by patients themselves. For children <12 years old, parents or caregivers filled out the survey. This study was approved by the Committee of Medical Ethics of Leiden University Medical Center (NL59114.058.17).

Survey

The survey included multiple topics with respect to desmopressin, namely whether a desmopressin test was ever performed, year of desmopressin testing (if performed), if there had been at least one treatment with desmopressin, which if answered positively, was followed by the following questions: the efficacy of desmopressin for certain bleeds (multiple answers possible, including an open text box), frequency of use in the last three years, its efficacy in general (only one answer possible), reported side effects after use of desmopressin, perceived advantages and disadvantages of desmopressin, patients' opinion on their knowledge on the effectiveness and side effects of desmopressin and patients' advice to increase the use of desmopressin. All patients were asked whether desmopressin was the first choice in the management plan in case of a bleed (for children only asked to adolescents between 12 and 18 years old) and what treatment was used in case of a bleed or as prophylaxis for a bleed (in general). Only answers relevant to the question were included in case of free text boxes. Side effects asked in the survey were: dizziness, nausea, fatigue, flushing, headache, unknown, stuffed nose and/or inflammation of nasal mucosa, other (free text). The severity of symptoms was asked on a scale from 1 to 5, "not that severe" to "very severe" respectively. Possible advantages asked in the survey were: none, easy to use, no need for FVIII concentrate or no need for an infusion (intranasal administration), home treatment (intranasal administration), treatment is fast, treatment is safe, treatment is cheap, unknown, other (free text). Possible disadvantages asked in the survey were: none, expensive, side effects, unknown, other (free text).

The management plan options were limited to only one choice, specifically: factor VIII concentrate, active prothrombin complex concentrate (aPCC), recombinant factor VII (rFVIIa), intranasal desmopressin or intravenous desmopressin.

The options for treatment in case of a bleed or for prophylaxis were: FVIII concentrate, activated prothrombin complex concentrate, recombinant FVIIa, intranasal desmopressin or intravenous desmopressin (multiple answer options possible). For this specific question, we only reported the patients who filled out the use of intranasal and/or intravenous desmopressin.

Statistical analysis
Categorical data are reported as frequencies and proportions. Continuous data are reported as median (interquartile range). All statistical analyses were performed in IBM Statistics SPSS v28.

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Results

Patient inclusion and reported desmopressin use

In total 706 non-severe male hemophilia A patients were included in the HiN6 study and responded to the survey; 589/706 (83%) were adults with an age range of 18 to 88 years and 117/706 (17%) were children with an age range of 0 to 17 years. About 50% (211/426) and 28% (23/82) of the adults and children respectively, reported to be treated with desmopressin at least once. Patient characteristics including data on historical desmopressin treatment and desmopressin testing are described in the Table. In total, 208/389 (54%) mild and 26/119 (22%) moderate hemophilia A patients who answered the question on historical desmopressin use, reported earlier desmopressin treatment. The age in children as well as the baseline FVIII:C in adults and children, seemed higher in patients who had been treated with desmopressin at least once in comparison to those who did not (Supplementary Table S1).

In 172 adults who reported desmopressin as current treatment for bleeds or prophylaxis (multiple answer options per patient possible), desmopressin was used intravenously in 72 (42%) and intranasally in 128 (74%). In 10 children between 12 and 18 years old, one used desmopressin intravenously (10%) and all 10 (100%) intranasally. In 21 children younger than 12 years old, 2 (10%) used desmopressin intravenously and 20 (95%) intranasally.

In total, 164 adults and 23 children answered general questions on the perceived effectivity of desmopressin, of whom 131 (80%) adults and 19 (83%) children stated it was effective, 19 (11.5%) adults and 2 (9%) children stated moderately effective and 14 (8.5%) adults and 2 (9%) children stated desmopressin was not effective.

Furthermore, 206 adults and 23 children answered specific questions on whether desmopressin was sufficiently effective to treat their bleeds, of whom 38 (19%) adults and 3 (13%) children stated that they did not know how effective desmopressin is. Ten (5%) adults and one (5%) child stated desmopressin was not effective at all for their bleedings, for 60 (29%) adults and 14 (61%) children, it was effective to treat mucosal bleedings (i.e. epistaxis) and for 96 (47%) adults and 12 (52%) children it was effective to treat larger bleedings. In addition, of the aforementioned 206 adults, other uses for desmopressin, such as after small trauma, were reported by 28 (13%) adults (Supplementary Table S2).

In 236 adults and 9 children, desmopressin was primarily mentioned as a part of the patients' management plan for a mild bleed by 85 (36%) adults and 6 (69%) children, for a moderate bleed by 28 (13%) adults and 5 (56%) children and for a life-threatening bleed by 5 (3%) adults and 2 (29%) children.

Table: Patient characteristics of all included patients

	Adults (≥18 years) Median (IQR) / n [%] (N = 589)	Children (<18 years) Median (IQR) / n [%] (N = 117)
Lowest historical measured FVIII:C (one-stage, IU/mL)	0.10 (0.05 - 0.19) (n = 457)	0.09 (0.03 - 0.15) (n = 77)
Age (years) at inclusion	51 (34 - 63) (n = 586)	9 (5 - 13) (n = 116)
Desmopressin test		
Performed	210 [48%]	40 [43%]
Not performed/Did not know	216 [52%]	53 [57%]
Age at desmopressin test[a] (years)	40 (26 - 52) (n = 138)	7 (4 - 9) (n = 20)
Hemophilia severity		
Mild	461 [78.3%]	83 [70.9%]
Moderate	128 [21.7%]	34 [29.1%]
Historical desmopressin treatment (n = 426 / n = 82)		
Yes	211 [49.5%]	23 [28%]
No	105 [24.7%]	49 [60%]
Do not know	110 [25.8%]	10 [12%]
Treatment frequency		
>10 times	11 [5%]	4 [17%]
1 - 10 times	135 [65%]	16 [70%]
Do not know	60 [30%]	3 [13%]
FVIII inhibitor in history	45 [9.9%] (n = 457)	1 [1%] (n = 77)
Negative inhibitor assay after an earlier measurable inhibitor	33 [83%] (n = 40)	1 [100%] (n = 1)

[a] Calculated by the difference between the reported year of desmopressin testing and birth date

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Reported side effects

Patients were asked whether they had experienced side effects while using desmopressin and if so, which. Two hundred and six adults and 22 children answered the questions on potential side effects after desmopressin: 86 (42%) adults and 7 (32%) children reported no side effects and side effects were unknown in 28 (14%) adults and 3 (14%) children. Ninety-two (45%) adults and 12 (55%) children had experienced at least one side effect. The most frequently reported side effects in adults were flushing in 54 (26%), headache in 36 (17%) and fatigue in 24 (12%). The most frequently reported side effects in children were flushing in 7 (31%), headache in 7 (31%) and fatigue in 5 (23%). The frequency and severity of the reported side effects are shown in Figure 1.
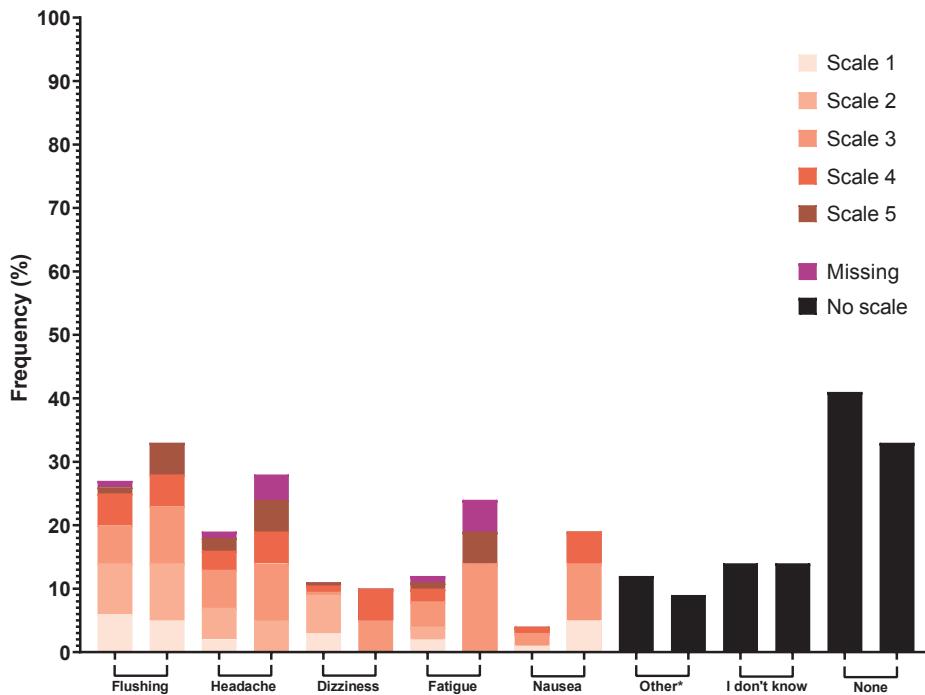
Figure 1: Reported side effects of desmopressin treatment in adults (first bar; *n* = 206) and children with non-severe hemophilia A (second bar; *n* = 22). Severity was reported on a scale from 1 (not that severe) to 5 (very severe), or missing (no severity reported). *The other reported side effects and severity (scale [s], 1-5) in adults were as follows: stuffed nose and/or mucosal inflammation (*n* = 1, s = 2), ureter clotting (*n* = 1, s = 5); thirst (*n* = 1, s = 3), flu-like symptoms (*n* = 1, s = 5), fluid retention (*n* = 2; s = 2 and 2), muscle cramp (*n* = 1, s = 4), shakiness (*n* = 1, s = 5), malaise (*n* = 1, s = 5), limited decrease in blood pressure (*n* = 1, s = 2), (sub)febrile temperature (*n* = 2, s = 1 and 5), difficult urination (*n* = 6, s = 2, 3, 1, 3, 1, and 3), chest pain (*n* = 1, s = 5), fluid restriction is not pleasant (*n* = 2, s = 5 and 3), sleepiness (*n* = 1, s = 1), polyuria (*n* = 1, s = 1), and feeling inebriated (*n* = 1, s = 4). The other reported side effects in children were as follows: (sub)febrile temperature (*n* = 1) and limiting fluid restriction (*n* = 1).

(Dis)advantages of desmopressin

Patients were asked what they perceived as advantages or disadvantages of desmopressin use. Of 205 adults and 23 children, 143 (70%) adults and 19 (83%) children reported at least one advantage of desmopressin, 22 (10%) adults and 2 (9%) no advantage and 40 (20%) adults and 2 (9%) children filled out unknown. The most reported advantages in both adults and children were the convenience of intranasal desmopressin in 108 (53%) and 18 (78%) respectively, followed by the possibility of home treatment in 80 (39%) and 12 (52%) respectively. Furthermore, 206 adults and 21 children reported on potential disadvantages of desmopressin. Ninety (44%) adults and 8 (38%) children reported no disadvantage,

in 44 (21%) adults and 3 (14%) children the question on possible disadvantages was answered as unknown. At least one disadvantage was reported by 72 adults (35%) and 10 children (48%). The most common disadvantages in both adults and children were the side effects in 38 (18%) and 6 (28%) respectively, followed by the expensive cost in 26 (12%) and 3 (14%) respectively. These advantages and disadvantages are depicted in Figures 2 and 3. In addition, the difference between mild and moderate patients are reported in Supplementary Tables S3 and S4 with comparable responses for both groups of patients.
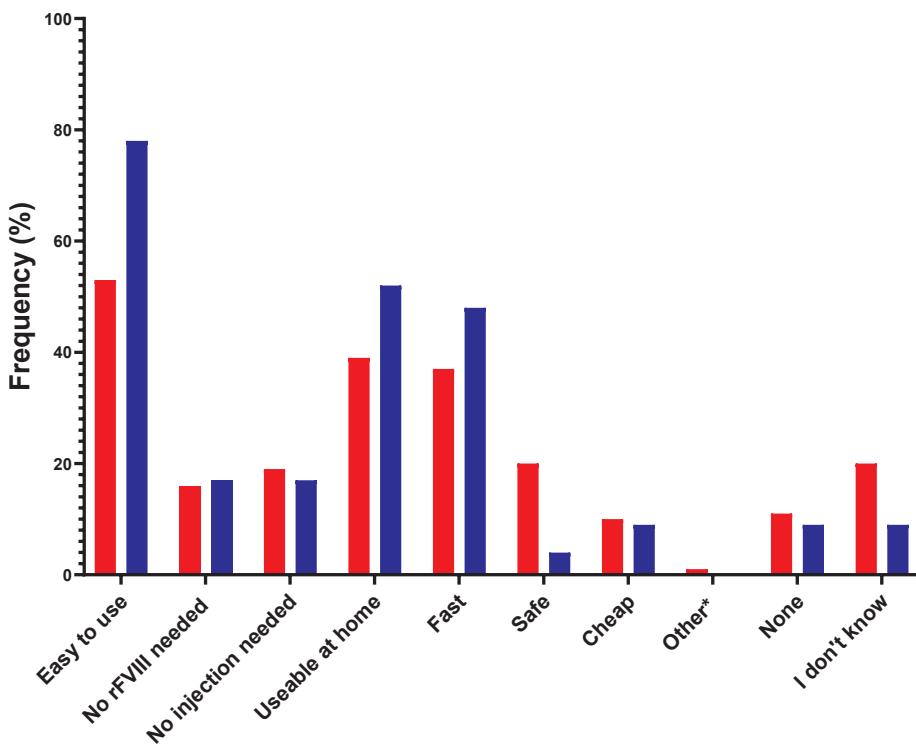
Figure 2: Reported advantages of desmopressin treatment in adults (red bars, *n* = 205) and children with non-severe hemophilia A (blue bars, *n* = 23). *The other reported advantages in adults were as follows: gives me inner peace and assurance for internal examinations (*n* = 1) and handy for travel (*n* = 1).

Information on desmopressin

Patients were asked if their knowledge on the efficacy and side effects of desmopressin was sufficient. In total, 310 adults and 31 children replied.
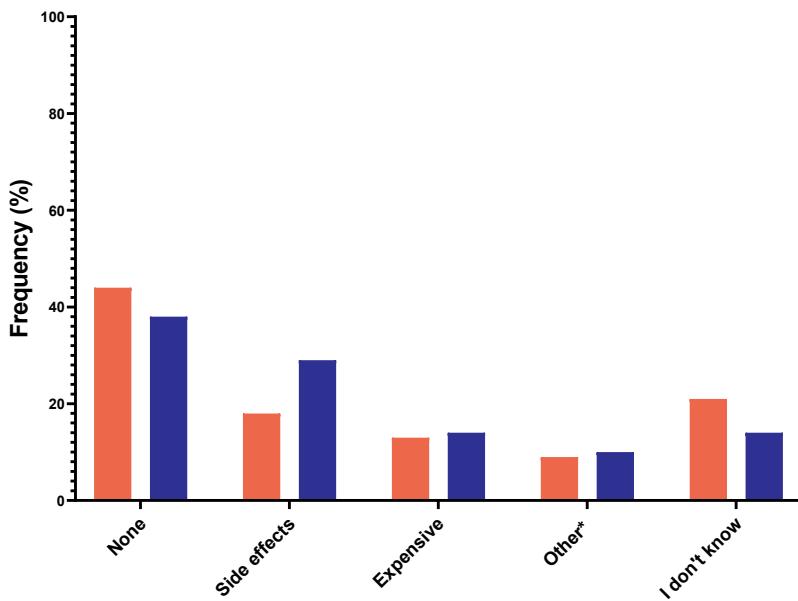
Figure 3: Reported disadvantages of desmopressin treatment in adults (red bars, *n* = 206) and children with non-severe hemophilia A (blue bars, *n* = 21). *The other reported disadvantages in adults were as follows: limited shelf-life (*n* = 3), storage temperature (*n* = 2), not usable after urologic procedure because of fluid intake (*n* = 1), fluid restriction (*n* = 4), seems to evaporate (*n* = 1), not usable anymore because of a higher risk of epilepsy (*n* = 1), not usable anymore because of atrial fibrillation (*n* = 1), occurrence of ureter clots (*n* = 1), deductible for nasal spray (*n* = 1), and limited efficacy (*n* = 4). The other reported disadvantages in children were as follows: fluid restriction (*n* = 2).

Of 204 adults and 21 children who also reported to have been treated with desmopressin, 101 (50%) adults and 9 (43%) children classified their knowledge as enough, 46 (23%) adults and 6 (28.5%) children as moderately satisfactory, 24 (12%) adults and four (19%) children classified their knowledge as not enough and 33 (16%) adults and 2 (9.5%) children did not know. Seven adults and two children who had been treated with desmopressin in the past, did not answer aforementioned question.

Of the 106 adults and 10 children who did not know if they had received desmopressin, 3 (3%) adults and 3 (30%) children classified their knowledge as enough, 7 (7%) adults and 4 (40%) children classified their knowledge as moderately satisfactory, 31 (29%) adults and none of the children as not enough and 65 (61%) adults and 3 (30%) children did not know. The difference between mild and moderate patients is depicted in Supplementary Table 5 with comparable responses between both groups.

Barriers and facilitators for desmopressin use

Adult patients and children were asked in the open question how the use of desmopressin could be improved and stimulated. In total, 48 adults answered. The reported answers were: the presence of a relative contraindication (i.e. chronic heart failure; n = 9), less side effects (n = 7), less costs (n = 6), more information on desmopressin (n = 7), more efficacy (n = 8), more availability of desmopressin (n = 4) (i.e. more easily obtainable nose spray), a longer shelf-life (of the intranasal administration; n = 4), the availability as a home treatment, reassuring the patient, (n = 1), good efficacy (n = 1) and its use for sports' participation (as facilitator). Interestingly, one patient reported that because of his work in a country with low FVIII concentrate resources, he was dependent on the use of intranasal desmopressin for emergencies. Among children, one child wanted more efficacy and less side effects. Two caretakers reported that they did not know what desmopressin was, one of them emphasizing that they would want more information. The findings are summarized in Supplementary Table S6, with quotes of interest depicted in Supplementary Table S7.

5

Discussion

In our study of 706 non-severe hemophilia A patients, of those who reported on historical desmopressin use, approximately 50% of the adults and children reported to have been ever treated with desmopressin and have undergone a desmopressin test. Additionally, 90% of the patients who had been treated with desmopressin reported that it was at least moderately effective enough to be used to prevent or treat bleedings. In 26% of the patients who had been treated with desmopressin, knowledge on desmopressin use was considered as not enough or unknown. The most reported barriers for desmopressin use was the presence of a contraindication, side effects and costs.

Multiple cohort studies have shown at least a partial response in 66% - 78%[13-16], up to 88%-98%[17,18], of the studied male patients with mild hemophilia A. In our study, the patient-reported efficacy was high as well, but no absolute desmopressin FVIII:C response was available, nor bleeding outcome measures. Therefore, a direct comparison cannot fully be made.

As patients were only allowed to fill out one treatment modality for the treatment in case of bleeding, most treatment plans listed FVIII concentrate as preferred treatment. These data are likely an underrepresentation of the general use of desmopressin: besides a (mild) bleeding, patients could be applying desmopressin prophylactically, i.e. before sports' participation, as was reported to be a facilitator. Despite a seemingly lower number of moderate hemophilia A patients who reported using desmopressin in comparison to mild hemophilia A patients in this study, previous research has shown the merits of desmopressin in moderate hemophilia A patients[19,20].

The most frequent reported side effects, flushing and headache, were also reported earlier in literature. In a study by Stoof et al.[10], the side effects of desmopressin were assessed in patients with a bleeding disorder who had just received desmopressin. Of 103 patients reporting side effects after one hour of intravenous desmopressin administration, itching eyes (68%), flushing (59%), headache (34%) and fatigue (40%) were reported as the most prevalent over multiple time points after desmopressin administration[10]. However, the frequency of the self-reported side effects in our study were lower than reported in Stoof et al.[10], which can possibly be explained by recall bias as most patients in the present study were not included in proximity of a recent desmopressin administration.

The most reported advantages of desmopressin were the convenience of intranasal administration and possibility of home treatment (intranasal of subcutaneous). The current World Federation of Hemophilia guideline states that the use of intranasal desmopressin can be difficult, possibly negatively influencing treatment. Although we did

not ask explicitly if a nasal spray was easy to use, it was not reported as a disadvantage by any of our patients[1]. Interestingly, the safety of desmopressin (i.e. no inhibitor formation, blood-borne diseases) and its use as an alternative for FVIII concentrate were less frequently considered as advantages than the aforementioned convenience.

The most reported disadvantage, namely side effects, was only reported in approximately one out of six patients. When choosing the route of administration, it is good to realize that subcutaneous or intranasal desmopressin results in less vasomotor side effects, such as flushing or headache, than intravenous administration[10,21]. However, at the time of the survey, subcutaneous desmopressin administration was not (yet) standard of care and therefore subcutaneous desmopressin was not added as a survey response option. Furthermore, the use of intranasal desmopressin can be challenging because of a slower rate of absorption and lesser magnitude of response in comparison to parenteral administration, in combination with other possible limiting factors such as epistaxis or nasal blockage[22,23]. One of the other reported disadvantages, the expensive cost of desmopressin, is related to the Dutch health care system: a yearly cumulative, minimum deductible €385 is demanded for medical care, including medication such as intranasal desmopressin. One container of intranasal desmopressin already charges this whole deductible. For non-severe hemophilia A patients with no other significant yearly healthcare costs who do not fully apply the whole deductible could consider this costly, especially in combination with the nasal spray's short shelf-life.

The knowledge of desmopressin was considered unsatisfactory or unknown by approximately a quarter of the respondents who had received desmopressin. Research on hemophilia education has shown that even a single education intervention on the knowledge and management of bleeding temporarily improved quality of life in persons with hemophilia A and parents of children with hemophilia A, albeit temporarily[24,25]. In other chronic disorders, such as diabetes mellitus type II, research on (self-care) education programs has also shown improvement on disease management and quality of life, with a longer program of five days leading to an improvement for up to two years [26,27]. More and frequent information and patient education on the use and self-management with desmopressin could, therefore, increase the quality of life in non-severe hemophilia A patients and answer possible unmet needs in this group related to our study-reported advantages of desmopressin such as home treatment. Unfortunately, however, as of the writing of this paper, intranasal desmopressin is not readily available worldwide.

Our study was the first large prospective study on the patients' perspectives of desmopressin use in adults and children with non-severe hemophilia A but was limited by some aspects of the survey. Not all patients who were included in the study, reported on

desmopressin use (74%). In addition, patients did not always fill out questions concerning treatment (i.e. management plan) and were sometimes limited to only one answer option, leading to missing data. This could have been caused by the use of different names for desmopressin used throughout the questionnaire (i.e. DDAVP, Octostim, Minrin). As no information on ethnicity was available in the HiN6 study, we were not able to assess its influence as a sociocultural determinant for the patients' perspectives on desmopressin. Recall bias could also have limited the reported number of desmopressin use in the survey. Furthermore, people who use desmopressin more often or who are more likely to use it, are also more likely to fill out the survey on desmopressin. This could lead to underreporting desmopressin use. The perceived efficacy of desmopressin could have been influenced by concomittant use of other medication such as antifibrinolytics, partially influencing the patients' opinion in favor of desmopressin. Additionally, as these questions were part of a larger survey, less attention could have been given to this specific category of question, leading to less overall response. We believe that all eligible non-severe hemophilia A patients should be informed on desmopressin's efficacy, use and side effects. After informing the patient (or caregiver), their perspective can be taken into account in order to decide with their health care provider whether desmopressin treatment is preferable. The main topics of discussion could be side effects, costs (if applicable) and the ability of home treatment.

Conclusions
Approximately half of the non-severe hemophilia A patients in this subanalysis of the HiN6 study reported to have ever received desmopressin, most often intranasally and 90% reported at least moderate effectiveness. Flushing was the most commonly reported side effect. The most frequent reported advantage was the convenience of intranasal administration and possibility of home treatment, and the most frequent reported disadvantages were the presence of side effects and high costs. More information and more education on desmopressin from healthcare providers can answer unmet needs in patients currently receiving desmopressin and the desmopressin-naive non-severe hemophilia A patients.

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Supplementary tables

Supplementary Table S1: Patient characteristics of patients who reported to have had desmopressin treatment versus those who reported not.

Supplementary Table S2: Questionnaire responses on efficacy of desmopressin for certain situations in hemophilia A patients.

Supplementary Table S3: Questionnaire responses on advantages for desmopressin use in moderate and mild hemophilia A patients.

Supplementary Table S4: Questionnaire responses on disadvantages for desmopressin use in moderate and mild hemophilia A patients.

Supplementary Table S5: Questionnaire responses on knowledge of efficacy and side effects of desmopressin use in moderate and mild hemophilia A patients.

Supplementary Table S6: Questionnaire responses on barriers and facilitators fordesmopressin use in moderate and mild hemophilia A patients.

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Supplementary Table S1: Patient characteristics of patients who reported to have had desmopressin treatment versus those who reported not.

	Adults (≥18 years) Median (IQR) / n [%]
	No treatment (n = 105)
Lowest historical measured FVIII:C (one-stage, IU/mL)	0.06 (0.02 - 0.10)
Age (years) at inclusion	54 (39 - 66)
Hemophilia severity	
Mild	65 [62%]
Moderate	40 [38%]

Supplementary Table S2: Questionnaire responses on efficacy of desmopressin for certain situations in hemophilia A patients.

Hemophilia A adults (n = 28)	n (%)
Before procedures to prevent bleeds	12 (43%)
Minor bleeds	10 (36%)
After trauma	2 (7%)
Minor bleeds and before procedures to prevent bleed	2 (7%)
Last part of treatment after clotting factor concentrate	1 (3.5%)
Joint bleed	1 (3.5%)

Adults (≥18 years) Median (IQR) / n [%] With treatment (n = 211)	Children (<18 years) Median (IQR) / n [%] No treatment (n = 49)	Children (<18 years) Median (IQR) / n [%] With treatment (n = 23)
0.13 (0.07 - 0.19) (n = 150)	0.07 (0.02 - 0.11) (n = 26)	0.12 (0.11 - 0.17) (n = 12)
50 (35 - 62) (n = 209)	5 (3 - 9) (n = 48)	11 (10 - 14)
186 [88%]	32 [65%]	22 [96%]
25 [12%]	17 [35%]	1 [4%]

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Supplementary Table S3: Questionnaire responses on advantages for desmopressin use in moderate and mild hemophilia A patients.

	Adults (≥18 years) n (%) Mild (n = 180)	Adults (≥18 years) n (%) Moderate (n = 25)	Children (<18 years) n (%) Mild (n = 22)	Children (<18 years) n (%) Moderate (n = 1)
None	17 (9%)	5 (20%)	2 (9%)	-
Easy to use	94 (52%)	14 (56%)	17 (77%)	1 (100%)
No rFVIII needed	31 (17%)	1 (4%)	4 (18%)	-
No injection needed	34 (19%)	4 (16%)	4 (18%)	-
Useable at home	71 (39%)	9 (36%)	12 (55%)	-
Fast	69 (38%)	7 (28%)	11 (50%)	-
Safe	36 (20%)	5 (20%)	1 (46%)	-
Cheap	16 (9%)	4 (16%)	2 (9%)	-
I don't know (unknown)	37 (21%)	3 (12%)	2 (9%)	-
Other*	2 (1%)	-	-	-

*The other reported advantages in adults were: gives me inner peace and assurance for internal examinations (n = 1), handy for travel (n = 1).

Supplementary Table S4: Questionnaire responses on disadvantages for desmopressin use in moderate and mild hemophilia A patients.

	Adults (≥18 years) n (%)	Adults (≥18 years) n (%)	Children (<18 years) n (%)	Children (<18 years) n (%)
	Mild (n = 181)	Moderate (n = 25)	Mild (n = 21)	Moderate (n = 0)
None	82 (45%)	8 (32%)	8 (38%)	-
Expensive	26 (14%)	-	3 (14%)	-
Side effects	30 (17%)	7 (28%)	6 (29%)	-
I don't know (Unknown)	37 (20%)	7 (28%)	2 (10%)	-
Limited efficacy	4 (22%)	-	-	
Limited shelf-life	3 (17%)	-	-	-
Storage temperature	2 (11%)	-	-	-
Fluid restriction	3 (17%)	1 (4%)	2 (10%)	-
Not usable after urological procedure because of fluid intake	1 (6%)	-	-	-
Not usable anymore because:				
higher risk for epilepsy	1 (6%)	-	-	-
atrial fibrillation	1 (6%)	-	-	-
Deductible for nasal spray	1 (6%)		-	-
Seems to evaporate	-	1 (4%)	-	-
Occurrence of ureter clot	-	1 (4%)	-	-

Supplementary Table S5: Questionnaire responses on knowledge of efficacy and side effects of desmopressin use in moderate and mild hemophilia A patients.

	Adults (≥18 years) n (%) Mild (n = 251)	Adults (≥18 years) n (%) Moderate (n = 59)	Children (<18 years) n (%) Mild (n = 29)	Children (<18 years) n (%) Moderate (n = 2)
Not enough	42 (17%)	13 (22%)	6 (21%)	1 (50%)
Moderately enough	91 (36%)	10 (17%)	10 (35%)	-
Enough	43 (17%)	13 (22%)	9 (31%)	-
I don't know (unknown)	75 (30%)	23 (39%)	4 (14%)	1 (50%)

Supplementary Table S6: Questionnaire responses on barriers and facilitators for desmopressin use in moderate and mild hemophilia A patients.

in adults with...	... mild hemophilia A (n = 37)	... moderate hemophilia A (n = 11)
Presence of a relative contraindication (e.g. epilepsy, chronic heart failure)	8	1
Less side effects	6	1
Less costs	6	-
More information on desmopressin is needed	5	2
More efficacy is needed	5	3
More availability of desmopressin	3	1
A longer shelf-life (of the intranasal administration)	2	2
The availability of desmopressin as a home treatment is reassuring	1	-
The efficacy of desmopressin is well	1	-
Use for sports' participation (is a facilitator)	-	1
in children with...	... mild hemophilia A (n = 3)	
More efficacy and less side effects	1	
No idea what desmopressin is	1	
No idea what desmopressin is: more information on desmopressin is needed	1	

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Supplementary Table S7: list of literal quotes concerning barriers and facilitators of desmopressin use.

Also storable outside of the refrigerator.

The past years I have kept some spare DDAVP after a few short treatments, just in case of a bleeding. Preventive DDAVP in my own home gives me inner peace and this pleases me.

I sometimes use it prophylactically when running more than twenty kilometers.

I only use DDAVP for emergencies. Not regularly. I have DDAVP in home i.e. for holidays. But usually I have to bin the nasal spray after its shelf-life has expired. A new spray immediately costs me my deductible and in my opinion that's a lot of money.

I have used it before, but because of heart failure I unfortunately cannot use it anymore.

More information is needed: my physicians told me that I'd rather not use DDAVP anymore considering my age (sixty-six years old). However, I regularly work in third world countries and therefore this is my only remedy, so I don't have a choice I think...?

I don't have any suggestions, as long as it is safe to use. I trust the physicians, I trust that it has been properly tested.
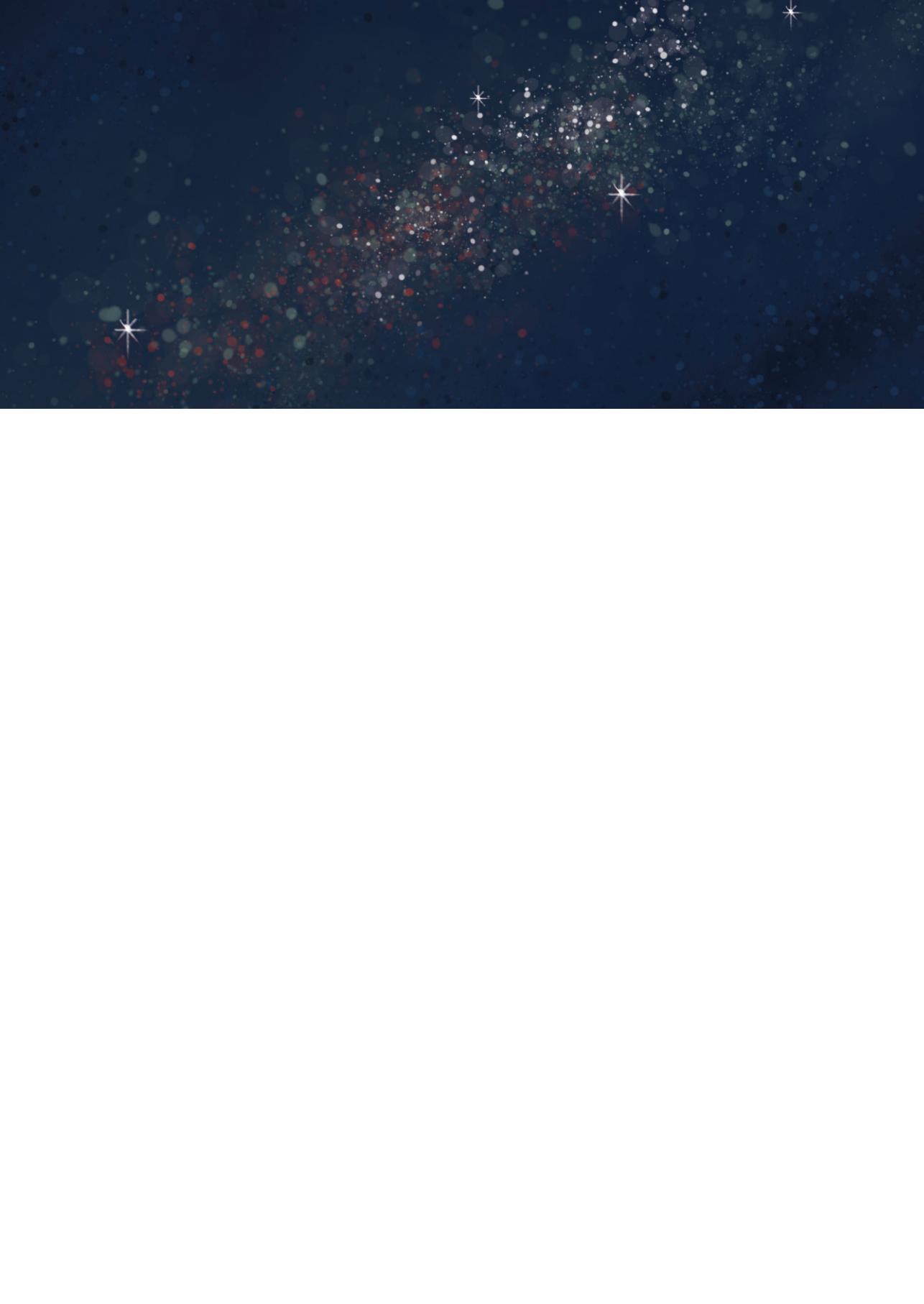
CHAPTER 6

DESMOPRESSIN TO PREVENT AND TREAT BLEEDING IN PREGNANT WOMEN WITH AN INHERITED BLEEDING DISORDER:
A SYSTEMATIC LITERATURE REVIEW

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Abstract

Background
Although desmopressin (DDAVP) is an accessible and inexpensive hemostatic drug, its use in pregnancy is still debated due to safety uncertainties.

Objectives
We aimed to review the safety and effectiveness of DDAVP in women with an inherited bleeding disorder during pregnancy and delivery.

Methods
Databases were searched for articles up to July 25, 2022, reporting maternal and/or neonatal outcomes. PRISMA methodology for systematic reviews and meta-analyses was followed (PROSPERO CRD42022316490).

Results
Fifty-three studies were included, comprising 273 pregnancies. Regarding maternal outcomes, DDAVP was administered in 73 women during pregnancy and in 232 during delivery. Safety outcome was reported in 245 pregnancies, with severe adverse events reported in 2 (1%, hyponatremia with neurologic symptoms). Overall, DDAVP was used as monotherapy in 234 pregnancies, with effectiveness reported in 153 pregnancies (82% effective; 18% ineffective). Regarding neonatal outcomes, out of 60 pregnancies with reported neonatal outcomes after DDAVP use during pregnancy, 2 children (3%) had a severe adverse event (preterm delivery n = 1; fetal growth restriction n = 1). Of the 232 deliveries, 169 neonates were exposed to DDAVP during delivery, and in 114 neonates, safety outcome was reported. Two children (2%) experienced a moderate adverse event (low Apgar score n = 1; transient hyperbilirubinemia not associated with DDAVP n = 1).

Conclusion
DDAVP use during pregnancy and delivery seems safe for the mother, with special attention to the occurrence of hyponatremia and for the child, especially during delivery. However, due to poor study designs and limited documentation of outcomes, a well-designed prospective study is warranted.

Introduction

Desmopressin, 1-deamino-8-D-arginine vasopressin (DDAVP), a synthetic analogue of antidiuretic hormone vasopressin, is an effective hemostatic drug that increases endogenous von Willebrand factor (VWF) and factor VIII (FVIII) plasma concentrations by stimulating the release of VWF/FVIII stores from vascular endothelial cells[1]. Desmopressin increases circulating VWF and FVIII levels three to five fold above basal levels, with plasma half-life of 5 to 8 hours and 8 to 10 hours for FVIII and VWF, respectively[2]. Due to interindividual variability in DDAVP response, a DDAVP test is warranted[3]. Desmopressin is regularly used in patients with inherited bleeding disorder including von Willebrand disease (VWD), mild to moderate hemophilia A and platelet function disorders (PFDs) or patients with a bleeding disorder of unknown cause to prevent and treat bleeding[4]. But it can also be used in other less common disorders with hemostatic defect such as factor XI (FXI) deficiency, Hermansky-Pudlak syndrome (HPS) and Ehlers Danlos syndrome (EDS)[5]. The common applied dose is 0.3 µg/kg intravenously or subcutaneously and this can be capped when body weight exceeds 100 kg with a maximum recommended dose of 30 mcg[6]. Intranasal administration of DDAVP is often used for milder bleeds in the home stetting. Repeated administration of DDAVP is limited due to depletion of VWF/FVIII stores, also described as tachyphylaxis[4]. Potential adverse effects of DDAVP are well characterized, compromising mild vasomotor effects (facial flushing, hypotension, and tachycardia), incidental antidiuretic effects (volume overload and hyponatremia with risk of seizures), and rare thrombotic complications (myocardial infarction and stroke). Fluid restriction can help avoid the antidiuretic effects. DDAVP is contradicted in patients with pre-existing cardiovascular disease.

Pregnancy and delivery represent a major hemostatic challenge in women with an inherited bleeding disorder. Prophylaxis or treatment of postpartum hemorrhage (PPH) might be required for these patients. In addition, hemostatic treatment may be necessary during pregnancy to prevent bleeding during pregnancy-related procedures (i.e. chorionic villus sampling, amniocentesis, cervical cerclage or pregnancy termination) or other medical interventions. Although DDAVP is effective in patients with an adequate response during DDAVP testing, its utilization during pregnancy and delivery is still debated due to safety concerns for the pregnant woman and her unborn child. DDAVP was initially used during pregnancy in women with diabetes insipidus because of its antidiuretic effects[7]. A review by Ray et al.[7] evaluated DDAVP treatment in diabetes insipidus during pregnancy and reported 53 cases which are described in a total of 20 publications. In these cases, DDAVP administration during pregnancy did not result in any DDAVP-related maternal or neonatal adverse events. However, there are several important differences in DDAVP use between inherited bleeding disorders and diabetes insipidus. In diabetes insipidus

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DDAVP is administered daily and usually orally or intranasally at lower doses (3 to 40 times lower doses per day in case of intranasal administration). Trigg et al.[8] conducted the first literature review on DDAVP in bleeding disorders and reported 212 pregnancies from a total of 30 publications[8]. Twenty-nine studies reported no significant maternal and neonatal adverse events. One study reported one case with water intoxication seizure and one case with premature labour after DDAVP infusion. No data was provided on the implementation of fluid restriction regime or risk factors for these two complications.

Despite the report of only 1 maternal and neonatal adverse event and limited information regarding potential contributing factors, such as the implementation of fluid intake restriction and the presence of other relevant conditions, safety concerns are still predominant in daily practice. This leads to an overall avoidance of DDAVP treatment during pregnancy and delivery. Therefore, additional data on safety are required. Moreover, evidence regarding effectiveness is also needed, as bleeding during pregnancy and delivery, especially PPH, lead to high morbidity and mortality rates[9]. Consequently, this systematic review aims to evaluate safety and effectiveness of DDAVP during pregnancy, delivery and postpartum period in women with an inherited bleeding disorder.

Methods

This systematic review was performed according to the PRISMA methodology for systematic reviews and meta-analysis and registered at PROSPERO, an international prospective register of systematic reviews (registration number CRD42022316490)[10].

Search strategy
A medical research library specialist co-designed and conducted the research strategy. The search was performed in EMBASE, Medline ALL, Cochrane Central Register of Controlled Trails, Web of science core Collection and Google Scholar. All databases were searched from inception until July 25th 2022. The search strategy included multiple medical heading terms and keyword for inherited bleeding disorders, desmopressin and pregnancy. The full details of the literature research including used key terms are available in the supplementary (Supplementary 1).

Study selection and eligibility criteria
Retrieved citations were independently assessed by two reviewers (W.A. and L.G.R.R) to identify studies that met inclusion criteria. Disagreements were discussed until consensus was reached. English articles reporting on maternal and/or neonatal outcomes during DDAVP treatment in pregnant women with an inherited bleeding disorder were included. Besides VWD, hemophilia carriership, PFD, also rare diseases such as FXI deficiency, ESD, HPS and Noonan syndrome were selected. DDAVP use in pregnancy was evaluated from reports if treatment was received during first, second and third trimester (antepartum), during delivery (intrapartum); and in the postpartum period (defined until 6 week after child delivery). For articles published more than once and were suspected of overlapping study populations, only the study with the largest number of pregnancies and most complete data was included. All review articles were screened to select case reports. Conference abstracts and articles concerning acquired bleeding disorders or articles missing any outcome related to DDAVP use were excluded. The reference lists of included studies identified by the literature search were alaso cross-checked for relevant citations.

Data extraction and assessment
For each included study, the following information was collected: study design, study population, treatment indication, details of DDAVP administration (route, dosage, and frequency), additional hemostatic treatment such as antifibrinolytic agents, factor concentrates, cryoprecipitate, platelet concentrates or other blood products, mode of delivery, and maternal and neonatal outcomes. Maternal outcomes included the effectiveness of DDAVP treatment to prevent or treat bleeding. DDAVP was considered effective if there was minimal bleeding, including PPH, or bleeding was resolved after

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DDAVP and no additional hemostatic agents were required. PPH was defined, according to World Health Organization (WHO) definition, as a blood loss of ≥ 500 mL within 24 hours after delivery, and for caesarean section a blood loss of ≥ 1000 mL according to commonly used definition[11,12]. Only DDAVP treatment without additional replacement therapies with factor concentrates or cryoprecipitate was evaluated for effectiveness. For safety evaluation, reported adverse events occurring in both mother and child were collected, with special attention to adverse effects related to DDAVP comprising vasomotor effects, antidiuretic effects and thrombotic complications[4]. For the child, adverse events were categorized into adverse events following (1) DDAVP exposure during pregnancy (antepartum exposure) and (2) DDAVP exposure during delivery (intrapartum exposure). Adverse events were graded according severity (mild, moderate, severe and life-threatening), using the Common Terminology Criteria for Adverse Events (CTCAE). Data on maternal and neonatal outcomes were defined as unknown if studies did not mentioned the outcome or authors reported outcomes as unknown.

Two reviewers (W.A. and L.G.R.R) independently extracted the data from each included study using a standardized data collection form. Discrepancies were resolved by consensus after a mutual discussion. Due to methodological heterogeneity of the studies, a descriptive review of all included studies was performed with summary of outcomes rather than a statistical analysis.

Risk of bias assessment

Risk of bias was assessed according to the National Institutes of Health (NIH) quality assessment tool for observational and cross-sectional studies [13]. Two reviewers (W.A. and L.G.R.R) independently assessed and rated each observational study. Quality rating conforming to the tool (good, fair or poor) was used to assess certainty of evidence. Inconsistencies were discussed and resolved by consensus. Studies were not excluded based on risk of bias assessment. The design of case reports was considered as a bias, therefore case reports were not assessed.

Results

Data retrieval

The systematic review yielded a total of 1029 unique references that were screened using predetermined criteria. A total of 53 unique articles met the inclusion criteria. The flow chart (Figure 1) shows the process of article selections from initial search to final inclusion or exclusion.
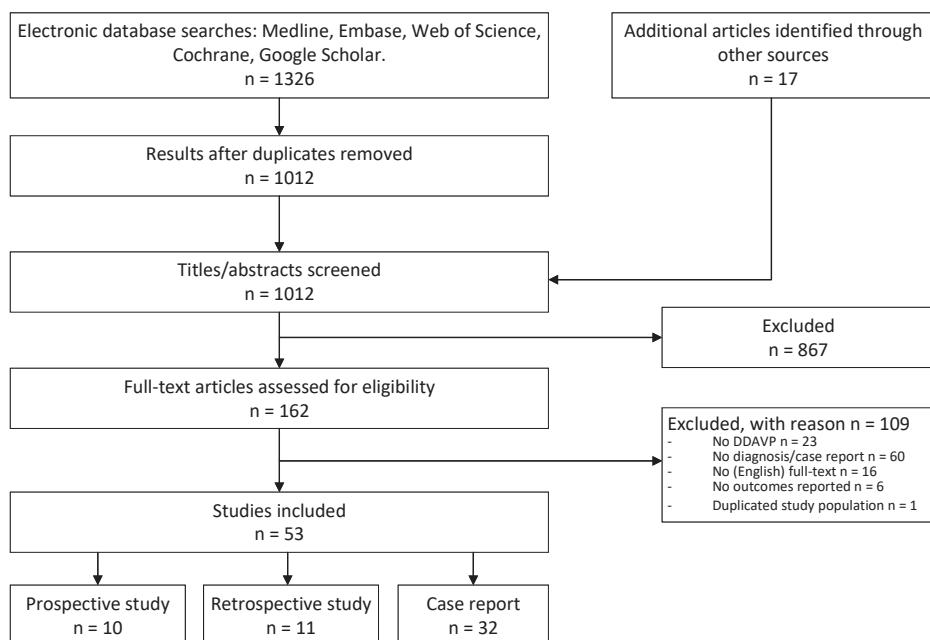

Figure 1: Flow chart for study identification, adapted from PRISMA Flow diagram. DDAVP, desmopressin; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

Study characteristics

All studies were published between 1986 and 2022 (Figure 2). Twenty-one articles were observational studies (prospective n = 10; retrospective n = 11)[14-34]. The remaining 32 articles were case reports or case series[35-66]. Most observational studies were conducted in the United States (n = 6) and Italy (n = 6), followed by United Kingdom (n = 3), and other European or Asian countries (n = 6). Case reports were conducted in different counties around the world, with the most reports in the United States (n = 15). The majority of observational studies focused on VWD (n = 17). Other studies included patient with hemophilia carriership (n = 2), PFD (n = 1) and FIX deficiency (n = 1). These disorders

were also studied in case reports among other rare inherited bleeding disorders including HPS, EDS and a combination of bleeding disorders. In nine included studies was the main objective to investigate the safety and, or effectiveness of DDAVP in pregnancy (5 observational studies[14,16,26,29,31]; 4 case reports[39,45,61,66]). Other studies mainly focused on the management of pregnancy and delivery and reported the use of DDAVP among other outcomes. Figure 3 presents the risk of bias assessment. Twelve studies were adjudicated as having poor quality, and nine fair quality. The most prevalent limitations were found in items related to sample size justification, reporting exposure and outcome measures and statistical analyses.
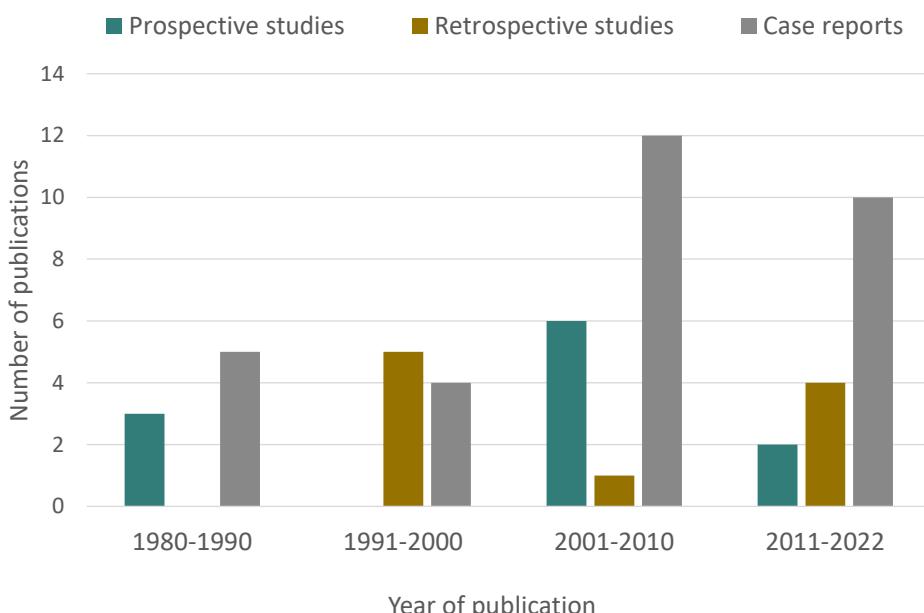

Figure 2: Publication year of included studies.

Studied pregnancies

A total of 273 pregnancies were studied (237 observational studies; 36 case reports). The exact number of women is unknown, as some studies did not report this information. VWD was most involved with 212 (78%) pregnancies, followed by hemophilia A carriership (31 pregnancies, 11%), PFD (14 pregnancies, 5%), HPS (8 pregnancies, 3%), FXI deficiency (3 pregnancies, 1%), EDS (2 pregnancies, 1%), and a combination of disorders (2 pregnancies, 1%). Regarding the type of disease in VWD, VWD type 1 was observed in 79 pregnancies (37%), VWD type 2 in 4 pregnancies (2%), VWD type 3 in 1 pregnancy, and platelet type VWD in 1 pregnancy. The type of VWD was not specified or reported as unknown in 127

pregnancies. Concerning PFD, patients with various disorders were treated with DDAVP: Gray platelet syndrome (2 pregnancies), Bernard-Soulier syndrome (4 pregnancies), platelet storage pool disorder (5 pregnancies), and Glanzmann's thrombasthenia (1 pregnancy). The type of PFD was not specified in the 2 pregnancies. Of the 273 pregnancies, DDAVP was administered in a total of 311 treatment episodes. This included 235 pregnancies with 1 treatment episode and 38 pregnancies with 2 treatment episodes. Specifically, DDAVP was administrated during pregnancy (antepartum period) in 73 pregnancies, during delivery in 232 pregnancies, and during the postpartum period in 6 pregnancies. During the antepartum period, DDAVP was given in all 3 trimesters (first trimester, 28 [38%] pregnancies; second trimester, 12 [16%] pregnancies; third trimester, 33 pregnancies [45%]). In these cases, the indication for DDAVP was to prevent bleeding (*n* = 71) and to treat (*n* = 2) obstetrical-related bleeding (7 studies[18,20,22,26,29,36,52]). In 232 pregnancies where DDAVP was given during delivery, the indication for DDAVP was PPH prophylaxis (*n* = 226) and PPH treatment (*n* = 6)[14-18,20,21,23-25,27-31,33-46,48-51,55,56,58-66]. In addition, in 6 pregnancies, DDAVP was initiated as treatment during the late postpartum period due obstetric-related bleeding[32,47,53,54,57]. In 38 pregnancies with 2 treatment episodes, DDAVP was given during the antepartum as well as during delivery[20,29,36]. In 32 of these pregnancies, DDVAP was administered from week 36 of gestational age until the postpartum period with an unknown dose frequency[20]. In relation to the use of tranexamic acid during delivery in combination with DDAVP (without replacement therapy), 2 observational studies and 2 case reports documented this combined treatment. This combined treatment approach was applied in 15 pregnancies (12 in VWD and 3 in PFD) as prophylaxis for PPH. Different dosing regimens were applied, commencing with intravenous (i.v.) administration and continuing with oral administration of tranexamic acid for different treatment durations. Concerning the route of administration for each treatment episode, DDAVP was given intravenously in 213 pregnancies (75%), intranasally in 67 pregnancies (24%), and subcutaneously in 2 (1%) pregnancies. The route of administration was unknown in 29 pregnancies. Regarding dose frequency, DDAVP was administered in 1 dose in the majority of cases (*n* = 152, 75%), followed by 2 doses (*n* = 32, 15%), and ≥3 doses (*n* = 19, 9%). In 108 treatment episodes, the dose frequency was not reported. In addition to the recommended dosage for i.v. DDAVP of 0.3 µg/kg, dosages of 0.2 and 0.4 µg/kg were also used.

Maternal and neonatal outcomes

Table 1 provides an overview of maternal and neonatal outcomes of included observational studies. For case reports Supplementary 2 summarizes the outcome of each case report.
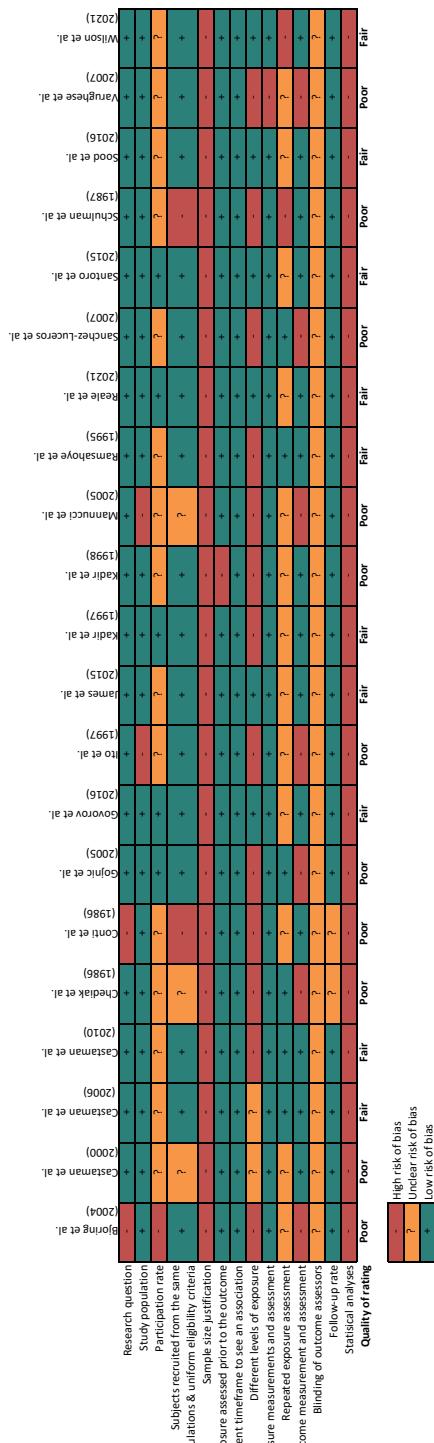
Figure 3. Risk of bias assessment using the National Institute of Health quality assessment tool for observational cohort and cross-sectional studies.

Maternal outcomes: effectiveness

Of the 73 pregnancies in which DDAVP was administered during antepartum, in 41 pregnancies DDAVP was given as monotherapy without replacement therapy. The indication for DDAVP in these pregnancies was prenatal diagnostic testing or other obstetrical-related intervention (planned abortion and cervical cerclage) (*n* = 38), DDAVP response test (*n* = 1), and treatment for an obstetrical-related bleeding (retroplacental hematoma and vaginal bleeding) (*n* = 2). DDAVP was reported to be effective in all pregnancies except in 1 with an unknown outcome. DDAVP was administered intravenously in all cases. Most pregnancies received 1 dose (29 of 41 pregnancies, 70%), and some received 3 or more doses (10 of 41 pregnancies, 24%). In the remaining 32 pregnancies reported in 1 study, DDAVP was indicated for PPH prevention and was initiated during the antepartum period (36 weeks of gestation) with intranasal administration until 6 weeks of the postpartum period. In addition, these women also received treatment with replacement therapy during delivery[20]. During the intrapartum period, DDAVP was administered without additional replacement therapy in 192 out of 232 pregnancies. PPH prophylaxis was the most frequent indication (185 pregnancies, 97%), followed by PPH treatment (6 pregnancies, 3%). The majority of women (165 pregnancies out of 192 pregnancies) had VWD. Moreover, in 87 of these 165 pregnancies, the effectiveness of treatment was reported. The treatment was effective in 74 pregnancies (85%) and ineffective in 13 pregnancies (15%). Table 2 summarizes the effectiveness outcome of each inherited bleeding disorder. Vaginal delivery was the most frequent mode of delivery in pregnancies with DDAVP monotherapy, with 84 (57%) pregnancies, 63 (43%) pregnancies with cesarean section, and in 44 pregnancies the mode of delivery was unknown. Concerning the combined treatment with DDAVP and tranexamic acid in 15 pregnancies for PPH prophylaxis, PPH was reported in 7 of these pregnancies and no PPH in 8 pregnancies.

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Table 1: An overview of pregnancies, treatment with DDAVP, and maternal and neonatal outcomes of each included observational study.

References (year)/country	Pregnancies using DDAVP/total pregnancies* (*n*/*n*)	Inherited bleeding disorder	Indication for DDAVP/ Timing of dosing (trimester/delivery/postpartum)
Bjoring et al.[14] *United States*	7/19	VWD • Type NS *n* = 6 • Type 3 *n* = 1	PPH prophylaxis, before delivery
Castaman et al.[15] *Italy*	3/6	VWD • Type 1 *n* = 3	PPH prophylaxis, after delivery
Castaman et al.[16] *Italy*	5/6	VWD • Type 1/Vicenza *n* = 5	PPH prophylaxis, after delivery
Castaman et al.[17] *Italy*	23/31	VWD • Type 1 *n* = 15 • Type 1/Vicenza *n* = 6 • Type 2 *n* = 2	PPH prophylaxis, after delivery
Chediak et al.[18] *United States*	2/8	VWD • Patient 1: type 1 • Patient 2: type NS	• Patient 1: PPH prophylaxis, after delivery • Patient 2: a test dose, third trimester
Conti et al.[19] *Italy*	1/5	VWD • Type 1	Secondary PPH treatment, postpartum

Treatment (route, dosage, dose frequency)	Mode of delivery	Maternal outcomes	Neonatal outcomes
DDAVP, 0.3 µg /kg, i.v., one dose	VD	• One patient had PPH. This was a patient with type 3 VWD who was responsive to DDAVP • No adverse effects or complications	No adverse events or effects
DDAVP i.v., 2 doses, dosage not specified	VD	• No Primary PPH • Adverse effects: not mentioned	NA
DDAVP, 0.3 µg/kg, i.v.; two doses every 24 hours	VD	• No primary PPH (≤ 300 mL) • No excessive blood in the late puerperium • Adverse effects: not mentioned	NA
DDAVP, 0.3 µg/kg, i.v.; one dose n = 5, two doses n = 13, and three doses n = 5	VD	• No primary PPH • One patient had on day 5 secondary PPH, treated with FVIII and VWF concentrate • Adverse effects: no signs of water intoxication or serum electrolyte abnormalities	NA
• Patient 1: DDAVP, 0.3 µg/kg, i.v.; 3 doses, every 18 hours, cryoprecipitate • Patient 2: DDAVP 0.4 µg/kg i.v.; one dose	Patient 1: CS Patient 2: NS	• PPH occurrence in both patients, not specified • *Patient 1*: Hyponatremia (108 mEq/L), water intoxication seizure • *Patient 2*: premature labor	• Patient 1: NA • Patient 2: Preterm newborn at 36 weeks • No other adverse events or effects
• DDAVP, 0.4 µg/kg, i.v., one dose; • 4x Packed cells	CS	• Bleeding stopped after DDAVP administration • Adverse effects: not mentioned	NA

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Table 1: Continued

References (year)/country	Pregnancies using DDAVP/total pregnancies* (*n*/*n*)	Inherited bleeding disorder	Indication for DDAVP/ Timing of dosing (trimester/delivery/postpartum)
Gojnic et al.[20] *Serbia*	32/32	VWD • Type 1 and type 2A, exact numbers NS	PPH prophylaxis, 36 weeks gestation until 4 weeks postpartum
Govorov et al.[21] *Sweden*	12/59	VWD • Type 1 *n* = 11 • Type 2 *n* = 1	PPH prophylaxis, timing of administration not specified
Ito et al.[22] *Japan*	1/14	VWD • Type 1	Induced abortion, first trimester
James et al.[23] *United States*	2/35	VWD • Type 1	PPH prophylaxis, before delivery
Kadir et al.[24] *United Kingdom*	4/82	HA-carriership	• PPH prophylaxis, after delivery *n* = 3 • Primary PPH treatment *n* = 1
Kadir et al.[25] *United Kingdom*	1/112	VWD • Type NS	Treatment primary PPH, after delivery

	Treatment (route, dosage, dose frequency)	Mode of delivery	Maternal outcomes	Neonatal outcomes
	• DDAVP, 300 µg i.n; dose not mentioned • 40-60 IU/kg Hemate P; 4 doses, every 24 hours • Cryoprecipitate and fresh frozen plasma, 4-6 doses	VD *n* = 26 CS *n* = 6	• No PPH • No adverse events, including DDAVP-related effects	No intracranial hemorrhage
	• DDAVP, one dose, further details not specified • Tranexamic acid, i.v. or oral, started from labor and continued every 8 hours and continued 2 to 10 days	NS	• Primary PPH *n* = 6 , no blood transfusion required • Secondary PPH *n* = 1 • No vaginal hematoma • Adverse effects: not mentioned	Not mentioned Exposure unknown
	DDAVP i.v., dose not specified	NA	• No abnormal bleeding • No complications	NA
	• DDAVP, one dose, dosage and route not specified • VWF concentrate, one dose *n* = 1, two doses *n* = 1	NS	• Primary PPH not specified • No excessive blood loss until 6 weeks postpartum • Adverse effects: not mentioned	Not mentioned
	DDAVP i.v., one dose, dosage not specified	VD *n = 1* NS *n = 3*	• No primary PPH in three patients with prophylaxis • After DDAVP, bleeding was controlled in the patient with primary PPH • Adverse effects: not mentioned	NA
	DDAVP i.v., dose and dosage not specified	VD	• Not mentioned if bleeding was controlled • Adverse effects: not mentioned	NA

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Table 1: Continued

References (year)/country	Pregnancies using DDAVP/total pregnancies* (n/n)	Inherited bleeding disorder	Indication for DDAVP/ Timing of dosing (trimester/delivery/postpartum)
Mannucci et al.[26] *Italy*	32/32	VWD $n = 5$ • Type 1 HA-carrier $n = 27$	• Chorionic villus sampling, 1st trimester $n = 20$ • amniocentesis, 2nd trimester $n = 12$ • Planned abortion $n = 12$
Ramsahoye et al.[27] *United Kingdom*	1/24	VWD • Type 1	PPH prophylaxis, after delivery
Reale et al.[28] *United States*	24/106	VWD • Type 1 $n = 20$ • Type unknown $n = 4$	PPH prophylaxis, before delivery $n = 24$
Sanchez et al.[29] *Argentina*	75/75	VWD • Type NS	• Retroplacental haematoma, first trimester $n = 1$ • Cervical cerclage, first trimester $n = 4$ • PPH prophylaxis, before delivery $n = 75$

	Treatment (route, dosage, dose frequency)	Mode of delivery	Maternal outcomes	Neonatal outcomes
	• DDAVP, 0.3 µg/kg, i.v., one dose n = 22 and 3-4 doses *n* = 10, additional dose for planned abortion *n* = 12	NA	• No abnormal bleeding *n* = 32 • Only headache and mild facial flushing reported (*n* = 1 to *n* = 32) • No clinical signs of water intoxication or significant increase in body weight	Healthy newborns *n* = 20
	DDAVP, 0.4 µg/kg, i.v., 3 doses	CS	• No primary or secondary PPH • No complications or adverse effects	NA
	DDAVP, 0.3 µg/kg, i.v., dose not specified	NS	• Three patients had primary PPH (>1000 mL); one of these patients received a second DDAVP dose to treat PPH • No neuraxial hematoma or thromboembolic events • Other adverse effects not mentioned	Not mentioned
	DDAVP, 0.3 µg/kg, i.v., • First trimester two doses *n* = 1, one dose *n* = 4 • Before delivery, one dose *n* = 75	VD *n = 30* CS *n = 45*	• First trimester: no bleeding reported, no increased uterine tone, or water intoxication • Delivery postpartum: hemorrhage not specified. • No hyponatremia or thromboembolic events. • No other adverse effects were reported	• 1st trimester: heathy newborn • Before delivery: no premature newborns, no neonatal bleeding, average weight reported *n = 63*

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Table 1: Continued

References (year)/country	Pregnancies using DDAVP/total pregnancies* (*n/n*)	Inherited bleeding disorder	Indication for DDAVP/ Timing of dosing (trimester/delivery/postpartum)
Santoro et al.[30] Italy	3/9	FXI deficiency	• PPH prophylaxis, during CS
Schulman et al.[31] *Sweden*	1/1	PFD	PPH profylaxis, after delivery
Sood et al.[32] *United States*	1/12	VWD • Type 1	Secondary PPH treatment, postpartum
Varughese & Cohen[33] *United States*	2/64	VWD • Type 1	PPH prophylaxis after delivery
Wilson et al.[34] *Australia*	6/23	VWD • Type 1 • Type 2, exact numbers NS	• PPH prophylaxis, after delivery *n* = 4 • Treatment of primary PPH *n* = 2

Not mentioned: authors did not report or define any outcome. If NS was added in the outcomes columns, it indicates that the authors reported one or more outcomes, but they did not provide any specific details about those outcomes.

CS, cesarean section; DDAVP, desmopressin; FIX deficiency, factor IX deficiency; HA-carriership, hemophilia A carriership; i.n. intranasal; i.v. intravenous; PFD inherited platelet function disorder;

	Treatment (route, dosage, dose frequency)	Mode of delivery	Maternal outcomes	Neonatal outcomes
	DDAVP, 0.3 µg/kg, dose and route not specified	CS	• No PPH • One patient with hyponatremia and transitory neurological complications	Not mentioned. Exposure unknown.
	• DDAVP, 0.2 µg/kg, i.v., dose not mentioned • Tranexamic acid	NS	• No hemorrhagic complication • Adverse effects: not mentioned	NA
	DDAVP, intranasal. Dose and frequency not mentioned.		• Self-administration at home • Adverse effects: not mentioned	NA
	DDAVP, further not specified	VD	• No primary PPH • Adverse effects: not mentioned	NA
	• DDAVP i.v., dose not specified *n* = 6 • Tranexamic acid for 5 days and Biostate, one dose (*n* = 1 with PPH prophylaxis)	• VD *n* = 2 • NS *n* = 4	• PPH prophylaxis: one patient developed primary PPH, treated with Biostate and blood transfusion • PPH treatment: not mentioned if bleeding was controlled • Adverse effects: not mentioned reported	NA

NA, not applicable; NS, not specified; PPH, postpartum hemorrhage; VD, vaginal delivery; VWD, Von Willebrand disease.

[a]Total pregnancies: number of pregnancies with or without DDAVP treatment.

[b]Only the type of disease reported by the authors is added.

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Table 2: Effectiveness outcome of desmopressin monotherapy during intrapartum period.

Inherited bleeding disorder	Total	Unknown	Effective	Ineffective
	Pregnancies, *n*	Pregnancies, *n*	Pregnancies, *n* (%)[a]	Pregnancies, *n* (%)[a]
VWD	165	84 (51)	74 (45)	7 (4)
Hemophilia carriership	4	0	4 (100)	0
PFD	8	0	2 (25)	6 (75)
FXI deficiency	3	0	3 (100)	0
HPS	8	0	3 (37)	5 (63)
ESD	2	0	1 (50)	1 (50)
Combined bleeding disorder	1	0	1 (100)	0
Total	191	84	88	19

Effective: studies reported "no postpartum hemorrhage" or blood loss ≤ 500 mL.
Ineffective: studies reported "postpartum hemorrhage" or a blood loss ≥ 500 mL, and in case cesarean section ≥ 1000 mL or additional unplanned hemostatic agents or blood products were needed.
Unknown: studies did not mention effectiveness outcomes or reported "unknown".
ESD, Ehlers-Danlos syndrome; FXI deficiency, factor XI deficiency; HPS, Hermansky-Pudlak syndrome; PFD, platelet function disorder; VWD, Von Willebrand disease.
[a]The percentages are derived from known outcomes.

In six pregnancies was DDAVP administration given in the late postpartum period to treat obstetric related complications. The treatment was in all cases effective.

Maternal outcomes: safety

In one observational study, it was reported that mild adverse events such as facial flushing and headache occurred in 1 to 32 pregnancies without further details[26]. In addition, one case report also described mild adverse events such as facial flushing in 1 pregnancy[48]. Two observational studies reported a severe adverse event in 2 (1%) pregnancies. One pregnancy was given DDAVP immediately after cesarean section for PPH prophylaxis[18]. A total of 3 doses were given every 18 hours and intravenously administered in a dosage of 0.3 µg/kg. Afterward, the patient developed a water intoxication seizure with hyponatremia (sodium plasma level, 108 mEq/L). Other details were not provided. The second patient received DDAVP during cesarean section for PPH prophylaxis[30]. One dose was given with a dosage of 0.3 µg/kg, route not specified. One dose of 0.3 µg/kg was given. Afterward, the patient developed transient neurologic symptoms; further details were not specified. In both cases, no information was available regarding the application of water restriction therapy and the monitoring during and after DDAVP administration. In 66 (21%) pregnancies, no information on safety outcomes was reported.

Neonatal outcomes

Of the 73 pregnancies with DDAVP during pregnancy, 60 resulted in the birth of a child. In one pregnancy, DDAVP was indicated to treat a planned abortion[22] In addition, in a single study, DDAVP was given to 12 pregnancies prior to undergoing prenatal diagnostic testing and eventually for subsequent planned abortion[26]. Two severe adverse events were reported. Due to preterm labor, 1 neonate was born at 36 weeks of gestation[18]. In this case, the pregnant woman received 1 dose of DDAVP (0.3 μg/kg, intravenously) at 36 weeks of gestation for a DDAVP test, and the authors mentioned that preterm labor was related to DDAVP administration. Besides the preterm labor, no other adverse events were mentioned, and no further details were provided. The second neonate with severe adverse events was born with fetal growth restriction at 36 weeks of gestation[52]. In this case, the women received DDAVP in the first trimester (route and dosage not specified) before amniocentesis. The authors linked preterm labor to the presence of insulin-dependent diabetes in pregnant women.

Of the 232 deliveries, 169 neonates were exposed to DDAVP during delivery (unknown exposure, 7 deliveries), ie, DDAVP was given to the women before the umbilical cord was clamped. Two (1%) neonates experienced a moderate adverse event. The first neonate had a low Apgar score and was admitted for 1 day in the intensive care for observation. In this case, the women received 0.3 ug/kg DDAVP intravenously, 2 doses every 12 hours for PPH prophylaxis. The second neonate had transient hyperbilirubinemia and received phototherapy[51]. DDAVP was intravenously administered for PPH prophylaxis with an unknown dosage and route. In 55 (33%) deliveries, no neonatal outcomes were mentioned. Table 3 summarizes the maternal and neonatal safety outcomes. Thirty-eight neonates were exposed to DDAVP twice, both during pregnancy and delivery[20,29,36]. Among them, 37 neonates had no adverse events, while the safety outcome for 1 neonate was not reported (unknown).

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Table 3: Safety outcomes after desmopressin administration during pregnancy and delivery.

Maternal outcomes - AE	
Antepartum & intrapartum/postpartum	Pregnancies, *n* (%)[a]
No AE	210 (68)
Mild AE	2-32 (1-10)[b]
Severe AE	2 (1)
Unknown	66 (21)
Total[c]	311
Neonatal outcomes - AE	
Antepartum exposure	Pregnancies, *n* (%)
No AE	58 (79)
Mild AE	0
Moderate AE	0
Severe AE	2 (3)
Unknown	0
Not applicable[d]	13 (18)
Total	73
Intrapartum exposure	Deliveries, *n* (%)
No AE	112 (66)
Mild AE	0
Moderate AE	2 (1)
Severe AE	0
Unknown	55 (33)
Total[e]	169

No AE: studies explicitly mentioned "no AE" or "no complications" or denied any specific AE.
Unknown: studies did not mention safety outcomes or reported them as "unknown.
Adverse events severity is graded according to Common Terminology Criteria for Adverse Events.
AE, adverse event.
[a]The percentages shown are derived from known outcomes.
[b]One study reported that 1 to 32 pregnancies experienced mild adverse events with no further details, and 1 case reported 1 mild adverse event.
[c]Total treatment episodes with DDAVP treatment during pregnancy (73 pregnancies), delivery (232 pregnancies), and the postpartum period (6 pregnancies).
[d]In 12 pregnancies, DDAVP was given for prenatal testing with a subsequently planned pregnancy abortion. In 1 pregnancy, DDAVP was given for a planned abortion.
[e]Total neonates from each delivery: 1 delivery resulted in 3 neonates.
Finally, 3 adverse events were reported (1 maternal and 2 neonatal adverse events) that were not correlated to DDAVP administration and/or had clearly other cause(s) (Supplementary 3).

Discussion

The aim of this systematic review was to review the safety and effectiveness of DDAVP during pregnancy, the intrapartum, and the postpartum period in women with an inherited bleeding disorder. DDAVP is an important choice of treatment in most patients with inherited bleeding disorders. Despite its advantages, such as easy accessibility, low costs, and patient-friendly intranasal administration, there are concerns regarding its safety for pregnant women and their children that require exploration. Furthermore, there is a need for effective data concerning bleeding during pregnancy and delivery, especially PPH, which has high morbidity and mortality rates. This systematic review provides a comprehensive summary of the available evidence regarding these concerns.

According to the findings of this review, monotherapy with DDAVP was effective in treating and preventing bleeding, especially in VWD. Seven studies reported DDAVP use during pregnancy for prenatal diagnostic testing but also for termination of pregnancy. No adverse bleeding events were reported in these pregnancies. In the majority of pregnancies, DDAVP was used during the intrapartum period and was indicated for PPH prophylaxis. In VWD, high effectiveness was reported for DDAVP monotherapy. However, the large amount of missing outcome data presents a challenge for drawing definitive conclusions. In other inherited bleeding disorders, only small numbers of pregnancies were reported with limited effectiveness, especially pregnancies with PFD and HPS. Therefore, these results should be interpreted with caution. DDAVP was mainly administered intravenously with 1 dose, but 5 included studies also reported the use of intranasal administration. Studies have shown that intranasal administration provides an equivalent improvement in hemostasis as 0.2 µg/kg i.v. DDAVP. Therefore, intranasal administration may not have a negative impact on effectiveness[67,68]. With respect to the combined treatment involving DDAVP and tranexamic acid, our review has identified a limited number of pregnancies that were treated with this combination. Consequently, drawing any conclusions about its effectiveness is not possible. However, guidelines recommend the use of tranexamic acid in combination with DDAVP or replacement therapy during delivery and postpartum period[3].

Severe adverse maternal outcomes after DDAVP administration were uncommon. Of the included 273 pregnancies with DDAVP administration, 2 pregnancies were accompanied by severe adverse events. One pregnancy was complicated by a water intoxication seizure due to severe hyponatremia, and the second pregnancy by transient neurologic symptoms; both women had a cesarean section. Antidiuretic effects of DDAVP are a common concern; therefore, a recommended fluid restriction after administration is applied for all patients[3]. Pregnant women might be at higher risk due to commonly used

i.v. fluids, especially in those with a cesarean section, and the physiological changes during pregnancy may aggravate water retention and changes in the pharmacokinetics of DDAVP[69]. In addition, the use of oxytocin is common during delivery, which may also contribute to hyponatremia[70]. In the majority of reviewed studies, there was a lack of information regarding the implementation of fluid restriction and monitoring measures. However, by incorporating fluid restriction, closely monitoring patients, and measuring sodium levels, it is possible to avoid these severe complications. In addition, an important question is whether the frequency of DDAVP administration is associated with maternal safety. Our review reveals that different dose frequencies were used, with a single DDAVP dose in the majority of pregnancies (75%). However, as different dose frequencies were applied in pregnancies where maternal adverse events occurred and data on both dose frequency and outcome were missing, drawing conclusions about the association between dose frequency and adverse events is not possible.

Regarding neonatal outcomes, current data suggest that the use of DDAVP is generally safe for children, especially when administered during delivery. During intrapartum DDAVP, 2 (2%) moderate adverse events were reported, with 1 neonate presenting with a lower Apgar score without consequences and the other neonate with transient hyperbilirubinemia. The latter adverse event is not associated with DDAVP. On the other hand, DDAVP administration during the antepartum period resulted in 2 (3%) neonatal adverse events, specifically preterm labor and fetal growth restriction. The incidence of preterm labor is approximately 10% in the general population[71]. Theoretically, DDAVP could lead to uterine contraction due to binding to the oxytocin receptor[72], while intrauterine growth restriction could be the consequence of poor placental flow due to the vasopressor effect of DDAVP. Nonetheless, due to the absence of information regarding other potential risk factors or causes that could contribute to these severe adverse events, it remains challenging to establish a direct causal relationship or association between DDAVP and these events. Moreover, in vitro models of placentae showed that DDAVP crosses the placenta but leads to minimal detectable concentrations[73]. Similar to data from DDAVP use in pregnant women with diabetes insipidus, no teratogenic effects in fetuses were reported[7]. It is important to note that more data are available on the use of DDAVP during delivery (114 deliveries with reported neonatal outcomes) compared with the antepartum period (60 pregnancies with reported neonatal outcomes). In relation to the dose frequency and neonatal safety, our review reveals that neonatal adverse events occurred in patients with different dose frequencies. Due to missing data on dose frequency and outcomes in studies, conclusions regarding the association between dose frequency and neonatal adverse events cannot be drawn.

Our systematic review contains additional evidence on the safety outcomes. Compared with the review by Trigg et al.[8], this review included 23 additional studies and 57 pregnancies. Regarding the safety outcomes, 1 severe maternal adverse event and 1 severe and 1 moderate neonatal adverse event were added.

This review has several limitations. First, the fact that only a few studies aimed to investigate the effectiveness and safety of DDAVP indicates that the remaining studies may have incomplete results on these outcomes. Second, due to heterogeneity in the included study population with lack of information about data that affect the effectiveness outcome, conclusions regarding effectiveness are difficult to draw. Thirdly, case reports were included, which may have contributed to biased results on outcomes, especially effectiveness outcomes. However, case reports are useful regarding safety, and they describe adverse events that remain unnoticed in observational studies or clinical trials. Finally, the majority of studies were poorly designed, potentially generating unreliable and/or biased results.

In conclusion, our review provides updated evidence on the effectiveness and safety outcome of DDAVP use during pregnancy and delivery in women with an inherited bleeding disorder. Based on current data, DDAVP use during pregnancy and delivery seems safe for the mother, with special attention to the occurrence of hyponatremia and for the child, especially during delivery. However, due to poor study designs and limited documentation of outcomes, a well-designed prospective study is still warranted.

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Supplementary material

Supplementary 1: search strategy with key terms
Embase

('desmopressin'/exp OR (DDAVP OR desmopressin* OR minrin OR octostim* OR deamino-arginine-vasopressin* OR 1-Desamino-8-arginine-Vasopressin* OR 1-Deamino-8-D-arginine*-Vasopressin* OR Adiuretin-SD OR Desmotab* OR Octim* OR Desmospray* OR Nocutil* OR Minurin OR Adiuretin OR Desmogalen):ab,ti,kw) **AND** ('congenital blood clotting disorder'/exp OR 'blood clotting disorder'/exp OR 'bleeding disorder'/de OR 'congenital blood dyscrasia'/exp OR 'Glanzmann disease'/exp OR 'Bernard Soulier disease'/exp OR 'blood clotting factor deficiency'/exp OR 'ocular albinism'/exp OR 'hermansky pudlak syndrome type 2'/de OR 'Ehlers Danlos syndrome'/de OR 'Noonan syndrome'/exp OR (VWD OR hemophil* OR haemophil* OR pseudohaemophil* OR pseudohemophil* OR ((willebrand* OR scott OR gray-platelet* OR glanzmann) NEAR/3 (disease* OR disorder* OR syndrom*)) OR ((congenital-hypoplastic* OR fanconi) NEAR/3 (anemia*)) OR ((bleed* OR blood* OR coagulat* OR clott* OR hemorrhag* OR thrombo* OR platelet* OR hematologic*) NEAR/3 (disease* OR disorder* OR syndrom* OR defect* OR tendenc* OR deficienc* OR dysfunct* OR anomal* OR dyscrasia)) OR hypercoagulabilit* OR hypocoagulabilit* OR coagulopath* OR thromboembolis* OR embolis* OR thrombophil* OR thrombosis* OR thrombus* OR thrombogenic* OR thrombocytos* OR thrombocytopen* OR Glanzmann* OR thrombasthen* OR thrombocytopath* OR Bernard-Soulier* OR dence-granule-deficienc* OR ((defect*) NEAR/3 (ADP OR adenosin*-diphosphat* OR thromboxane-A2 OR thromboxane-A-2 OR TxA2)) OR ((prothrombin* OR antithrombin* OR 2 OR 5 OR 7 OR 8 OR 9 OR 10 OR 13 OR II OR V OR VII OR VIII OR IX OR X OR XIII) NEAR/3 (deficien*)) OR ((ocular OR eye OR hemorrhagic-diathes* OR haemorrhagic-diathes*) NEAR/3 (albinism*)) OR ((Hermansk*) NEAR/3 (Pudlak*)) OR ((ehler*) NEAR/3 (danlos)) OR ((Noonan* OR familial-Turner* OR male-Turner* OR noman* OR Turner-like) NEAR/3 (syndrom* OR diseas*))):ab,ti,kw) **AND** ('pregnancy'/exp OR 'pregnant woman'/de OR 'pregnancy disorder'/exp OR 'newborn'/de OR 'birth'/de OR 'childbirth'/exp OR 'obstetric procedure'/exp OR 'fetus'/de OR (pregnan* OR gestat* OR delivery OR child-bear* OR childbear* OR gravidit* OR labor OR labour OR maternal* OR neonat* OR birth* OR childbirth* OR parturit* OR prematur* OR newborn* OR fetus OR foetus OR fetal OR fetopath* OR fetomaternal* OR placenta* OR postpartum* OR prepartum* OR post-partum* OR pre-partum* OR perinatal* OR obstetric* OR prenatal* OR postnatal* OR peri-natal* OR pre-natal* OR post-natal* OR stillbirth* OR antenatal* OR ante-natal* OR partus):ab,ti,kw)

Medline

(Deamino Arginine Vasopressin/ OR (DDAVP OR desmopressin* OR minrin OR octostim* OR deamino-arginine-vasopressin* OR 1-Desamino-8-arginine-Vasopressin* OR 1-Deamino-8-D-arginine*-Vasopressin* OR Adiuretin-SD OR Desmotab* OR Octim* OR Desmospray*

OR Nocutil* OR Minurin OR Adiuretin OR Desmogalen).ab,ti,kf.) AND (exp Blood Coagulation Disorders, Inherited/ OR Blood Coagulation Disorders/ OR Hematologic Diseases/ OR Thrombasthenia/ OR Bernard-Soulier Syndrome/ OR Hermanski-Pudlak Syndrome/ OR Ehlers-Danlos Syndrome/ OR Noonan Syndrome/ OR (VWD OR hemophil* OR haemophil* OR pseudohaemophil* OR pseudohemophil* OR ((willebrand* OR scott OR gray-platelet* OR glanzmann) ADJ3 (disease* OR disorder* OR syndrom*)) OR ((congenital-hypoplastic* OR fanconi) ADJ3 (anemia*)) OR ((bleed* OR blood* OR coagulat* OR clott* OR hemorrhag* OR thrombo* OR platelet* OR hematologic*) ADJ3 (disease* OR disorder* OR syndrom* OR defect* OR tendenc* OR deficienc* OR dysfunct* OR anomal* OR dyscrasia)) OR hypercoagulabilit* OR hypocoagulabilit* OR coagulopath* OR thromboembolis* OR embolis* OR thrombophil* OR thrombosis* OR thrombus* OR thrombogenic* OR thrombocytos* OR thrombocytopen* OR Glanzmann* OR thrombasthen* OR thrombocytopath* OR Bernard-Soulier* OR dence-granule-deficienc* OR ((defect*) ADJ3 (ADP OR adenosin*-diphosphat* OR thromboxane-A2 OR thromboxane-A-2 OR TxA2)) OR ((prothrombin* OR antithrombin* OR 2 OR 5 OR 7 OR 8 OR 9 OR 10 OR 13 OR II OR V OR VII OR VIII OR IX OR X OR XIII) ADJ3 (deficien*)) OR ((ocular OR eye OR hemorrhagic-diathes* OR haemorrhagic-diathes*) ADJ3 (albinism*)) OR ((Hermansk*) ADJ3 (Pudlak*)) OR ((ehler*) ADJ3 (danlos)) OR ((Noonan* OR familial-Turner* OR male-Turner* OR noman* OR Turner-like) ADJ3 (syndrom* OR diseas*))).ab,ti,kf.) AND (exp Pregnancy/ OR Prenatal Care/ OR Maternal-Fetal Relations/ OR Pregnant Women/ OR exp Pregnancy Complications/ OR exp Infant, Newborn/ OR exp Obstetric Surgical Procedures/ OR exp Fetus/ OR (pregnan* OR gestat* OR delivery OR child-bear* OR childbear* OR gravidit* OR labor OR labour OR maternal* OR neonat* OR birth* OR childbirth* OR parturit* OR prematur* OR newborn* OR fetus OR foetus OR fetal OR fetopath* OR fetomaternal* OR placenta* OR postpartum* OR prepartum* OR post-partum* OR pre-partum* OR perinatal* OR obstetric* OR prenatal* OR postnatal* OR peri-natal* OR pre-natal* OR post-natal* OR stillbirth* OR antenatal* OR ante-natal* OR partus).ab,ti,kf.)

Cochrane

((DDAVP OR desmopressin* OR minrin OR octostim* OR (deamino NEXT/1 arginine NEXT/1 vasopressin*) OR (1 NEXT/1 Desamino NEXT/1 8 NEXT/1 arginine NEXT/1 Vasopressin*) OR (1 NEXT/1 Deamino NEXT/1 8 NEXT/1 D NEXT/1 arginine* NEXT/1 Vasopressin*) OR (Adiuretin NEXT/1 SD) OR Desmotab* OR Octim* OR Desmospray* OR Nocutil* OR Minurin OR Adiuretin OR Desmogalen):ab,ti,kw) **AND** ((VWD OR hemophil* OR haemophil* OR pseudohaemophil* OR pseudohemophil* OR ((willebrand* OR scott OR (gray NEXT/1 platelet*) OR glanzmann) NEAR/3 (disease* OR disorder* OR syndrom*)) OR (((congenital NEXT/1 hypoplastic*) OR fanconi) NEAR/3 (anemia*)) OR ((bleed* OR blood* OR coagulat* OR clott* OR hemorrhag* OR thrombo* OR platelet* OR hematologic*) NEAR/3 (disease* OR disorder* OR syndrom* OR defect* OR tendenc* OR deficienc* OR

dysfunct* OR anomal* OR dyscrasia)) OR hypercoagulabilit* OR hypocoagulabilit* OR coagulopath* OR thromboembolis* OR embolis* OR thrombophil* OR thrombosis* OR thrombus* OR thrombogenic* OR thrombocytos* OR thrombocytopen* OR Glanzmann* OR thrombasthen* OR thrombocytopath* OR (Bernard NEXT/1 Soulier*) OR (dence NEXT/1 granule NEXT/1 deficienc*) OR ((defect*) NEAR/3 (ADP OR (adenosin* NEXT/1 diphosphat*) OR (thromboxane NEXT/1 A2) OR (thromboxane NEXT/1 A NEXT/1 2 OR TxA2))) OR ((prothrombin* OR antithrombin* OR 2 OR 5 OR 7 OR 8 OR 9 OR 10 OR 13 OR II OR V OR VII OR VIII OR IX OR X OR XIII) NEAR/3 (deficien*)) OR ((ocular OR eye OR hemorrhagic-diathes* OR haemorrhagic-diathes*) NEAR/3 (albinism*)) OR ((Hermansk*) NEAR/3 (Pudlak*)) OR ((ehler*) NEAR/3 (danlos)) OR ((Noonan* OR familial-Turner* OR male-Turner* OR noman* OR Turner-like) NEAR/3 (syndrom* OR diseas*))):ab,ti,kw **AND** ((pregnan* OR gestat* OR delivery OR (child NEXT/1 bear*) OR childbear* OR gravidit* OR labor OR labour OR maternal* OR neonat* OR birth* OR childbirth* OR parturit* OR prematur* OR newborn* OR fetus OR foetus OR fetal OR fetopath* OR fetomaternal* OR placenta* OR postpartum* OR prepartum* OR (post NEXT/1 partum*) OR (pre NEXT/1 partum*) OR perinatal* OR obstetric* OR prenatal* OR postnatal* OR (peri NEXT/1 natal*) OR (pre NEXT/1 natal*) OR (post NEXT/1 natal*) OR stillbirth* OR antenatal* OR (ante NEXT/1 natal*) OR partus):ab,ti,kw)

Web of Science

TS=(((DDAVP OR desmopressin* OR minrin OR octostim* OR deamino-arginine-vasopressin* OR 1-Desamino-8-arginine-Vasopressin* OR 1-Deamino-8-D-arginine*-Vasopressin* OR Adiuretin-SD OR Desmotab* OR Octim* OR Desmospray* OR Nocutil* OR Minurin OR Adiuretin OR Desmogalen)) AND ((VWD OR hemophil* OR haemophil* OR pseudohaemophil* OR pseudohemophil* OR ((willebrand* OR scott OR gray-platelet* OR glanzmann) NEAR/2 (disease* OR disorder* OR syndrom*)) OR ((congenital-hypoplastic* OR fanconi) NEAR/2 (anemia*)) OR ((bleed* OR blood* OR coagulat* OR clott* OR hemorrhag* OR thrombo* OR platelet* OR hematologic*) NEAR/2 (disease* OR disorder* OR syndrom* OR defect* OR tendenc* OR deficienc* OR dysfunct* OR anomal* OR dyscrasia)) OR hypercoagulabilit* OR hypocoagulabilit* OR coagulopath* OR thromboembolis* OR embolis* OR thrombophil* OR thrombosis* OR thrombus* OR thrombogenic* OR thrombocytos* OR thrombocytopen* OR Glanzmann* OR thrombasthen* OR thrombocytopath* OR Bernard-Soulier* OR dence-granule-deficienc* OR ((defect*) NEAR/2 (ADP OR adenosin*-diphosphat* OR thromboxane-A2 OR thromboxane-A-2 OR TxA2)) OR ((prothrombin* OR antithrombin* OR 2 OR 5 OR 7 OR 8 OR 9 OR 10 OR 13 OR II OR V OR VII OR VIII OR IX OR X OR XIII) NEAR/2 (deficien*)) OR ((ocular OR eye OR hemorrhagic-diathes* OR haemorrhagic-diathes*) NEAR/2 (albinism*)) OR ((Hermansk*) NEAR/2 (Pudlak*)) OR ((ehler*) NEAR/2 (danlos)) OR ((Noonan* OR familial-Turner* OR male-Turner* OR noman* OR Turner-like) NEAR/2 (syndrom* OR diseas*)))) AND

((pregnan* OR gestat* OR delivery OR child-bear* OR childbear* OR gravidit* OR labor OR labour OR maternal* OR neonat* OR birth* OR childbirth* OR parturit* OR prematur* OR newborn* OR fetus OR foetus OR fetal OR fetopath* OR fetomaternal* OR placenta* OR postpartum* OR prepartum* OR post-partum* OR pre-partum* OR perinatal* OR obstetric* OR prenatal* OR postnatal* OR peri-natal* OR pre-natal* OR post-natal* OR stillbirth* OR antenatal* OR ante-natal* OR partus)))

Google Scholar
desmopressin "congenital|inheritance|heritage blood|coagulation" pregnancy|pregnant
desmopressin 'congenital|inheritance|heritage blood|coagulation' pregnancy|pregnant

6

Supplementary 2: An overview of pregnancies, treatment with DDAVP and maternal and neonatal outcome of each included case report.

References (year)/ country	Pregnancies using DDAVP/ total pregnancies* (*n/n*)	Inherited bleeding disorder/Type**	Indication for DDAVP/ Timing of dosing (trimester/delivery/postpartum)
Adnyana et al., (2020) *Indonesia*	1/1	VWD • Type NS	PPH prophylaxis, before delivery
Agarwal et al., (2011) *United Kingdom*	1/2	GPS	• Vaginal bleeding treatment, 1st trimester (week 7) • PPH prophylaxis, before delivery
Asatiani et al., (2007) *United States*	2/2	Patient 1: VWD type 1, EHS and FXI and FXII deficiency Patient 2: VWD type 1 and FIX deficiency	Patient 1: PPH treatment, at delivery Patient 2: PPH prophylaxis, before delivery
Bachmann et al., (2014) *Germany*	1/1	HPS	PPH prophylaxis, before delivery

Treatment (route, dosage, dose, frequency)	Mode of delivery	Maternal outcomes	Neonatal outcomes
• DDAVP i.n. 300 mcg, 2 doses before delivery and for 5 days postpartum (dose not specified) • Cryoprecipitate, 5 units before delivery, and for 5 days postpartum • FVIII concentrate, 1000 units before delivery and 5 days postpartum (dose not specified)	CS	• No excessive bleeding • No meaningful complications • Adverse effects: not specified	No meaningful complications
• DDAVP 0.3 µg/kg, i.v., one dose. Packed cells, 2 units (1st trimester) • DDAVP 0.3 µg/kg, i.v., one dose (delivery)	CS	• 1st trimester: not mentioned if there was still bleeding • PPH prophylaxis: No PPH (500 mL) • Adverse effects not mentioned regarding DDAVP administration • At 27 weeks mother developed fever due to chorioamnionitis.	• 1st trimester: Normal growing fetus • At delivery: Preterm delivery at week 27 gestation due to chorioamnionitis, no bleeding complication, adverse effects not mentioned
Patient 1 and 2: DDAVP, further not specified	Patient 1: NS Patient 2: CS	• Patient 1: severe PPH not responding to DDAVP, additional treatment with Haemate P and aminocaproic acid • Patient 2: bleeding not specified, uneventful cesarean section • Adverse effects: not mentioned	• Patient 1: NA • Patient 2: not mentioned
DDAVP two doses further not specified	VD	• No PPH (300 mL) • No complications	Not mentioned

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Supplementary 2: Continued

References (year)/ country	Pregnancies using DDAVP/ total pregnancies* (*n/n*)	Inherited bleeding disorder/Type**	Indication for DDAVP/ Timing of dosing (trimester/delivery/postpartum)
Beesley et al., (2008) *United States*	2/2	HPS	Patient 1 and 2: PPH prophylaxis, before delivery
Butwick et al., (2007) *United States*	1/1	VWD • Type 1	PPH prophylaxis, before delivery
Cata et al., (2009) *United States*	1/1	VWD • Type 2M	PPH prophylaxis, after delivery
Clements et al., (2011) *United Kingdom*	1/1	GPS	PPH prophylaxis, before delivery
Cohen et al., (1989) *United States*	1/1	VWD • Type NS	PPH prophylaxis, before delivery
Denholm et al., (2008) *New Zealand*	1/1	PT-VWD	PPH prophylaxis, before delivery
Greinacher et al., (1993) *Unknown*	1/1	BSS	PPH prophylaxis, before delivery
Harris et al., (2013) *United States*	1/1	HPS	PPH prophylaxis, before delivery

Treatment (route, dosage, dose, frequency)	Mode of delivery	Maternal outcomes	Neonatal outcomes
Patient 1: DDAVP, one dose, dosage and route not specified Patient 2: DDAVP 0.3 μg/kg, i.v., one dose	VD	• Patient 1: PPH (1500 mL), additional treatment with Packed cells and platelets concentrate • Patient 2: PPH (1000 mL) • Adverse effects not mentioned in both cases	Not mentioned
DDAVP 0.3 μg/kg, i.v., one dose	CS	• PPH (1000 mL) • Uneventful recovery • Adverse effects: not mentioned	Two healthy newborns
DDAVP 0.3 μg/kg, i.v., 4 doses, every 12 hours	CS	• No PPH • Normal neurological examinations • Other adverse effects not mentioned	NA
DDAVP 0.3 μg/kg, i.v., one dose	CS	• No PPH (600 mL) • Uneventful recovery • Adverse effect: not mentioned	Healthy newborn
DDAVP 0.3 μg/kg, i.v., one dose	VD	• No PPH • Adverse effects: not mentioned	Not mentioned
DDAVP 0.3 μg/kg, i.v. one dose	CS	• Excessive incision site bleeding, patient was treated with Biostate • Uneventful recovery • Adverse effects: not mentioned	Healthy newborn
DDAVP i.v., two doses, dosage not specified	CS	• No PPH (500 mL) • Adverse effects: not mentioned	• Healthy preterm newborn (week 36) • No electrolyte abnormalities
DDAVP 0.3 μg/kg, i.v. two doses before delivery	CS	• No PPH (800 mL) • No complications, including hyponatremia	• One of three newborns had intraventricular hemorrhage with normal developmental milestones • No other adverse events reported

6

Supplementary 2: Continued

References (year)/ country	Pregnancies using DDAVP/ total pregnancies* (*n/n*)	Inherited bleeding disorder/Type**	Indication for DDAVP/ Timing of dosing (trimester/delivery/postpartum)
Heslop et al., (1986) *New Zealand*	1/1	BSS	Treatment superficial wound (at day 7 and 11 postpartum) and secondary PPH (one month postpartum)
Jewell et al., (2003) *Australia*	1/1	PSPD	PPH prophylaxis, before delivery
Kailash et al., (2009) *United States*	1/1	VWD • Type 1	PPH prophylaxis, after delivery
Khakwani et al., (2021) *United Kingdom*	1/1	PSPD	PPH prophylaxis, before delivery
Kleiman et al., (1991) *Canada*	1/1	PFD • Type NS	PPH prophylaxis, before delivery
Milaskiewicz et al., (1990) *United Kingdom*	1/1	VWD • Type 1	1ste trimester for amniocentesis
Oukkache et al., (2012) *Morocco*	1/1	Combined FV and FVIII deficiency	Secondary PPH prophylaxis, postpartum

Treatment (route, dosage, dose, frequency)	Mode of delivery	Maternal outcomes	Neonatal outcomes
DDAVP 0.4 µg/kg, i.v., two doses for treatment wound and one dose for secondary PPH	CS	• Normal wound closure • Secondary PPH, bleeding stops after DDAVP • Adverse effects: not mentioned	NA
• DDAVP 0.4 µg/kg, i.v., one dose • Platelet concentrate, 2 units	CS	• PPH (1000 mL) • Facial flushing and malaise • Uneventful postpartum period	Not mentioned
DDAVP 0.3 µg/kg, i.v., one dose	CS	• No PPH (700 mL) • No neurological symptoms • Uneventful follow-up visits until 3 weeks postpartum	NA
• DDAVP 0.3 µg/kg s.c., one dose • Tranexamic acid i.v., 1000 mg, one dose before delivery until 4 days postpartum	CS	• No PPH (500 mL) • No (surgical) complications • Follow-up: patient was asymptomatic	Healthy newborn
DDAVP i.v., dose not specified	VD	• No PPH (450 mL) • Uncomplicated course	• Neonate received phototherapy for transient hyperbilirubinaemia • No apnoea or bradycardia
DDAVP, futher not specified	CS	• No bleeding complications mentioned • Adverse effect not mentioned	• Intrauterine growth retardation, preterm delivery at 36 week of gestation
DDAVP 0.3 µg/kg s.c., for 2 days postpartum	CS	• No PPH • Adverse effects: not mentioned	NA

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Supplementary 2: Continued

References (year)/ country	Pregnancies using DDAVP/ total pregnancies* (*n/n*)	Inherited bleeding disorder/Type**	Indication for DDAVP/ Timing of dosing (trimester/delivery/postpartum)
Paeceman et al., (1989) *United States*	1/1	BSS	Secondary PPH treatment, 3 weeks postpartum
Pillia et al., (2019) *Oman*	1/1	GT	PPH prophylaxis, before delivery
Poddar et al., (2007) *United Kingdom*	1/1	HPS	Primary PPH treatment, after delivery
Prabu et al., (2006) *United Kingdom*	1/2	BSS	Secondary PPH treatment, 10 days after delivery
Rahman et al., (2008) *United Kingdom*	1/1	PSPD	PPH prophylaxis, before delivery

Treatment (route, dosage, dose, frequency)	Mode of delivery	Maternal outcomes	Neonatal outcomes
• DDAVP 0.3 µg/kg, i.v., 3 doses every 24 hours; • Platelet concentrate, 16 units in 5 days • Immune globuline 400 mg/kg, daily for 4 days	CS	• Bleeding stopped • Adverse effects: not mentioned	NA
• DDAVP i.n., dose not specified • Platelet concentrates, 5 units • Tranexamic acid i.v., 1000 mg, one dose	VD	• No primary PPH (350 mL), secondary PPH 2 weeks postpartum, patient was admitted and treated for hematometra with blood products • Adverse effects: not mentioned	Not mentioned
DDAVP 0.3 µg/kg, i.v., one dose	VD	• Minimal blood loss • Adverse effects: not mentioned	NA
• DDAVP 0.4 µg/kg, i.v., one dose • Packed cells, 8 units • Platelet concentrate, 10 units • tranexamic acid 4g daily, duration not specified	VD	• Bleeding stopped after 48 hours • Adverse effects: not mentioned	NA
• DDAVP 0.3 µg/kg, one dose, route not specified • Tranexamic acid i.v., 1000 mg, one dose	VD	• Severe PPH (8 liter), treated with packed cells, fresh frozen plasma, platelet concentrate and cryoprecipitate • Adverse effects: not reported	Adverse effects not mentioned

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Supplementary 2: Continued

References (year)/ country	Pregnancies using DDAVP/ total pregnancies* (*n/n*)	Inherited bleeding disorder/Type**	Indication for DDAVP/ Timing of dosing (trimester/delivery/postpartum)
Rajpal et al., (2011) *United States*	1/1	PSPD	PPH prophylaxis, before delivery
Roberson et al., (2018) *United States*	1/1	VWD • Type 1	PPH prophylaxis, before delivery
Rochelson et al., (1991) *United States*	1/1	EDS	PPH prophylaxis, before delivery
Saif et al., (2001) *United States*	1/1	VWD • Type 1	PPH prophylaxis, before delivery
Snow et al., (2020) *United States*	1/1	PSPD	PPH prophylaxis, before delivery

Treatment (route, dosage, dose, frequency)	Mode of delivery	Maternal outcomes	Neonatal outcomes
DDAVP i.v., two doses, dosage not specified	VD	• Primary PPH (3500 mL), treated with second dose DDAVP and 6 units platelet concentrates • Uneventful postpartum course, no bleeding, no neurological sequelae	Not mentioned
DDAVP 0.3 µg/kg, i.v., two doses, every 12 hours	CS	• No orimary PPH 750 mL, minimal lochia • Adverse effects: not mentioned	• Due to low Apgar scores (1 at 1 minute, 3 at 5 minutes, and 6 at 10 minutes) one day admitted to the intensive care, after 3 days discharged • No other adverse effects or events mentioned
DDAVP, 20 mg***, route not specified, before delivery and until 5 days postpartum	VD	• Normal blood loss • Adverse effects: not mentioned	Not mentioned
DDAVP 0.3 µg/kg, i.v., one dose and at 3 days postpartum DDAVP i.n., dose not specified	VD	• No primary PPH • At day 3 secondary postpartum bleeding (150 mL), treated with intranasal DDAVP • Adverse effects: not mentioned	Not mentioned
• DDAVP 0.3 µg/kg, two doses, every 24 hours, route not specified • Tranexamic acid 1 gram • Recombinant factor VIIa 50 µg/kg before and after delivery	CS	• No primary PPH (800 mL) • No complications	Healthy newborn

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Supplementary 2: Continued

References (year)/ country	Pregnancies using DDAVP/ total pregnancies* (*n/n*)	Inherited bleeding disorder/Type**	Indication for DDAVP/ Timing of dosing (trimester/delivery/postpartum)
Tong et al., (2006) *United States*	1/1	HPS	PPH prophylaxis, before delivery
Weinbaum et al., (1987) *United States*	1/1	EDS	PPH prophylaxis, before delivery
Zatik et al., (2002) *Hungary*	2/2	HPS	PPH prophylaxis, before delivery

*Total pregnancies: number of pregnancies with or without DDAVP treatment.

**Only the reported type of disease by the authors is added.

***The reported dosage of "20 mg" is not reliable as it appear unusually high compared to typical dosages. This could be a reported mistake by the authors but it cannot be verified. Therefore, this dosage should be interpret with caution.

Treatment (route, dosage, dose, frequency)	Mode of delivery	Maternal outcomes	Neonatal outcomes
DDAVP 0.3 µg/kg i.v., one dose	VD	• Bleeding from perineal area with dropped haemoglobin and platelets, treated with 12 units platelet concentrates • Adverse effects: not mentioned	Not mentioned
DDAVP, i.v., 12 µg, one dose	CS	• No primary PPH 500-750 mL • Discharged in good condition • Adverse effects not mentioned	Discharged in good condition
DDAVP 0.3 µg/kg, one dose, route not specified	CS	• Pregnancy 1: Primary PPH (1600 mL), treated with Packed cells and platelet concentrates; • Adverse effects: not mentioned • Pregnancy 2: no bleeding requiring blood products, uneventful recovery; adverse effects: not mentioned	Pregnancy 1 and 2: healthy newborns

Not mentioned: authors did not report or define any outcome. NS, Not specified: If NS was added in the outcomes columns, it indicates that the authors reported one or more outcomes, but they did not provide any specific details about those outcomes.

Abbreviations: DDAVP, desmopressin; VWD, Von Willebrand disease; PT-VWD, platelet type Von Willebrand disease; PFD Inherited platelet function disorder; GPS, Grey Platelet Syndrome; BSS, Bernard Soulier syndrome; HPS, Hermansky-pudlak syndrome; EDS, Ehlers-Danlos syndrome; PSPD, Platelet storage pool disorder; GT, Glanzmann's Thrombasthenia; VD, vaginal delivery; CS, caesarean section; i.n. intranasal; i.v. intravenous; s.c., subcutaneous; PPH, postpartum hemorrhage; NA, not applicable.

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Supplementary 3: Adverse events not related to DDAVP administration.

References (year)/country	Pregnancies using DDAVP/total pregnancies* (*n/n*)	Inherited bleeding disorder	Indication for DDAVP/ Timing of dosing (trimester/delivery/postpartum)
Agarwal et al., (2011) *United Kingdom*	1/2	GPS	• Vaginal bleeding treatment, 1st trimester (week 7) • PPH prophylaxis, before delivery
Harris et al., (2013) *United States*	1/1	HPS	PPH prophylaxis, before delivery

*Total pregnancies: number of pregnancies with or without DDAVP treatment.

Not mentioned: authors did not report or define any outcome. Not specified: authors had reported one or more outcomes, but did not describe any details.

Treatment (route, dosage, dose frequency)	Mode of delivery	Maternal outcomes	Neonatal outcomes
• DDAVP 0.3 µg/kg, i.v., one dose. Packed cells, 2 units (1st trimester) • DDAVP 0.3 µg/kg, i.v., one dose (delivery)	CS	• 1st trimester: not mentioned if there was still bleeding • PPH prophylaxis: No PPH (500 mL) • Adverse effects not mentioned regarding DDAVP administration. • At 27 weeks mother developed fever due to chorioamnionitis. **Adverse event not related to DDAVP**: (1) time of DDAVP administration is not related to the occurrence of adverse event.	• 1st trimester: Normal growing fetus • At delivery: Preterm delivery at week 27 gestation due to chorioamnionitis, no bleeding complication, **Adverse event not related to DDAVP**: (1) time of DDAVP administration is not related to the occurrence of adverse event (2) chorioamnionitis is probably the cause of preterm delivery (3) first trimester a normal growing fetus was observed
DDAVP 0.3 µg/kg, i.v. two doses before delivery	CS	• No PPH (800 mL) • No complications, including hyponatremia	• One of three newborns had intraventricular hemorrhage with normal developmental milestones • No other adverse events reported **Adverse event not related to DDAVP**: (1) the occurrence of intraventricular hemorrhage is not related to DDAVP (2) multiple gestation is probably the cause.

Abbreviations: DDAVP, desmopressin; GPS, Grey Platelet Syndrome;HPS, Hermansky-pudlak syndrome; VD, vaginal delivery; CS, caesarean section; i.v. intravenous; PPH, postpartum hemorrhage; NA, not applicable.
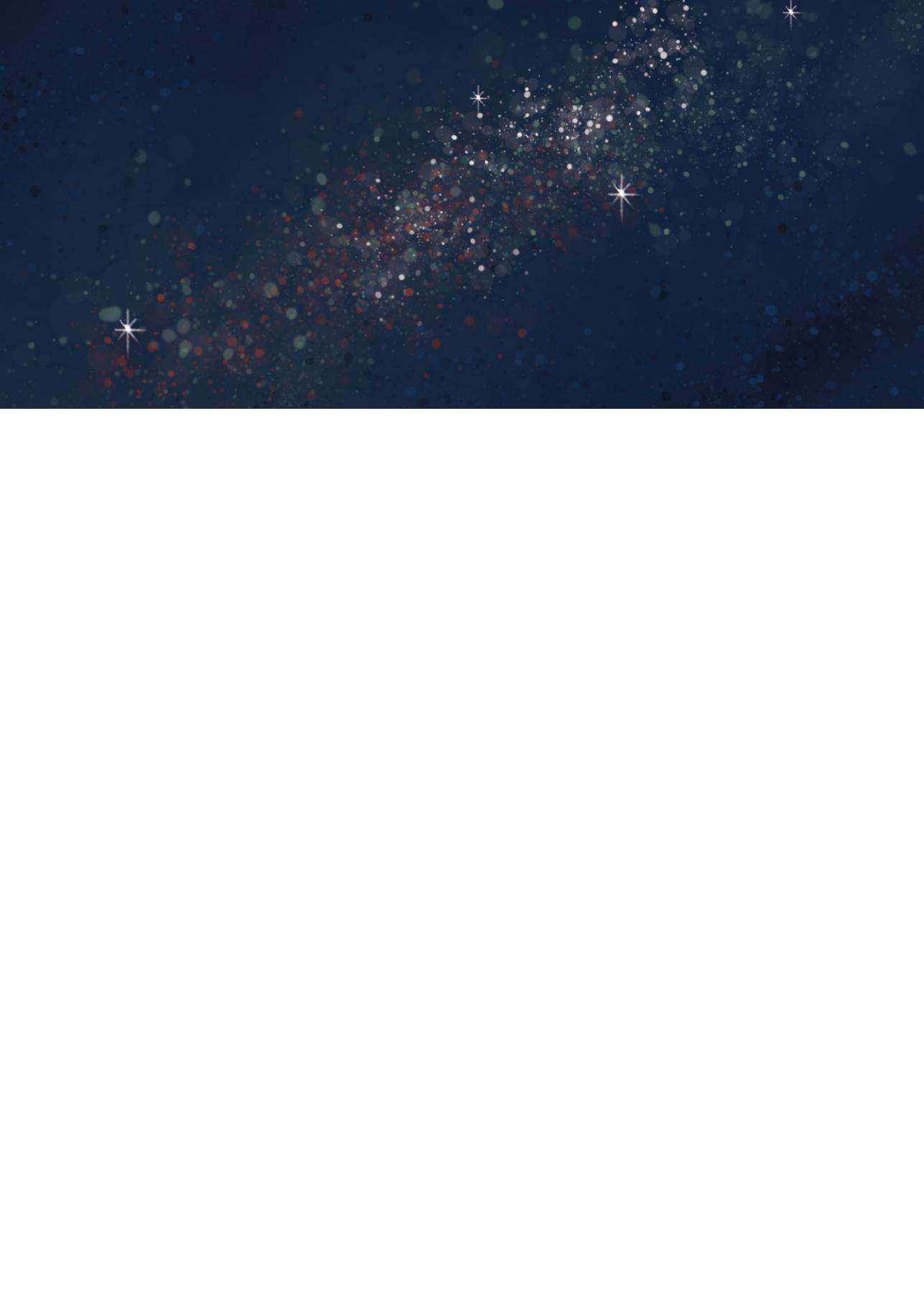
CHAPTER 7

DIAGNOSTIC EVALUATION OF THE FIRST MACROSCOPIC HEMATURIA EPISODE IN ADULT HEMOPHILIA PATIENTS

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Abstract

Introduction
Patients with hemophilia may experience spontaneous macroscopic hematuria. Various international guidelines concerning evaluation of macroscopic hematuria are available for the general population. These guidelines advise urinalysis followed by imaging and/or cystoscopy. The necessity of diagnostic evaluation and hemostatic treatment of hematuria in hemophilia patients is still a topic of debate.

Aim
To evaluate the diagnostic strategy and treatment of hemophilia patients at the first lifetime episode of macroscopic hematuria in a single hemophilia treatment center.

Materials and methods
Adult hemophilia patients with at least one spontaneous macroscopic hematuria episode in their medical history were included. Patient characteristics, consecutive diagnostic hematuria evaluation, etiology and treatment data were collected. Diagnostic evaluation consisted of urinalysis, imaging (CT, ultrasonography or plain radiography), and/or cystoscopy.

Results
Thirty-five hemophilia (A/B) patients were included. Median age at first episode of macroscopic hematuria was 33 years (IQR 20 - 41; n = 33) with no significant difference between severe and non-severe patients. In total, thirty-one patients underwent diagnostic evaluation to establish the hematuria etiology during or after the first episode, revealing a cause in 26% (8/31). In about half of patients, therapy consisted of multiple hemostatic treatments (9/16; 56%).

Conclusion
Spontaneous macroscopic hematuria in hemophilia patients can be the first sign of underlying pathology. In approximately a quarter of the evaluated patients, the etiology was found. Therefore, diagnostic evaluation at first lifetime episode including urinalysis and imaging is indicated. Referral to a urologist should be considered in case of a high risk for malignancy.

Introduction

Hemophilia is an inherited X-linked bleeding disorder characterized by a deficiency in clotting factor VIII (FVIII) or clotting factor IX (FIX). Recent retrospective cohort studies in hemophilia patients report prevalences of macroscopic hematuria ranging from 3.0% to 10.4%[1,2] . In three studies, 69% of hemophilia patients of all ages, 66% of adult hemophilia patients and 51.5% of hemophilia patients older than 40 years reported lifetime event of macroscopic hematuria[3-5].

The World Federation for Hemophilia (WFH) recently updated their guideline for diagnosis and treatment of macroscopic hematuria in hemophilia patients[6,7]. In case of urinary tract hemorrhage, site of bleeding should be identified and treatment should immediately be administered. In case of mild painless hematuria, treatment can consist of complete bed rest and vigorous hydration (3 liters/m^2 body surface area per day), together with clotting factor replacement for two days. In case of renal or cardiac impairment with fluid restriction, vigorous hydration is not advised. Administration of desmopressin should be avoided in case of vigorously hydrating patients because of the risk of water retention. Antifibrinolytics are contraindicated to prevent obstruction of the urinary tract by clots. Furthermore, in case of renal bleeding, treatment should continue until bleeding is resolved. In case of recurrent or persistent macroscopic hematuria, patients should be referred to a urologist to assess a possible local etiology.

Various international guidelines are available for the diagnostic evaluation of (macroscopic) hematuria in the general population[8-10]. Of these, the Dutch guideline is considered most cost-effective with the lowest risk for radiation-induced sequelae and the least use of diagnostic evaluation[11]. This guideline for macroscopic hematuria advises sequential steps, starting with assessment of a possible renal etiology. If a urological and/or renal cause for hematuria is not found by urine analysis, further urological evaluation is advised: cystoscopy and imaging (urological CT scan or ultrasound, depending on patient's age). According to the guideline, patients older than 50 years are considered to have a higher risk for urogenital malignancy, warranting a more thorough evaluation. An overview of some hematuria guidelines for diagnosis and therapy is shown in Supplementary Appendix 1.

Despite the recent World Federation of Hemophilias guideline, which is based on limited studies, worldwide clinical practice in case of macroscopic hematuria in hemophilia differs[7]. Furthermore, reported outcomes of diagnostic evaluation and treatment in hemophilia patients with macroscopic hematuria are limited in literature despite its high frequency.

Therefore, we initiated the present study to evaluate outcomes of diagnostic evaluation and treatment of hemophilia patients at first lifetime episode of spontaneous macroscopic hematuria. Subsequent outcomes were also evaluated in light of current macroscopic hematuria guidelines for hemophilia patients and for the general population.

Materials and methods

Patients
Adult hemophilia (A and B) patients treated at the hemophilia treatment center of the Erasmus University Medical Center, Rotterdam, the Netherlands, from January 2015 until December 2020 were screened for inclusion in the study. Patients with at least one reported macroscopic hematuria episode in their medical history were included. In our study period, 271 adult hemophilia A patients, of whom 46 (17%) severe, 46 (17%) moderate and 179 (66%) mild and 33 hemophilia B patients, of whom nine (27%) severe, eight (24%) moderate and sixteen (49%) mild patients were treated and under hemophilia care in our hemophilia treatment center. Based on medical record review, forty-one (13%) had reported at least one episode of macroscopic hematuria of whom six of iatrogenic etiology; prostatectomy, mechanical manipulation of urinary catheter (two patients), prostate biopsy, urethra stricture dilation. These patients were not included as they did not have spontaneous macroscopic hematuria.

Baseline characteristics, information on hematuria episodes, diagnostic evaluation from the referring general practitioner, hemophilia treatment center and hemostatic treatment were collected from available medical files of included patients. The study was not subject to the Medical Research Involving Human Subjects Act (WMO) and was approved by the Committee of Medical Ethics of the Erasmus University Medical Centre Rotterdam, the Netherlands, for data collection and analysis (MEC-2020-0683).

Assessment methods and definitions
Persistent macroscopic hematuria was defined as a new episode of macroscopic hematuria within 3 months after first presentation; recurrent macroscopic hematuria was defined as a new presentation of macroscopic hematuria after 3 months of first presentation. Data on diagnostic procedures including performed urinalysis, imaging (CT, ultrasonography or plain radiography) and/or cystoscopy were collected. Hematuria etiology was based on imaging or cystoscopy, urine culture and/or histopathological analysis. Data on hematuria etiology, defined by clinical judgment after evaluation, were also collected. Data on pain assessment, management of bleeding consisting of watchful waiting, and treatment with clotting factor concentrate or desmopressin were also collected. Hemostatic treatment before cystoscopy (as prophylaxis for procedure-related bleeding) was not considered as hematuria treatment.

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Statistical methods

Categorical data are reported as frequencies and proportions. Continuous data are reported as median and interquartile range. Descriptive statistics were reported for age at first episode, type and severity of hemophilia, etiology found at first and subsequent episode(s) of hematuria, diagnostic evaluation performed and treatment. Kaplan-Meier analysis with log-rank test was performed to assess difference in age at first lifetime episode of macroscopic hematuria between severe and non-severe hemophilia with alpha < 0.05 for statistical significance.

Results

In total, 35 patients had spontaneous hematuria and were included; 30 (86%) had hemophilia A and 5 (14%) had hemophilia B. Patient inclusion is further described in Figure 1. One patient with hemophilia A also had mild type 1 von Willebrand disease. Eleven out of 35 (31%) of included patients had severe hemophilia, eight out of 35 (23%) moderate and sixteen out of 35 (46%) mild. In patients with severe hemophilia, eight of the eleven (73%) of the patients were on prophylactic treatment with clotting factor concentrate before start of the hematuria episode. Patient characteristics are described in Table 1.
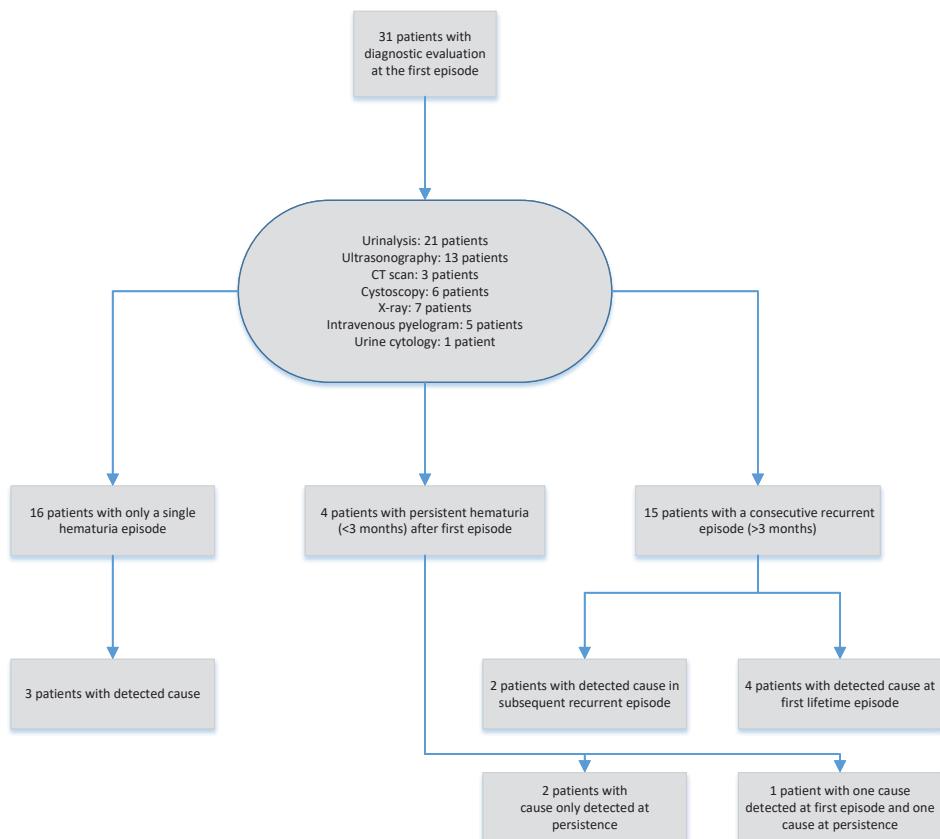

Figure 1: Diagnostic evaluation of first spontaneous macroscopic hematuria and at subsequent persistence or recurrence.

Table 1: Patient characteristics (*n* = 35).

Characteristics	Number (%) / median [IQR]
Hematuria episode	
Single lifetime	16 (46%)
Recurrent/persistent	19 (54%)
Severity hemophilia	
Severe	11 (31%)
Moderate	8 (23%)
Mild	16 (46%)
Age at first episode of hematuria (n = 33[†])	
Severe hemophilia (*n* = 10), years	24 [20 - 37]
Moderate hemophilia (*n* = 8), years	35 [22 - 47]
Mild hemophilia (*n* = 15), years	33 [21 - 41]
Etiology found with diagnostic evaluation at first lifetime episode? (n = 31[‡])	
Yes	8 (26%)
No	23 (74%)
Painless hematuria (n = 28[§])	19 (68%)

[†]Two patients had missing data on age of first episode.
[‡]Four patients had missing data on evaluation at first episode.
[§]Seven patients had missing data on pain at first episode.

A single episode of macroscopic hematuria was reported in sixteen out of 35 (45%), the remaining patients had recurrent or persistent hematuria. In twenty-eight patients, data on pain with the first hematuria episode was available. Of these, nineteen of 28 (68%) reported painless hematuria. Median age at first episode of macroscopic hematuria was 33 years (IQR 20 - 40; n = 33), which did not differ significantly between severe and non-severe hemophilia patients (24 years versus 33 years; p = 0.126). However, all severe hemophilia patients had their first documented lifetime episode before 40 years of age. Of two patients, age at first macroscopic hematuria episode could not be retrieved. Age at first time macroscopic hematuria episode per patient is depicted in Figure 2.

Diagnostic evaluation at first episode
In 31 of 35 (89%) patients diagnostic evaluation was performed at the first episode of macroscopic hematuria of whom eight of 31 (26%) had an identifiable cause (figure 1 and table 2). Nineteen (61%) patients underwent more than one diagnostic test. Specifically, evaluation mainly consisted of urinalysis (21/31; 68%), imaging (19/31; 62%), including ultrasonography (13/19; 42%), and cystoscopy (6/31; 19%). Imaging detected an etiology

in eight of nineteen (42%), of which four out of thirteen by ultrasonography (31%; 4/13). Cystoscopy or urinalysis only detected an etiology in one of six (16%) and one of 21 (5%) respectively. In twelve of 31 (39%) of patients both urinalysis and ultrasonography were performed. Ultrasonography also revealed one medullary sponge kidney which may have been related to hematuria. In case of evaluation by CT, performed in three patients (3/21; 14%), only one cortical kidney cyst was found of which the association with the hematuria etiology was also unclear. About half of the patients who underwent diagnostic evaluation (16/31; 52%) were referred to a urologist at time of the first hematuria episode. In these 16 patients, seven (44%) hematuria causes were found. In one patient, diagnosis was found without referral to a urologist (renal contusion on CT imaging).
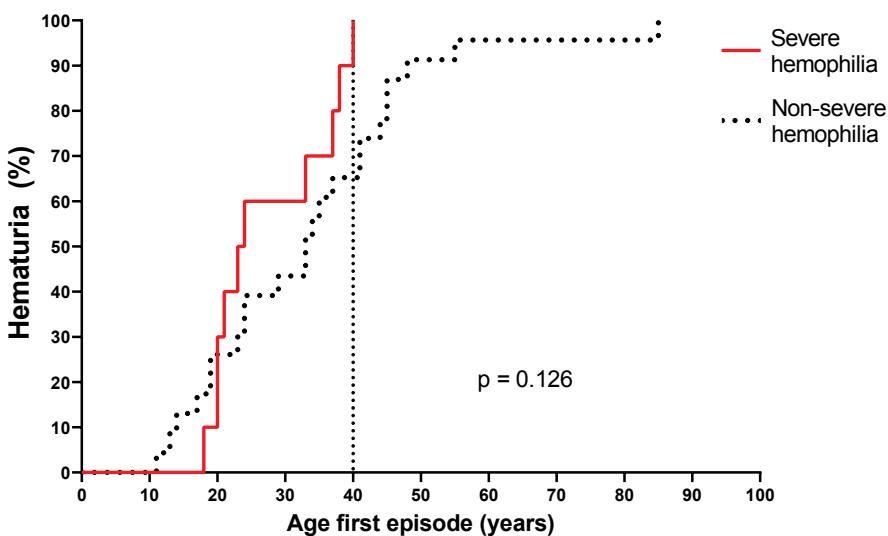
Figure 2: Age at first lifetime macroscopic hematuria episode.

Diagnostic evaluation at persistence or first recurrence of hematuria

Of all 35 patients, nineteen patients (54%) had either persistent hematuria (four patients) after the first episode or at least one recurrent hematuria episode (fifteen patients). In four patients (4/19; 21%), hematuria etiology was found with diagnostic evaluation performed at persistence or first recurrence. In one other patient, an additional cause for hematuria was found at persistence (1/19). At persistence, three patients (3/4) were referred to the urologist and the fourth patient to a nephrologist (1/4). Performed diagnostic evaluation and hematuria etiology of these patients are also described in table 2 and case descriptions are found in the Supplementary Appendix 2. In case of patients with recurrent hematuria, an etiology was found in the first recurrent episode in three patients (2/15; 13%): a urinary tract infection (one patient) and nephrolithiasis (one patient).

Table 2: Diagnostic evaluation of first macroscopic hematuria episode and at time of persistence of hematuria (*n* = 35).

First episode and patients with reported findings

Evaluation	Performed	Findings	Description (number)	Severity	Age
Urinalysis (+ culture)	21	1	Pyelonephritis[†]	Mild[†]	44[†] years
Urine cytology	1	0	-	-	-
Ultrasonography	13	5	Urolithiasis (2)[†]	Mild[†], mild	44[†], 41 years
			Testicular torsion	Severe	17 years
			Prostatitis	Mild	23 years
			Medullary sponge kidney[‡]	Mild	24 years
CT scan	3	2	Renal contusion	Severe	33 years
			Cortical kidney cyst[‡]	Mild	45 years
Intravenous pyelogram	5	1	Urolithiasis	Moderate	37 years
Plain radiography	7	2	Urolithiasis (2)[§]	Mild[§], severe	85[§], 40 years
Cystoscopy	6	1	Urolithiasis / bladder lesion[§]	Mild[§]	85[§] years

Persistence after first episode

Evaluation	Performed	Findings	Description (number)	Severity	Age
Urinalysis (+ culture)	2	1	Urinary tract infection	Severe	20 years
Cystoscopy	2	1	Bladder lesion[§]	Mild	85 years
Ultrasonography	1	0	-	-	-
CT	2	0	-	-	-
Clinical diagnosis	1	1	Nephrolithiasis (urination of debris at emergency department)	Severe	25 years

[†]One patient had both urolithiasis and pyelonephritis simultaneously at time of evaluation.

[‡]Of unknown significance as a hematuria etiology, possibly related.

[§]One patient had urolithiasis at time of first episode, urothelial carcinoma at persistence (possibly related to bladder lesion).

Treatment of macroscopic hematuria to reach hemostasis

In 26 out of 35 patients (74%), information on hemostatic treatment was available. The treatment at first event of the macroscopic hematuria episode is shown in table 3. Hemostatic treatment with clotting factor concentrate was administered to sixteen of 26 (62%). Nine out of sixteen (56%) of these patients received multiple administrations of clotting factor concentrate, seven out of sixteen (44%) received a single treatment dose. Pain was present in only four out of sixteen (25%) of treated patients. In case of painless hematuria, seven out of eightteen (39%) received multiple treatment administrations in order to stop hematuria.

Table 3: Hemostatic treatment at first episode of macroscopic hematuria.

Severity and treatment (*n* = 26)[†]	Number		
	Single dose (*n* = 7)	Repeated doses (*n* = 9)	No treatment (*n* = 10)
Severe hemophilia (*n* = 9)	4	3	2[‡]
Moderate hemophilia (*n* = 7)	3	3	1
Mild hemophilia (*n* = 10)	0	3	7
Hematuria with pain (*n* = 8)	2	2	4
Painless hematuria (*n* = 18)	5	7	6

[†]Nine patients had missing data on treatment.
[‡]One patient had no access to factor concentrate suppletion at the first episode in penitentiary. One other patient had his first lifetime episode before the introduction of FVIII concentrate prophylaxis.

7

Discussion

Spontaneous macroscopic hematuria was reported in 12% of our study cohort of adult patients with hemophilia, of whom 89% underwent diagnostic evaluation. In 26% of these patients a cause could be detected. Of all diagnostic tools, imaging was the most effective in detecting hematuria etiology. Half of the evaluated patients were referred to a urologist. Fifty-six percent of patients received multiple administrations with clotting factor concentrate in order to stop hematuria. In case of painless hematuria, 39% needed multiple administrations of clotting factor concentrate to stop the bleeding.

Recently, three retrospective studies on hematuria in hemophilia patients were published. One cohort included hemophilia patients over five years, in which 3.0% (101/3422) of these patients had been diagnosed with a kidney bleed (according to ICD-9)[2]. During a 6 year follow-up, a significant number of patients with a kidney bleed (4/31; 12.9%) developed chronic renal failure (according to ICD-9). The cause of the kidney bleed was not specified. Concerning chronic renal failure, a second European cross-sectional study in hemophilia patients with hematuria did not show an association between renal clearance and macroscopic hematuria episodes[5]. In this study, 56.3% (282/503), had a lifetime history of at least one hematuria episode. In a third study with a cohort included over seven years, 10.4% (49/474) of the hemophilia patients had at least one episode of hematuria. No information was given on evaluation at the first hematuria episode. However, extensive analysis with urinalysis, plain radiography and ultrasonography in recurrent hematuria revealed that 33% (6/18) was caused by urolithiasis[1]. Of these 18 patients, five (28%) had moderate hemophilia, all other 13 (72%) severe hemophilia. In our study, 14.3% (5/35) at the first hematuria episode and 3% at persistence (1/35) were diagnosed with urolithiasis, less than in the reported study (33%) from literature. Because of the small number of patients in both these studies, these data should be cautiously interpreted. Administration of clotting factor was not specified for all three studies[1,2,5].

In patients with hemophilia, macroscopic hematuria is considered to be benign and is usually attributed to the underlying bleeding disorder[12]. However, in a quarter of the evaluated patients in our study a cause for the hematuria was found, including malignancy in one patient. The current WFH guideline states that elderly patients with hematuria have a higher risk for malignancy, but does not specify agebor method for bleeding site identification in case of a urinary tract haemorrhage[7]. Considering the ubiquity of urinalysis and the effectiveness of imaging (ultrasonography) in our study we suggest to consider these tests in all hemophilia patients with a first macroscopic hematuria episode. This strategy is comparable to the multiple strategies used for macroscopic hematuria in the general population. In addition, this strategy could prevent persistent

hematuria leading to extensive use of clotting factor concentrate with a risk for inhibitor formation. The WFH guidelines only advise referral to a urologist in case of persistence or recurrence[7]. In our study, the hematuria etiology was detected in 44% of the patients with a referral to the urologist. This may be due to the fact that patients who had a high chance to find a detectable hematuria cause (e.g. with pain, no resolution after a single administration of FVIII concentrate) were all referred. We therefore suggest that the initial diagnostic evaluation at the first episode should include referral to a urologist in case of a high risk for malignancy, in case of a high risk for malignancy, such as age above 50 years and smoking, in accordance with guidelines for the general population. A supporting finding for this strategy is that in patients with macroscopic hematuria using anticoagulants - with a higher bleeding risk - have an equal risk in finding a malignancy after diagnostic evaluation when compared to the general population[13,14]. Consequently, sequelae of late diagnosis of a malignancy can be prevented. In our study, only one patient was eventually diagnosed with a malignancy. This is probably related to the relatively young age of the included patients, as a urinary tract malignancy is usually diagnosed in patients older than 40 years. Additionally, this strategy could prevent persistent hematuria leading to extensive use of clotting factor concentrate in case of treatable hematuria etiologies, such as urinary tract infection. However, our study was not performed with a cost-effectiveness analysis in mind.

The most common cause of hematuria in our study was urolithiasis. A possible hypothesis for this is the increased urinary calcium excretion caused by repeated joint bleeds[1]. The use of older imaging modalities in our cohort such as plain radiography, could have led to an underreporting as non-radiopaque stones might have been missed. In addition, this could explain the early age of the first lifetime hematuria episode in the severe hemophilia patients, as (spontaneous) joint bleeds are more common in severe than non-severe hemophilia. In the current WFH guideline, the proposed difference in treatment strategy between a urinary tract hemorrhage, renal bleeding and a mild painless hematuria episode is not further explained: only the latter does not need clotting factor suppletion[7]. On the other hand, in our study, a significant proportion of painless hematuria patients received at least one treatment administration. In addition, the use of hyperhydration in combination with absolute bed rest in order to treat painless hematuria can be challenging for patients. Interestingly, all ten mild hemophilia patients with known treatment had not received clotting factor concentrate at the first lifetime episode. Possibly, this could reflect that for the treating physicians, the first macroscopic hematuria episode would be self-limiting for these mild hemophilia patients. Nonetheless, one mild hemophilia patient developed persistent hematuria, caused by a urinary tract infection that was undetected at first episode (see Supplementary Appendix 2).

Our study has certain limitations related to its retrospective character. Information bias could have been introduced as our cohort is based on retrospective data. However, we expect an underreporting of hematuria causes as not all patients in our cohort had received a diagnostic evaluation. Nonetheless, a majority of our patients still underwent further analysis including urology referral.

Conclusion

Spontaneous macroscopic hematuria in hemophilia patients can be the first sign of underlying pathology. In approximately a quarter of the evaluated patients, the etiology was found. Therefore, evaluation at first episode including urinalysis and ultrasonography is indicated to treat accordingly, comparable to guidelines for the general population. Referral to a urologist should be considered in case of a high risk for malignancy.

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Supplementary Appendix

Supplementary Appendix 1: Comparison of guidelines related to hematuria: evaluation (for all) and treatment (in hemophilia)[†].

	American (KP, U.S.A.)	**Canadian[‡]**
Evaluation first episode	Non-smoking females <50 years: • No evaluation Smokers, non-smoking females ≥50 years or males with hematuria: • Cystoscopy and ultrasonography	• Full investigation of macroscopic hematuria, not further specified
Evaluation subsequent persistent or recurrent episode	Recurrent or persistent macroscopic hematuria in the last 6 months: • Cystoscopy and CT	Recurrent macroscopic hematuria after asymptomatic microscopic hematuria: • (Repeat) urological evaluation
Treatment	N/A	N/A

[†]Adapted after KP guideline[1], Wollin et al.[2], van der Molen et al.[3], and Srivastava et al.[4].
[‡]Mainly a guideline for asymptomatic microscopic hematuria[2].

Dutch	Hemophilia (WFH)
Macroscopic: • Urinalysis • Blood pressure (if <50 years) • Renal function assessment (eGFR[§]) • Referral to urologist If (urin)analyses negative (no etiology found): <50 years: • Ultrasonography and cystoscopy ≥50 years • CT and cystoscopy If signs nephrologic disease present: • Referral to nephrologist	In urinary tract hemorrhage: • Identify site of bleeding Urinary tract bleeding: • Possibly first sign of malignancy (particularly older patients)
If smoking and persistent microscopic hematuria, even after earlier analysis: • CT scan and urine cytology If persistent macroscopic hematuria: • CT scan If imaging/cystoscopy negative: • Urine cytology If signs nephrologic disease present: • Referral to nephrologist	In persistent or recurrent hematuria: • Referral to urologist
N/A	• No antifibrinolytics. • Urinary tract hemorrhage: immediately start clotting factor suppletion. • Renal bleeding: give clotting factor concentrate until bleeding subsides, treatment is urgent. • Mild painless hematuria: complete bed rest and vigorous hydration (3 l/m^2 body surface area) for 48 hours. • Avoid desmopressin during hydration.

[§]Estimated glomerular filtration rate, i.e. calculated by using the CKD-EPI formula.

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Supplementary Appendix 2: Diagnostic evaluation of four patients with hematuria persistence after first episode.

1) The first patient with persistent hematuria and severe hemophilia A, a cystoscopy and a plain abdominal radiography showed a bladder stone at first macroscopic hematuria episode. A second cystoscopy at persistence also showed a bladder lesion, considered as benign and was assumed to be caused by a previously placed urinary catheter. After persistent hematuria episodes occurred, which were repeatedly treated with clotting factor treatment, the patient developed an inhibitor to factor VIII. Eventually, a fatal bladder bleeding occurred despite treatment with extensive clotting factor treatment, rescue therapy with recombinant FVIIa and plasma. Autopsy revealed urothelial cell carcinoma of the bladder.

2) The second patient with mild hemophilia A developed persistent hematuria after his first episode, requiring extensive clotting factor suppletion for multiple days. No urinalysis nor imaging had been performed at first episode. After referral to urology, diagnostic procedures were performed and a urinary tract infection with *Klebsiella oxytoca* was diagnosed. Hematuria swiftly resolved after antibiotic treatment.

3) The third patient with persistent hematuria, severe hemophilia A and FVIII inhibitors, had a first hematuria episode with urinalysis revealing hematuria and proteinuria. Renal function assessment revealed stage 3b chronic kidney disease and bleeding resolved with FEIBA. Patient was also referred to nephrology for assessment of hematuria and possible chronic kidney disease. The persistent hematuria episode occurred one month later with urination of debris and clots at the emergency department, possibly related to nephrolithiasis. Repeat urinalysis revealed hematuria, leukocyturia and proteinuria. A plain radiography at the emergency department revealed old calcifications, attributed to earlier muscle bleeds. Multiple FEIBA administrations lead to bleeding resolution. An additional urological CT advised by the nephrologist, performed after earlier persistent hematuria, did not reveal any nephrolithiasis. After resolution, patient eventually was diagnosed with C3 glomerulonephritis, confirmed by renal biopsy.

4) The fourth persistent hematuria patient with severe hemophilia B had no access to factor concentrate suppletion at the first episode in penitentiary. Urinalysis at first episode confirmed hematuria. With persistent hematuria after first episode, patient was referred to urology and urinalysis was repeated. Extensive analysis was performed by urology, including a urological CT and cystoscopy and clotting factor suppletion was started. Eventually, no hematuria cause was found.

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CHAPTER 8

A FACTOR IX VARIANT THAT FUNCTIONS INDEPENDENTLY OF FACTOR VIII MITIGATES THE HEMOPHILIA A PHENOTYPE IN PATIENT PLASMA

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Abstract

Background
Recombinant factor (F)IX-FIAV has previously been shown to function independently of activated FVIII (FVIIIa) and ameliorate the hemophilia A (HA) phenotype *in vitro* and *in vivo*.

Objectives: To assess the efficacy of FIX-FIAV in HA patient plasma using thrombin generation (TG) and intrinsic clotting activity (APTT) analyses.

Methods: Plasma obtained from 21 patients with HA (>18 years; 7 mild, 7 moderate, and 7 severe) was spiked with FIX-FIAV. The FXIa-triggered TG lag time and APTT were quantified in terms of FVIII-equivalent activity using FVIII calibration for each patient plasma.

Results: The linear, dose-dependent improvement of the TG lag time and APTT reached its maximum with approximately 400-600% FIX-FIAV in severe HA plasma and with approximately 200-250% FIX-FIAV in non-severe HA plasma. The cofactor-independent contribution of FIX-FIAV was therefore suggested and confirmed by addition of inhibitory anti-FVIII antibodies to non-severe HA plasma, resulting in a FIX-FIAV response similar to severe HA plasma. Addition of 100% (5 μg/mL) FIX-FIAV mitigated the HA phenotype from severe to moderate (from <0.01% to 2.9% [IQR 2.3-3.9%] FVIII-equivalent activity), from moderate to mild (3.9% [IQR 3.3-4.9] to 16.1% [IQR 13.7-18.1%] FVIII-equivalent activity), and from mild to normal (19.8% [IQR 9.2-24.0%] to 48.0% [IQR 34.0-67.5%] FVIII-equivalent activity). No substantial effects were observed when combining FIX-FIAV with current HA therapies.

Conclusion: FIX-FIAV is capable of increasing the FVIII-equivalent activity and coagulation activity in plasma from HA patient, thereby mitigating the HA phenotype. Hence, FIX-FIAV could serve as a potential treatment for HA with or without inhibitors.

Introduction

Hemophilia A (HA) is an inherited bleeding disorder characterized by a deficiency of coagulation factor (F)VIII. Treatment consists of on-demand administration of FVIII concentrate in case of bleeding, or of regular FVIII infusions as prophylaxis to prevent bleeding. Emicizumab, a bispecific monoclonal antibody that mimics FVIII[1], can also be used for prophylaxis. FVIII replacement therapy may be associated with the development of anti-FVIII inhibitory antibodies (FVIII inhibitors), which develop in 13-39% of HA patients[2,3]. In inhibitor patients, treatment options are emicizumab and bypassing agents such as recombinant FVIIa (rFVIIa) and activated prothrombin complex concentrate (aPCC)[4-7]. Although these treatments are effective in most patients, the use of rFVIIa may lead to thrombotic complications in rare cases and more often in combination with aPCC[8]. Furthermore, thrombotic microangiopathy has been observed in patients receiving emicizumab and aPCC simultaneously[9]. In addition, in up to 50% of patients in prospective cohort studies emicizumab treatment did not prevent breakthrough bleeds, although most of these were trauma-associated bleeds[10].

To further expand current HA treatment strategies, we validated a novel bypassing agent. This agent is a modified FIX variant, FIX-FIAV, that functions independently of activated FVIII (FVIIIa) and was previously shown to ameliorate the HA phenotype *in vitro* and *in vivo*[11,12]. FIX-FIAV is named after its four amino acid substitutions: L6F, V181I, K265A, and I383V. These modifications have been implicated to be important for substrate recognition and binding and together lead to FVIII-independent FIX activity. The biochemical characteristics of FIX-FIAV were previously assessed[11]. In short, in a plasma-free system FIXa-FIAV presented up to 5-fold increased factor X (FX) activation in the absence of FVIIIa compared to wild-type FIXa(-WT).

Here we have assessed the prohemostatic effect of FIX-FIAV in a preclinical setting in plasma of patients with severe, moderate, or mild HA with and without FVIII inhibitor. This allowed us to not only evaluate the FIX-FIAV response regardless of residual and baseline FVIII levels, but to also study potential enhanced effects of FIX-FIAV in combination with the current HA therapies FVIII, emicizumab, FVIIa, or aPCC.

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Methods

Patient inclusion

Overall, 21 adult (>18 years) male HA patients were included: 7 severe (historically lowest FVIII:C <0.01 IU/mL), 7 moderate (historically lowest FVIII:C 0.01–0.05 IU/mL), and 7 mild (historically lowest FVIII:C >0.05–0.40 IU/mL). Patients with an additional bleeding disorder, chronic liver failure, or having received prophylactic treatment <48 hours within study participation or bypassing therapy in general, including prophylaxis with bypassing agents, (eg emicizumab, FVIIa, aPCC) were excluded. The median patient age was 38 years (interquartile range (IQR), 30–49 years). Included patients received a last dose of FVIII concentrate at least 3 days prior to inclusion, and none of the patients were treated with extended half-life FVIII products. Historically lowest measured FVIII:C levels and known *F8* mutations were collected from patients' medical files. The study was approved by the local Medical Ethics Committee (MEC-2019-0617), and all patients gave written informed consent.

Human plasma

Blood was collected in vacuum tubes containing 0.129 M sodium citrate with and without 25 µg/mL of the FXIIa inhibitor Thermostable Inhibitor of Contact Activation (TICA, provided by prof. T.M. Hackeng, CARIM, Maastricht University)[13]. Platelet-poor plasma was prepared at room temperature by 2500 x g centrifugation for 15 minutes, followed by 10 minutes 14000 x g centrifugation of the supernatant, and aliquots were stored at -80°C. Normal pooled human plasma (NPP) comprising platelet-poor plasma from ≥20 male and female donors (18–66 years) was from Precision Biologic. FVIII- or FIX-immuno-depleted plasma was from Diagnostica Stago.

Reagents and proteins

Benzamidine and Poly-D-lysine hydrobromide were from Sigma–Aldrich, all tissue culture reagents from Thermo Fisher Scientific. Calibrator and fluorescent substrate (FluCa) were from Thrombinoscope, TriniCLOT automated activated partial thromboplastin time (APTT) reagent, and Owren–Koller buffer (isotonic saline) from Diagnostica Stago. Phospholipid TGT containing phosphatidylserine, phosphatidylcholine, and sphingomyelin was from Rossix, an anti-FVIII inhibitory antibody (GMA-8015) from Green Mountain Antibodies. Human plasma-derived FIX, FIXa, and FXIa were from Prolytix, recombinant FVIII (NovoEight®) and FVIIa (NovoSeven®) from Novo Nordisk A/S, aPCC (FEIBA®) from Takeda Pharmaceutical Company Limited, and emicizumab (HemLibra®) from Roche. Molecular weights (Da) and extinction coefficients ($E_{0.1\%}$, 280 nm) of the proteins used were taken as follows: FIX, 55,000 and 1.32; FIXa, 45,000 and 1.40; FXIa, 160,000 and 1.34. For FIX-FIAV values

for the human protein were used. All functional assays were performed in Hepes-buffered Saline (20 mM Hepes, 0.15 M NaCl, pH 7.5) (HBS buffer) supplemented with 0.1% (w/v) PEG8000 (dilution buffer) and 5 mM $CaCl_2$ (assay buffer).

Plasma analysis

The APTT, FVIII:C using a one-stage FVIII-specific APTT assay (one-stage assay [OSA]) and chromogenic substrate assay (CSA), FIX:C (OSA and CSA), FVIII inhibitors according to the Nijmegen modification of the Bethesda assay, antithrombin, prothrombin, FX, and von Willebrand antigen (VWF:Ag) were measured in citrated plasma without the addition of TICA employing a Sysmex CS5100 (Siemens) using corresponding reagents according to the manufacturer. Bethesda Units (BU) of >0.5 were considered clinically relevant. FVIII:Ag and FIX:Ag were assessed in TICA-comprising plasma employing enzyme-linked immunosorbent assays as described by the manufacturer (Cedarlane).

Construction and expression of recombinant FIX

Constructs encoding for human wild-type FIX (FIX-WT) and FIX comprising L6F, V181I, K265A, and I383V substitutions (FIX-FIAV) were provided by uniQure Biopharma B.V. Human embryonic kidney 293 (HEK293, CRL-1573; ATCC) cell lines stably expressing FIX were obtained following cotransfection of pcDNA3.1-FIX-WT or pcDNA3.1-FIX-FIAV with pcDNA3.1-Furin vectors employing Lipofectamine2000 per the manufacturer instructions and essentially as described previously for FX[14]. In brief, FIX expression of transfectants was assessed by conditioning individual clones for 24 hours in Dulbecco's modified Eagle's medium/F-12 without phenol red supplemented with 2 mM L-glutamine, 100 U/mL penicillin, 0.1 mg/mL streptomycin, 0.25 μg/mL amphotericin B, 100 μg/mL geneticin, 10 μg/mL insulin-transferrin-sodium selenite, and 6 μg/mL vitamin K (Konakion®, Roche) (FIX-specific expression media) and subsequently measuring the FIX-specific APTT clotting activity in a modified one-stage assay by mixing conditioned media with FIX-depleted human plasma in a 1:1 ratio. A reference curve of NPP serially diluted in Owren–Koller buffer mixed in a 1:1 ratio with FIX-depleted human plasma was used to calculate the equivalent FIX Units per mL plasma, with 1 mL of NPP comprising 1 Unit of FIX activity. To monitor FIX-FIAV expression, up to twelve transfectants per variant with the highest FIX expression were assessed for FVIII-equivalent APTT clotting activity using FVIII-depleted human plasma to calculate the equivalent FVIII Units per mL plasma, with 1 mL of NPP comprising 1 Unit of FVIII activity. The transfectants with the highest FIX expression (and FVIII-equivalent expression for FIX-FIAV) were expanded into a 6320 cm^2 cell factory that was pretreated with Poly-D-lysine hydrobromide (5 mg for 1 hour at room temperature) and conditioned for 24 hours in FIX-specific expression media. Conditioned media was collected for 10 consecutive days, filtered over an 0.45 μm polyethersulfone membrane, and supplemented with 10 mM benzamidine prior to storage at −20 °C.

Purification of FIX

Conditioned media (15 L) was thawed at 37 °C, applied to a size 6 A ultrafiltration hollow fiber cartridge using an Äkta flux 6 instrument (Cytiva), diafiltrated to ~500 mL in 20 mM Hepes, 0.15 M NaCl, 10 mM benzamidine, pH 7.4, and stored at −20 °C. After thawing at 37 °C, the concentrate was applied at room temperature to a 4.8 × 4 cm Q-Sepharose Fast Flow column (Cytiva) equilibrated in 20 mM Tris, 0.15 M NaCl, 10 mM benzamidine, pH 7.4. After washing with the same buffer, bound protein was eluted with a linear 0.15 to 0.75 M NaCl gradient. Fractions containing FIX activity were stored at −80 °C. After thawing at 37 °C, the fractions were pooled and dialyzed at 4 °C, first for 3 hours to 1 mM EDTA, 20 mM Tris, 10 mM benzamidine, pH 7.0 (5 L), next for 3 hours to 40 mM Na_2HPO_4/NaH_2PO_4, 10 mM benzamidine, pH 6.8 (5 L), followed by overnight dialysis to the same buffer. The dialysate was centrifuged at 10,000 × g for 20 minutes at 4 °C, and the supernatant was applied at room temperature to a Bio-Scale CHT20-I hydroxyapatite column (Bio-Rad) equilibrated in 40 mM Na_2HPO_4/NaH_2PO_4, 10 mM benzamidine, pH 6.8. After washing with the same buffer, bound protein was eluted with a linear 40 to 400 mM Na_2HPO_4/NaH_2PO_4 gradient at a flow rate of 3 mL/min. Fractions containing FIX activity were analyzed employing sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis, stored at −80 °C, pooled upon thawing at 37 °C, precipitated with solid $(NH_4)_2SO_4$ (80% saturation) at 4 °C, collected by centrifugation (10,000 × g for 30 minutes at 4 °C), dissolved in HBS-buffer and 50% (v/v) glycerol, and stored at −20 °C. The typical yield of fully γ-carboxylated recombinant FIX was 0.15 to 0.20 mg/L conditioned medium. Purified products were visualized by SDS-PAGE analysis employing Quick Coomassie Stain (Protein Ark), indicating homogeneous protein preparations of >95% purity (Supplementary Figure S1).

Anti-FVIII inhibitory antibody

All plasma samples were measured with and without an anti-FVIII inhibitory antibody. Plasma was pretreated with 0.5 µg/mL antibody (corresponding to approximately 8.0 BU) and incubated for 2 hours at 37 °C prior before addition of FIX-FIAV, rFVIII, rFVIIa, aPCC, or emicizumab, as described below. A Nijmegen Bethesda Assay was performed (n = 2) to determine the BU of 0.5 µg/mL inhibitory antibody. In short, NPP was buffered with 0.1M imidazole (pH 7.4) and supplemented with 0.5 µg/mL FVIII antibody. After 2 hours of incubation at 37 °C serial dilutions were prepared in FVIII-deficient plasma and the APTT was measured using the following ratios: one part diluted treated NPP sample, one part untreated NPP, one part APTT reagent, and one part $CaCl_2$.

Calibrated automated thrombography and APTT measurements

Thrombin generation (TG) was adapted from protocols using low plasma volumes as previously described[15,16]. All analyses involved supplementing FVIII-deficient plasma or HA patient plasma with 0-600% (0-30 µg/mL) FIX-FIAV, or 0% to 160% (1-160 IU/dL)

rFVIII, or 1.5 μg/mL FVIIa, or 1 IU/mL aPCC, or 7 to 55 μg/mL emicizumab, or the same volume of dilution buffer. Different ranges of FIX-FIAV were tested per phenotype: 0% to 600% (0-30 μg/mL) for severe HA, 0% to 250% (0-12.5 μg/mL) for moderate, and 0% to 200% (0-10 μg/mL) for mild HA. Sufficient volumes of supplemented plasma samples were prepared to assess both TG and APTT. To convert FIX-FIAV protein concentrations to percentages, 5 μg/mL FIX-FIAV was assumed to be equivalent to 100%[17]. NPP was used as a control. Pre-heated (37 °C) FluCa was added to the plasma prior to addition of the FXIa-trigger. The concentration of the FXIa trigger was optimized in pilot experiments in which FIX-deficient plasma was reconstituted with FIX-WT or FIX-FIAV to obtain full TG correction (Supplementary Figure S2). The final reaction volume was 60 μL, with 40 μL being supplemented plasma, 10 μL phospholipid TGT (20 μM final)/FluCa mix, and 10 μL FXIa (5 nM). Thrombin formation was determined every 20 seconds for 90 to 120 minutes and corrected for the calibrator using Thrombinoscope software. The lag time, thrombin peak, endogenous thrombin potential (ETP), time to peak (TTP), and velocity index (VI) were calculated from duplicate measurements. The FVIII-equivalent activity (expressed in %) of FIX-FIAV was quantified using FVIII reference curves generated individually for each HA patient plasma in which the FVIII concentration was plotted versus the TG lag time. Plasma was supplemented with recombinant FVIII, and baseline FVIII:C (CSA) values determined the lowest value of the reference curve. Subsequently, employing a Start4 coagulation instrument (Diagnostica Stago) the APTT was measured in the same plasma sample as prepared for TG that was stored on ice. One part of the prepared plasma sample was incubated with an equal part of APTT reagent (micronized silica) and incubated for 180 seconds at 37 °C. The reaction was started by adding one part pre-warmed $CaCl_2$.

Data analysis
All continuous data are presented as median and interquartile range (IQR), unless otherwise stated. Categorical data are presented as frequency and proportions. A Wilcoxon signed rank test (IBM SPSS statistics version 28) was performed to analyze continuous data before and after treatment with 100% FIX-FIAV. The level of significance was set at a *p*-value of <0.05.

Results

Patient plasma protein levels

FVIII:C analysis confirmed the severe HA patients having <0.01 IU/mL plasma FVIII activity, the moderate patients 0.03-0.05 IU/mL, and the mild patients 0.07-0.21 IU/mL (Table 1). For all HA phenotypes circulating FVIII protein was detected (FVIII:Ag, Table 1). FVIII inhibitor assessment indicated the presence of a clinically relevant inhibitor towards exogenous FVIII in two patients with moderate HA (0.8 and 5.2 BU) and in two patients with mild HA (1.4 and 2.8 BU). No FVIII inhibitor was detected for the severe patients. The plasma proteins VWF, FIX, FX, prothrombin, and antithrombin were within the normal range (Table 1).

FXIa-triggered thrombin formation detects FVIII activity <1%

To assess the FVIII-bypassing effect of FIX-FIAV in HA plasma we made use of a FXIa-triggered TG assay for sensitive assessment of very low FVIII activity levels (<1%). Analysis of the TG lag time demonstrated a dose-dependent correlation with recombinant FVIII added to HA patient plasma (Figure 1) and allowed for the detection of FVIII levels as low as 0.05% in FVIII-deficient plasma (Supplementary Figure S3). Considering the well-described large inter-individual variation in TG response[18], the FVIII-equivalent activity following supplementation with FIX-FIAV was quantified in percentage using reference curves of known FVIII concentrations versus the TG lag time generated individually for each HA plasma (Figure 1D-F), where 100% FIX-FIAV equals 5 µg/mL.

FIX-FIAV improves thrombin formation in HA plasma

Next, we demonstrated that the addition of purified recombinant FIX-FIAV to severe, moderate, or mild HA plasma improved TG, which was indicated by a shortening of the TG lag time corresponding with increasing concentrations of FIX-FIAV (Figure 2A-C, Supplementary Table S1). In contrast, supplementation of HA plasma with FIX-WT did not affect the TG parameters (Supplementary Figure S4), thereby confirming the FVIII-bypassing potency of FIX-FIAV. Interestingly, conversion of the TG lag times obtained in the presence of FIX-FIAV to FVIII-equivalent activity demonstrated a linear correlation of FIX-FIAV levels with FVIII-equivalent activity (Figure 2D-F).

Table 1: Coagulation parameters and plasma protein levels

	Severe (*n* = 7)	Moderate (*n* = 7)	Mild (*n* = 7)
APTT (sec)	86.1 [65.7 − 88.2]	43.5 [38.8 − 46.1]	37.5 [33.8 − 39.3]
FVIII:C, OSA (IU/mL)	<0.01	0.04 [0.03 − 0.05]	0.14 [0.07 − 0.20]
FVIII:C, CSA (IU/mL)	<0.01	0.03 [0.03 − 0.05]	0.13 [0.07 − 0.22]
FVIII:Ag (%)	4.3 [1.7 − 5.0]	5.0 [3.8 − 8.2]	10.9 [8.6 − 22.8]
FVIII inhibitors (BU)	0.0 [0.0 − 0.1]	0.0 [0.0 − 0.5]	0.1 [0.0 − 0.8]
FIX:C, OSA (IU/mL)	1.13 [1.02 − 1.21]	0.96 [0.89 − 1.06]	0.99 [0.96 − 1.02]
FIX:C, CSA (IU/mL)	1.21 [1.05 − 1.35]	1.03 [0.99 − 1.13]	1.12 [1.00 − 1.20]
FIX:Ag (%)	85.0 [79.0 − 93.0]	79.0 [76.0 − 83.0]	88.0 [78.5 − 94.5]
AT (IU/mL)	1.02 [0.97 − 1.14]	1.08 [0.99 − 1.17]	1.06 [0.93 − 1.08]
FII (IU/mL)	1.13 [1.02 − 1.20]	1.09 [0.96 − 1.17]	1.11 [1.03 − 1.13]
FX (IU/mL)	1.10 [1.07 − 1.20]	0.98 [0.87 − 1.21]	1.02 [0.92 − 1.09]
VWF:Ag (IU/mL)	1.41 [0.91 − 2.23]	1.22 [0.98 − 1.34]	1.76 [1.36 − 2.26]
Days since last FVIII concentrate dose	4 [3 − 5]	198 [118 − 262]	50 [39 − 614]
Gene mutation			
p.Arg2169His	-	6 (86%)	2 (28.5%)
Intron 22 inversion	4 (57%)	-	-
p.LLE1213Phefs*5	2 (29%)	-	-
p.Gln802X	1 (14%)	-	-
p.Leu504Leu and p.Asp1241Glu	-	1 (14%)	
p.Arg612Cys	-	-	1 (14.5%)
p.Phe698Ser	-	-	1 (14.5%)
p.Arg2178Cys	-	-	1 (14.5%)
p.Pro149Arg	-	-	1 (14.5%)
p.Ala563Gly	-	-	1 (14.5%)

Reported in median [IQR] or number (percentage). Abbreviations: APTT, activated partial thromboplastin time; C, clotting activity; Ag, antigen; BU, Bethesda units; OSA, one-stage assay; CSA, chromogenic substrate assay, AT, antithrombin; FII, prothrombin; FX, factor X; VWF, von Willebrand factor.

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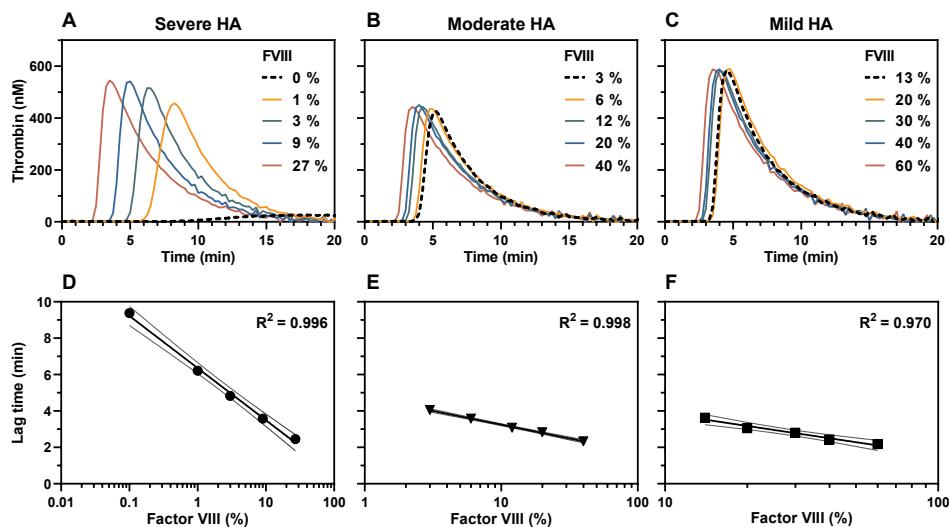
Figure 1. Correlation between factor VIII levels and the thrombin generation lag time. Thrombin generation was initiated with 5 nM FXIa in severe (A,D), moderate (B,E), or mild (C,F) HA plasma in the absence or presence of increasing concentrations of recombinant FVIII (%) (NovoEight) as indicated.

A-C: Representative curves are shown, with thrombin generation monitored in patient plasma with endogenous FVIII:C levels of <0.01 IU/mL (<1%) for severe HA, 0.03 IU/mL (3%) for moderate HA, or 0.13 IU/mL (13%) for mild HA.

D-F: The FVIII concentration was plotted versus the lag time of thrombin generation, and the data were fitted using a semilog fit and are displayed as mean ± 95% confidence interval.

HA, hemophilia A.

This dose-dependent response was consistently observed for all severe, moderate, and mild HA plasmas (Figure 3). The maximum response of FIX-FIAV differed per HA phenotype and was attained upon supplementation with approximately 600% FIX-FIAV for severe HA, resulting in a median FVIII-equivalent activity of 26% (IQR 19%–31%). For moderate HA the maximum response was observed at 250% FIX-FIAV leading to 33% (IQR 27%-37%) FVIII-equivalent activity, and for mild HA at 200% FIX-FIAV producing 44% (IQR 45%-97%) FVIII-equivalent activity (Figure 3, Supplementary Table S1). This is likely because of the plasma FVIII:C levels as this augments the FVIII-equivalent activity of FIX-FIAV in a dose-dependent manner (Supplementary Figure S5).
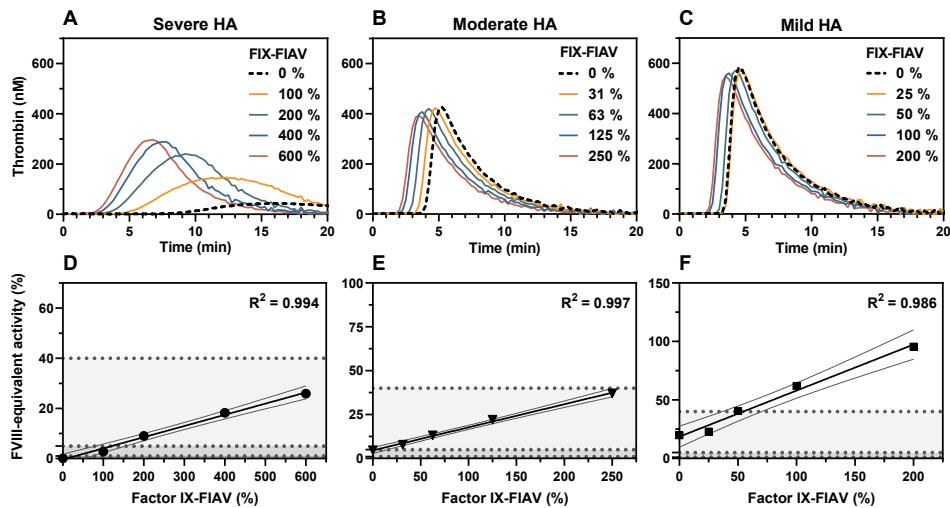
Figure 2. Factor IX-FIAV improves thrombin generation in hemophilia A plasma. Thrombin generation was initiated with 5 nM FXIa in severe (A,D), moderate (B,E), or mild (C,F) HA plasma supplemented with 0 to 600%, 0% to 250%, or 0% to 200% FIX-FIAV, respectively (100% equals 5 μg/mL FIX-FIAV).

A-C: Representative thrombin generation curves are shown.

D-F: The FIX-FIAV concentration was plotted versus the FVIII-equivalent activity, with the latter calculated employing the thrombin generation lag time as described in the "Methods" section. The data were fitted using linear regression and are displayed as mean ± 95% confidence interval. The grey areas bordered by the dotted lines represent the FVIII activity cut-off values indicating severe (<1%), moderate (1-5%), or mild (5-40%) HA.

HA, hemophilia A.

The APTT of HA plasma was also shortened with increasing FIX-FIAV concentrations (Figure 4D-F) in a similar manner as observed for the TG lag time (Figure 4A-C). In contrast, the TG parameters ETP, thrombin peak, and velocity index did not show a dose-dependent response and plateaued at approximately 200% to 400% FIX-FIAV in severe HA, while in both moderate and mild HA, similar values were observed for all FIX-FIAV concentrations tested (Supplementary Table S1). Collectively, these data demonstrate that FIX-FIAV improves thrombin formation in HA plasma.
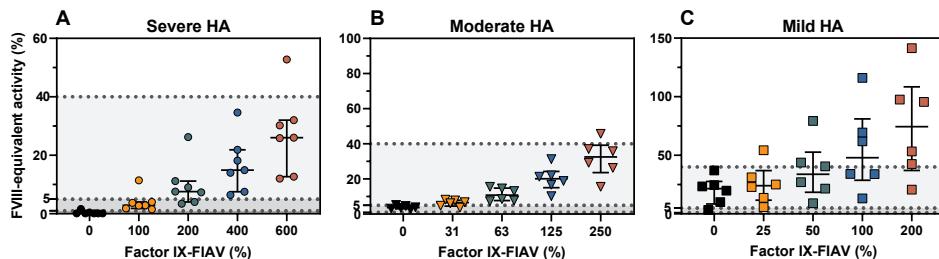
Figure 3. FIX-FIAV generates FVIII-equivalent activity in hemophilia A plasma. Thrombin generation was initiated with 5 nM FXIa in HA plasma supplemented with FIX-FIAV as indicated. The FIX-FIAV concentration (100% equals 5 μg/mL FIX-FIAV) of 0% to 600% for severe HA (A), 0% to 250% for moderate HA (B), or 0% to 200% for mild HA (C) was plotted versus the FVIII-equivalent activity, which was calculated individually for each HA plasma employing the thrombin generation lag time as described in the "Methods" section. Analyses in plasmas with a FVIII inhibitor titer ≥2.8 BU were excluded, as the inhibitor did not allow for quantification of the FVIII-equivalent activity due to interference with the FVIII reference curves. All outliers in Figure 3A can be ascribed to one individual who presented with FVIII antigen levels of 2.2% which can be explained by FVIII prophylaxis 3 days before inclusion. The grey areas bordered by the dotted lines represent the FVIII activity cut-off values indicating severe (<1%), moderate (1-5%), or mild (5-40%) HA. Median [IQR] values are provided in Supplementary Table S1.
HA, hemophilia A.

FIX-FIAV ameliorates the HA phenotype in patient plasma

To gain more insight on how FIX-FIAV affects the HA phenotype in plasma *in vitro*, we specifically focused on the addition of 100% (5 μg/mL) FIX-FIAV to HA plasma, as this results in an overall 1:1 ratio of endogenous FIX versus FIX-FIAV. For severe HA, a median FVIII-equivalent activity of 2.9% (IQR 2.3-3.9%) was observed following supplementation with 100% FIX-FIAV (Figure 5A-C), which corresponds to a moderate laboratory HA phenotype. For moderate HA, the median FVIII-equivalent activity increased to 16.1% (IQR 13.3-18.1%) upon adding 100% FIX-FIAV, leading to a mild laboratory HA phenotype. In mild HA, a median FVIII-equivalent activity of 48.0% (IQR 34.0-67.5%) was observed in the presence of 100% FIX-FIAV, corresponding to normal FVIII plasma levels. These findings indicate that FIX-FIAV levels equimolar to those of endogenous FIX are sufficient to improve the hemostatic potential and thereby mitigate the HA phenotype in patient plasma.
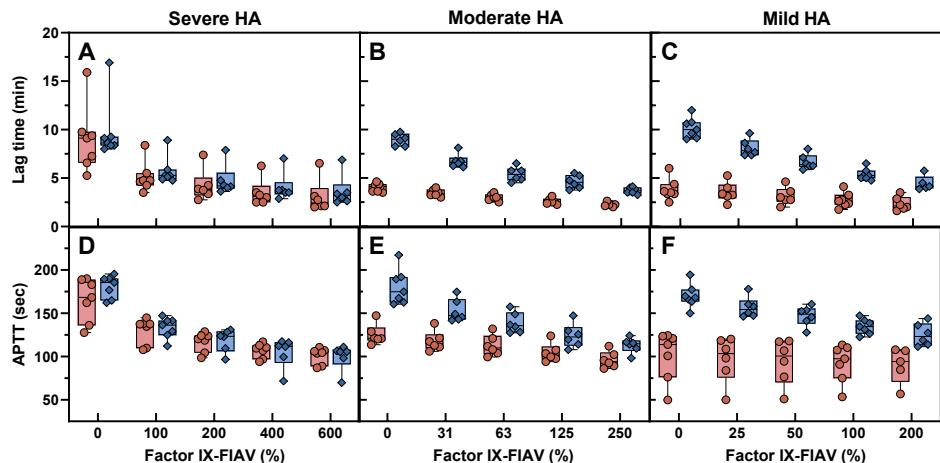
Figure 4. Cofactor-independent effect of FIX-FIAV on coagulation parameters in hemophilia A plasma. The lag time of FXIa-triggered thrombin generation (A-C) or the APTT (D-F) was determined in severe (A,D), moderate (B,E), or mild (C,F) HA plasma supplemented with 0% to 600% for severe HA, 0% to 250% for moderate HA, and 0% to 200% for mild HA FIX-FIAV (100% equals 5 µg/mL FIX-FIAV) in the absence (red circles) or presence of 5 µg/mL anti-FVIII antibody (blue diamonds) as described in the "Methods" section. Analyses in plasmas with a FVIII inhibitor titer ≥2.8 BU were excluded, as the inhibitor did not allow for quantification of the FVIII-equivalent activity because of interference with the FVIII reference curves. Median (IQR) values are provided in Supplementary Tables S1 and S2.

HA, hemophilia A.

The cofactor-independent activity of FIX-FIAV improves coagulation in HA plasma

The baseline FVIII activity in plasmas of both moderate and mild HA patients hampers the interpretation of the cofactor-independent effect of FIX-FIAV. We therefore performed the same experiments as described above in the presence of an inhibitory anti-FVIII antibody to abolish all endogenous FVIII activity. In the absence of FIX-FIAV but with 5 µg/mL of the anti-FVIII antibody present, both the TG lag time and APTT clotting time were not affected in severe HA plasma given its low baseline FVIII activity (Figure 4, Supplementary Table S2). In contrast, analyses of moderate and mild HA plasma in the presence of the anti-FVIII antibody demonstrated prolonged TG lag times and APTT clotting times relative to conditions without the antibody, generating values similar to those observed for severe HA. Furthermore, the addition of increasing concentrations of FIX-FIAV to the antibody-spiked moderate and mild HA plasmas resulted in TG and APTT responses comparable to those observed for severe HA plasma. These data further substantiate that FIX-FIAV displays cofactor-independent FIX activity in a range of plasmas from HA patients.
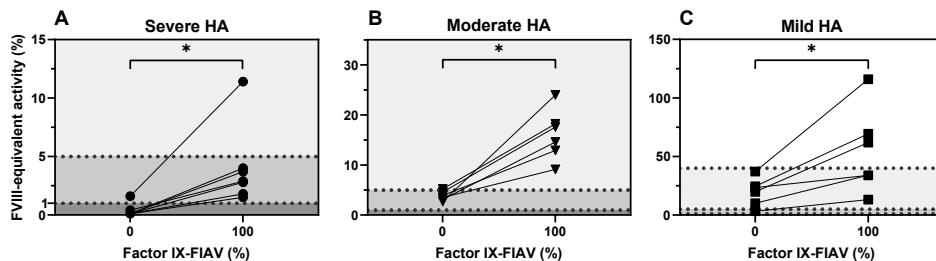
Figure 5. FIX-FIAV mitigates the hemophilia A phenotype in severe, moderate, and mild patient plasma. The lag time of FXIa-triggered thrombin generation in the absence or presence of 100% (5 µg/mL) FIX-FIAV determined in severe (A), moderate (B), or mild (C) HA plasma was converted to FVIII-equivalent activity as described in the "Methods" section. For moderate HA, the FVIII-equivalent activity at 100% FIX-FIAV was extrapolated from the individual FIX-FIAV response plots. Analyses in plasmas with a FVIII inhibitor titer ≥2.8 BU were excluded, as the inhibitor did not allow for quantification of the FVIII-equivalent activity due to interference with the FVIII reference curves. The grey areas bordered by the dotted lines represent the FVIII activity cut-off values indicating severe (<1%), moderate (1-5%), or mild (5-40%) HA. Statistical significance: *$p \leq 0.05$. Median (IQR) values are provided in Supplementary Table S1.

HA, hemophilia A.

We next assessed the efficacy of FIX-FIAV in the setting of circulating clinically relevant inhibitors towards exogenous FVIII, which were detected in two moderate (0.8 and 5.2 BU) and in two mild (1.4 and 2.8 BU) HA plasmas. As expected, a FVIII titration revealed a minimal shortening of the TG lag time in plasmas with an inhibitor titer of ≥1.4 BU, while an up to 1.7-fold shortened lag time was observed in plasma with an inhibitor titer of 0.8 BU, with a visible difference between high and low titer inhibitor patients (cut-off value 5 BU) (Figure 6A). In contrast, a dose-dependent shortening of the TG lag time was found for all inhibitor plasmas following FIX-FIAV addition (Figure 6B). Collectively, these findings indicate that the cofactor-independent FIX-FIAV activity is not affected by exogenous or endogenous inhibitory FVIII antibodies, thus allowing FIX-FIAV to function as a bypassing agent to improve hemostasis in HA plasma.

FIX-FIAV combined with various HA treatment modalities shows a minor enhanced effect
To investigate a potential enhanced effect of bypassing agents on FIX-FIAV function, we have performed spiking experiments with the FVIII-bypassing agents aPCC or FVIIa in FIX-FIAV-treated HA plasmas (three per phenotype). Although supplementation with 100% FIX-FIAV shortened the median TG lag time up to 1.5-fold compared to baseline, supplementation with 1 IU/mL aPCC or 1.5 µg/mL FVIIa, both peak plasma concentrations of respective drugs in typical clinical use, displayed a minimal effect on the TG lag time (maximum 1.2-fold reduced) (Figure 7A, Supplementary Table S3). Given that we made use of FXIa-triggered TG and as such primarily monitored the intrinsic coagulation

system, none of the TG parameters were affected upon supplementation with FVIIa, corroborating previous observations[19]. When compared to supplementation with FIX-FIAV alone, the combination of aPCC or FVIIa with FIX-FIAV did not affect the TG lag time, while the combination of aPCC and FIX-FIAV resulted in an up to 2.2-fold enhanced ETP. This was mostly caused by an aPCC-related increase in ETP of up to 2.3-fold relative to supplementation with FIX-FIAV. Similar results were obtained following APTT analyses (Supplementary Table S3). Hence, no substantial enhanced effect was observed when combining FIX-FIAV with the bypassing agents aPCC or FVIIa.
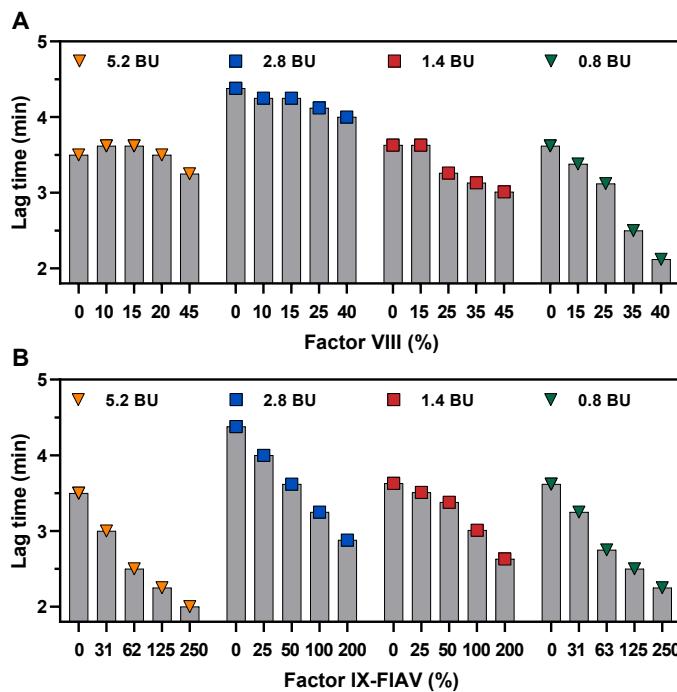
Figure 6. FIX-FIAV enhances thrombin generation in hemophilia A plasma of patients with clinically relevant circulating FVIII inhibitors. The lag time of FXIa-triggered thrombin generation was determined in HA plasmas with clinically relevant FVIII inhibitors, which were supplemented with either 0% to 45% FVIII (A) or 0% to 250% (0-12.5 μg/mL) FIX-FIAV (B) as described in the "Methods" section. The plasmas assessed were moderate HA with an inhibitor titer of 5.2 BU, FVIII:C (CSA) 0.07 IU/mL, FVIII:Ag 4% (yellow triangles), moderate HA with an inhibitor titer of 0.8 BU, FVIII:C 0.05 IU/mL, FVIII:Ag 27% (green triangles), mild HA plasma with an inhibitor titer of 2.8 BU, FVIII:C 0.10 IU/mL, FVIII:Ag 9% (blue squares), and mild HA plasma with an inhibitor titer of 1.4 BU, FVIII:C 0.07 IU/mL, FVIII:Ag 4% (red squares).

HA, hemophilia A.

A

B

C
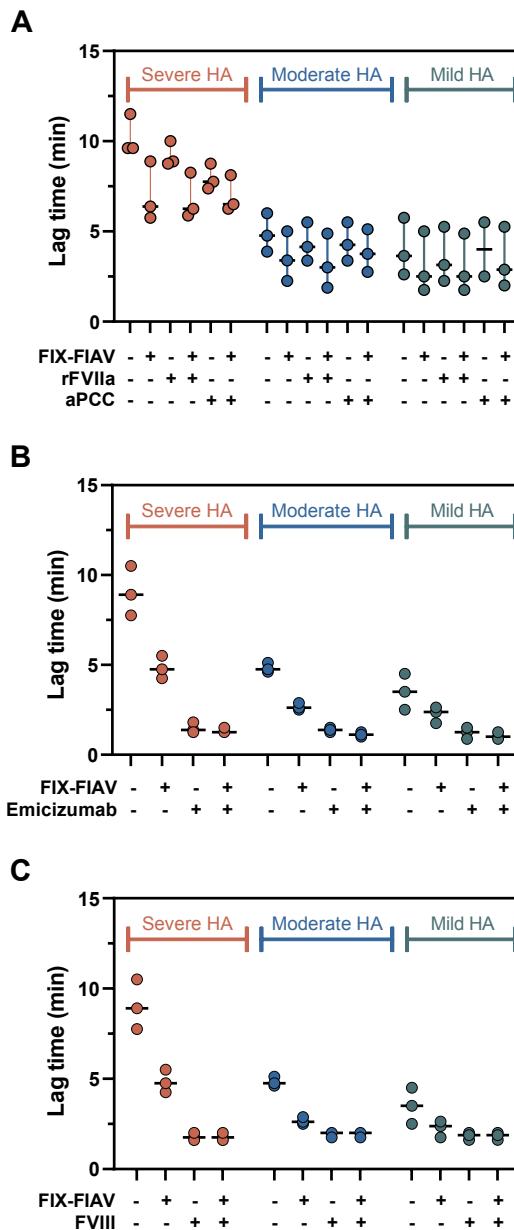
Figure 7. The effect of FIX-FIAV combined with conventional HA therapies. Severe (red), moderate (blue) and mild (green) hemophilia A patient plasma with and without 100% (5 µg/mL) FIX-FIAV was tested with and without (A) 1.5 µg/mL of bypassing agents activated recombinant factor VII (FVIIa) or 1 U/mL activated prothrombin complex concentrates (aPCC), (B) 55 µg/mL emicizumab, or (C) 1 U/mL FVIII. Data are depicted as median and range of thrombin generation lag time and represent two to three independent experiments.

HA, hemophilia A.

Similar experiments in which hemostatic levels of recombinant FVIII (1 U/mL) (Figure 7B) or emicizumab (55 μg/mL) (Figure 7C) were added to HA patient plasma presented a 2- to 6-fold shortened TG lag time compared to baseline, with the largest effect observed for severe HA plasma (Figure 7B-C, Supplementary Table S4). Addition of either FVIII or emicizumab to FIX-FIAV-spiked plasma shortened the TG lag time by 2- to 4-fold relative to FIX-FIAV alone. The latter was primarily because of the effect of supplementation with either FVIII or emicizumab on the TG lag time. No substantial effects were seen on other TG parameters for the combination of current HA treatments and FIX-FIAV (Supplementary Table S4). Collectively, using FXIa-triggered TG assessment our findings indicate that no severe procoagulant effects were observed for the combination of FIX-FIAV with conventional HA therapeutics.

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Discussion

To our knowledge, this is the first preclinical *in vitro* study on the effect of FIX-FIAV in HA patient plasma. Previously, FIX-FIAV has been shown to improve hemostasis in *in vitro* and murine models of HA, with the specific substitutions introduced into FIX-FIAV giving rise to its FVIII-independent activity[11,12]. Here, we assessed the prohemostatic effects of FIX-FIAV alone and in combination with the currently available HA therapies employing FXIa-triggered TG assays. We demonstrated that FIX-FIAV improves hemostasis in HA regardless of residual FVIII, baseline FVIII, or FVIII inhibitor status. In addition, minimal enhanced effects were observed when FIX-FIAV and currently available HA therapies or FVIII-bypassing agents were combined.

To establish this, we made use of a modified FXIa-triggered calibrated automated thrombography assay to monitor thrombin formation in HA patient plasma. The ISTH SSC subcommittee has proposed to use low tissue factor for hemostatic monitoring in HA[20]. Using a low amount of tissue factor as trigger, not only the extrinsic FX-mediated pathway in coagulation is initiated but also the intrinsic pathway through FIX activation, known as the Josso loop[21,22]. However, low tissue factor-triggered TG has been reported to inadequately differentiate between moderate and severe HA[23,24]. To circumvent this, TG using a low FXIa trigger has previously been assessed, which allowed for enhanced sensitivity in quantifying FVIII/FIX levels in HA/B compared to the tissue factor-driven TG methods[23-26]. Here, we have built upon this earlier work and made use of a relatively high FXIa concentration to initiate thrombin formation. Consequently, as higher amounts of FIX are activated and considered to be in excess, the limiting factor in this assay set-up is (activated) FVIII. We have shown that this approach allows for assessment of FVIII(-equivalent) activity even at low FVIII plasma concentrations using the TG lag time as parameter. The TG lag time consistently displayed a dose-dependent correlation with FVIII levels, specifically in plasma from HA patients, while this was less apparent for the ETP and thrombin peak, corroborating previous observations[27]. Accordingly, the high FXIa trigger allowed us to quantify the FVIII-independent activity of FIX-FIAV in terms of FVIII-equivalence.

In light of its FVIII-independent activity, we have demonstrated that FIX-FIAV is capable of improving thrombin formation in severe, moderate, and mild HA patient plasma. Moreover, we have revealed that 100% FIX-FIAV resulted in a phenotype shift in FVIII-equivalent activity: addition of 100% FIX-FIAV to severe, moderate, or mild HA plasma increased the median FVIII-equivalent activity to 3%, 16%, or 48%, respectively, compared to baseline. The higher efficacy observed for FIX-FIAV in moderate and mild HA suggests that this is related to FVIII-dependent FIX activity, while the lack of (detectable) FVIII in

severe HA limits FIX-FIAV to its FVIII-independent function. Increasing the concentration of FIX-FIAV up to 600% in severe HA enhanced the FVIII-equivalent activity, although the maximum effect in non-severe HA plasma was reached at 200% to 250% FIX-FIAV. The latter may be due to limited availability of the substrate FX as a result of consumption by FVIIIa-FIXa-FIAV. Interestingly, whereas shortened TG lag times correlated with increased FIX-FIAV, a trend towards a reduction in thrombin peak and ETP was observed for high FIX-FIAV in both mild and moderate HA. We hypothesize that this may result from competition between the added FIX-FIAV and endogenous FIX for interaction with residual functional FVIII. This is confirmed by the addition of a neutralizing FVIII-inhibitory antibody to moderate/mild HA patient plasma, revealing results comparable to those observed for severe HA patient plasma.

The FVIII-independent effect of FIX-FIAV was repeatedly confirmed by adding a neutralizing FVIII-inhibitory antibody to all patient plasmas, thereby diminishing residual FVIII and improving the FVIII-equivalent activity of FIX-FIAV in all HA phenotypes in a similar manner. Interestingly, plasma of three severe HA patients (OSA/CSA <1% FVIII baseline) who had received prophylaxis 48 to 72 hours before inclusion displayed increased TG parameters compared with other severe HA patient plasmas. After adding the FVIII-inhibitory antibody the TG parameters resembled those of the other severe HA plasmas, suggesting the former resulted from residual traces of FVIII. Furthermore, the improved FVIII-equivalent activity observed with a FVIII-inhibitory antibody present suggests that FIX-FIAV could serve as a potential treatment in HA with inhibitors. This is further underlined by the FIX-FIAV-induced shortened lag times observed for inhibitor patient plasma (BU> 0.5).

For the combination of recombinant FVIII or the FVIII-bypassing agents emicizumab, aPCC or FVIIa with FIX-FIAV minimal enhanced effects were observed based on the TG parameters. When comparing aPCC or FVIIa combined with FIX-FIAV to the effect of FIX-FIAV alone, similar TG lag times were obtained for all patient samples. Furthermore, for concentrations of emicizumab as low as 7 µg/mL a minimal and non-significant enhanced effect was found when combined with FIX-FIAV. Concordantly, the combination of recombinant FVIII with FIX-FIAV resulted in a modest enhanced effect on lag time. More extensive analyses would be required to establish whether administration of FIX-FIAV affects current HA therapies. Our study was adequately powered to ascertain the differences before and after the addition of FIX-FIAV and the primary endpoint of our study, namely FIX-FIAV's effect on TG lag time. Furthermore, employing a FXIa-driven TG allowed us to solely assess the intrinsic route of coagulation without interference of the extrinsic route to evaluate the effect of FIX-FIAV, also with and without emicizumab, FVIIIa, FVIIa, or aPCC. However, one disadvantage of using the FXIa-driven TG is that it

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might underestimate the effect of aPCC and FVIIa. The small sample size of n = 3 per phenotype on the comparison and combination of FIX-FIAV with FVIII, emicizumab and bypassing agents was chosen as a proof of concept. Of note, this could be considered a limiting factor; the results should therefore be interpreted with caution. Future clinical studies would be necessary to evaluate FIX-FIAV as potential treatment for HA patients.

Conclusion

FIX-FIAV significantly improved the FVIII-equivalent activity in severe, moderate, and mild HA patient plasma. The combination of current HA treatments with FIX-FIAV showed minor or modest effects on the TG lag time. As such, FIX-FIAV could serve as a potential treatment for HA patients, regardless of current treatment, inhibitor status, or FVIII levels. Clinical studies are needed to assess the hemostatic effects of FIX-FIAV in HA patients with or without inhibitors to FVIII.

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Supplementary Figures and Tables
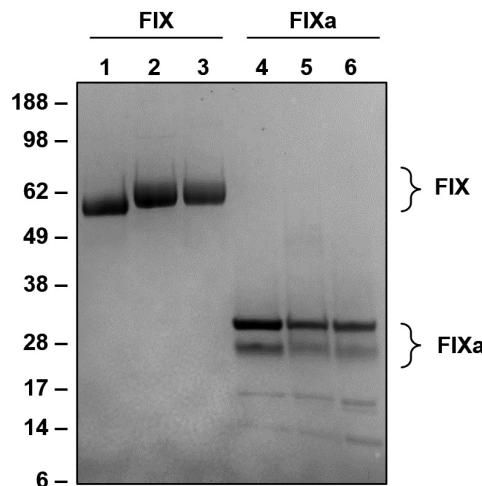
Supplementary Figure S1

Fully purified factor IX variants. SDS-PAGE of fully purified (activated) FIX-variants (3 µg/lane) under reducing conditions and visualized by staining with Coomassie Brilliant Blue R-250. Lane 1, pd-FIX; lane 2, FIX-WT; lane 3, FIX-FIAV; lane 4, pd-FIXa; lane 5, FIX-WT; lane 6, FIX-FIAV. Relevant FIX fragments (FIX, FIXa) of the standards are indicated. pd = plasma-derived.

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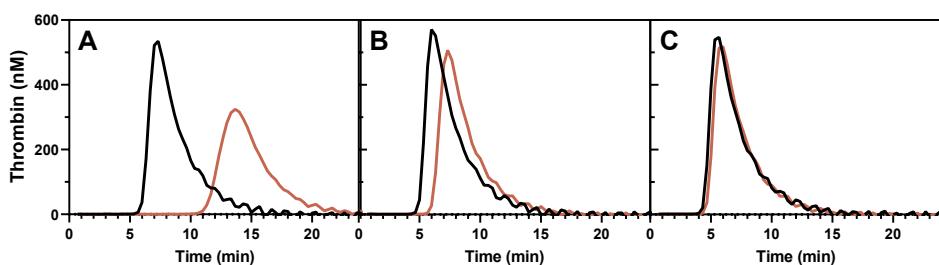
Supplementary Figure S2

Factor XIa titration in factor IX-deficient plasma supplemented with FIX-FIAV or FIX-WT. Thrombin generation was initiated with (A) 0.22 nM, (B) 2 nM or (C) 5 nM factor XIa in factor IX-deficient plasma supplemented with 100% (5 µg/mL) FIX WT (black lines) or FIX-FIAV (red lines). The highest concentration of FXIa revealed similar thrombin generation profiles for both FIX variants.

Supplementary Table S1. Coagulation and thrombin generation parameters of HA patient plasma in the presence of increasing concentrations of FIX-FIAV.

Severe HA (n = 7)			
FIX-FIAV (%)	**FVIII-equivalent activity** (%)	**Lag time** (min)	**Thrombin peak** (nM)
0	0.1 [0.1–0.3]	9. [6.9–9.6]	57 [34–355]
100	2.9 [2.3–3.9]	4.8 [4.5–5.3]	172 [156–349]
200	7.6 [5.7–10.1]	3.9 [3.6–4.1]	239 [231–346]
400	14.9 [10.8–20.0]	3.3 [2.8–3.4]	289 [260–336]
600	26.0 [19.3–31.1]	2.8 [2.1–2.9]	297 [269–324]
Moderate HA (n = 7)			
FIX-FIAV (%)	**FVIII-equivalent activity*** (%)	**Lag time** (min)	**Thrombin peak** (nM)
0	3.7 [3.2–4.4]	4.1 [3.6–4.3]	531 [493–542]
31	6.5 [5.2–7.6]	3.5 [3.3–3.9]	521 [490–541]
63	10.8 [8.0–14.2]	3.0 [2.9–3.4]	523 [492–539]
125	20.1 [17.3–21.8]	2.5 [2.4–2.9]	511 [479–523]
250	32.5 [27.0–36.7]	2.3 [2.2–2.4]	494 [472–511]
Mild HA (n = 7)			
FIX-FIAV (%)	**FVIII-equivalent activity*** (%)	**Lag time** (min)	**Thrombin peak** (nM)
0	21.6 [12.5–24.2]	3.6 [3.4–4.0]	559 [514–592]
25	24.0 [16.0–29.7]	3.4 [3.2–3.8]	537 [507–578]
50	33.9 [22.9–43.0]	3.0 [2.8–3.5]	539 [510–572]
100	48.0 [33.9–67.5]	2.6 [2.3–3.1]	527 [499–565]
200	74.4 [45.0–97.0]	2.3 [1.9–2.8]	522 [493–552]

The data are presented as median [IQR]. 100% equals 5 µg/mL FIX-FIAV.

ETP (nM*min)	Velocity index (nM/min)	Time to peak (min)	APTT (sec)
801 [490–1667]	8 [5–150]	16.3 [9.3–17.6]	168 [159–187]
1669 [1596–1714]	28 [25–145]	10.8 [7.1–11.4]	137 [130–141]
1787 [1711–1799]	49 [46–145]	8.3 [6.3–9.1]	120 [117–126]
1790 [1659–1820]	72 [63–142]	6.6 [5.7–7.3]	109 [106–114]
1731 [1620–1740]	82 [71–139]	6.0 [5.1–6.4]	104 [101–106]

ETP (nM*min)	Velocity index (nM/min)	Time to peak (min)	APTT (sec)
2060 [1969–2304]	354 [329–338]	5.6 [5.1–5.8]	122 [120–129]
2048 [1981–2285]	358 [338–367]	5.0 [4.8–5.3]	117 [112–122]
2006 [1956–2245]	349 [328–367]	4.5 [4.3–4.9]	112 [106–117]
1912 [1876–2138]	350 [331–367]	3.9 [3.8–4.4]	103 [99–107]
1935 [1851–2124]	338 [320–346]	3.6 [3.6–3.9]	95 [91–101]

ETP (nM*min)	Velocity index (nM/min)	Time to peak (min)	APTT (sec)
2282 [2096–2448]	440 [389–457]	4.9 [4.7–5.4]	116 [107–122]
2310 [2098–2493]	374 [353–412]	4.9 [4.6–5.1]	109 [103–117]
2293 [2024–2417]	379 [365–389]	4.5 [4.2–4.9]	107 [98.9–113.8]
2219 [1983–2416]	413 [376–418]	4.0 [3.6–4.5]	97 [93.9–107.9]
2015 [1888–2234]	360 [339–390]	3.8 [3.4–4.1]	94 [88.4–103.6]

*Analyses in plasma from one moderate and one mild HA patient were excluded as a FVIII inhibitor titer ≥2.8 BU did not allow for quantification due to interference with the individual FVIII reference curves.

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Supplementary Table S2. Coagulation and thrombin generation parameters of HA patient plasma in the presence of an anti-FVIII inhibitory antibody and increasing concentrations of FIX-FIAV.

Severe HA (n = 7)

FIX-FIAV (%)	FVIII-equivalent activity (%)	Lag time (min)	Thrombin peak (nM)
0	0.1 [0.0–0.2]	9.1 [8.5–92]	17 [15–21]
100	2.0 [1.3–2.7]	5.3 [5.0–5.8]	137 [122–148]
200	5.6 [3.5–6.8]	4.3 [4.0–4.8]	212 [201–229]
400	11.4 [7.2–12.9]	3.8 [3.5–3.9]	259 [245–280]
600	18.2 [10.9–20.9]	3.4 [2.8–3.6]	267 [261–297]

Moderate HA (n = 7)

FIX-FIAV (%)	FVIII-equivalent activity* (%)	Lag time (min)	Thrombin peak (nM)
0	<0.1	9.1 [8.6–9.6]	64 [58–78]
31	0.1 [0.1–0.1]	6.8 [6.4–7.4]	132 [94–152]
63	0.7 [0.4–0.8]	5.8 [4.9–6.1]	185 [142–224]
125	2.2 [1.8–3.1]	4.8 [4.2–5.4]	227 [202–267]
250	5.3 [4.4–6.8]	3.9 [3.5–4.0]	391 [266–296]

Mild HA (n = 7)

FIX-FIAV (%)	FVIII-equivalent activity* (%)	Lag time (min)	Thrombin peak (nM)
0	<0.1	10.0 [9.5–10.7]	104 [86–109]
25	0.2 [0.1–0.5]	8.0 7.– 8.3]	135 [131–188]
50	0.8 [0.5–2.1]	6.5 [6.3–7.0]	186 [174–258]
100	2.5 [2.3–4.9]	5.3 [5.0–5.9]	256 [219–316]
200	6.7 [4.5–10.2]	4.3 [4.0–4.9]	315 [263–340]

The data are presented as median [IQR]. 100% equals 5 μg/mL FIX-FIAV.

ETP (nM*min)	Velocity index (nM/min)	Time to peak (min)	APTT (sec)
285 [264–331]	1.8 [1.3–2.6]	18.6 [16.6–19.9]	186 [175–193]
1445 [1401–1657]	20.7 [18.7–23.9]	11.9 [11.1–12.4]	136 [129–144]
1757 [1637–1869]	42.8 [40.3–44.6]	9.4 [8.9–9.8]	123 [115–127]
1769 [1574–1850]	65.0 [55.3–70.1]	7.8 [7.4–7.9]	111 [104–114]
1722 [1596–1791]	71.1 [65.2–90.4]	6.6 [6.6–7.0]	105 [100–108

ETP (nM*min)	Velocity index (nM/min)	Time to peak (min)	APTT (sec)
769 [716–894]	11.7 [8.9–14.0]	15.0 [14.3–15.8]	175 [167–191]
1363 [1164–1519]	25.1 [14.8–28.4]	12.3 [11.6–13.8]	149 [144–157]
1658 [1522–1690]	35.3 [23.5–53.5]	11.0 [9.3–12.2]	135 [131–143]
1684 [1626–1822]	41.8 [39.1–66.7]	10.1 [8.2–10.5]	121 [117–127]
1711 [1623–1846]	78.9 [61.6–91.4]	7.6 [6.8–8.4]	114 [109–116]

ETP (nM*min)	Velocity index (nM/min)	Time to peak (min)	APTT (sec)
1119 [915–1158]	18.5 [16.4–21.7]	15.3 [14.6–15.9]	168 [165–173]
1416 [1304–1674]	27.0 [25.7–41.0]	13.0 [12.1–13.5]	158 [147–163]
1673 [1561–1862]	40.2 [35.9–63.5]	11.1 [10.4–11.9]	152 [140–157]
1766 [1691–1952]	68.1 49.9–90.7]	9.4 [8.4–10.1]	139 [129–142]
1785 [1717–2000]	94.1 [69.9–114.4]	7.9 [6.9–8.7]	127 [113–134]

*Analyses in plasma from one moderate and one mild HA patient were excluded as a FVIII inhibitor titer ≥2.8 BU did not allow for quantification due to interference with the individual FVIII reference curves.

8

Supplementary Table S3. Coagulation and thrombin generation parameters of HA patient plasma in the presence of FIX-FIAV and bypassing agents aPCC or FVIIa.

Severe HA (*n* = 3)

	Lag time (min)	Thrombin peak (nM)
Plasma only	9.6 [9.6–11.5]	59 [17–143]
FIX-FIAV (100%)	6.4 [5.8–8.9]	135 [128–145]
aPCC (1 U/mL)	7.8 [7.4–8.8]	187 [82–214]
FIX-FIAV (100%) **+ aPCC** (1 U/mL)	6.5 [6.3–8.1]	210 [203–273]
FVIIa (1.5 µg/mL)	8.9 [8.8–10.0]	69 [23–143]
FIX-FIAV (100%) **+ FVIIa** (1.5 µg/mL)	6.3 [5.9–8.3]	149 [122–149]

Moderate HA (*n* = 3)

	Lag time (min)	Thrombin peak (nM)
Plasma only	4.8 [3.9–6.0]	388 [225–483]
FIX-FIAV (100%)	3.4 [2.3–5.0]	368 [214–470]
aPCC (1 U/mL)	4.3 [3.4–5.5]	671 [444–748]
FIX-FIAV (100%) **+ aPCC** (1 U/mL)	3.8 [2.8–5.1]	680 [422–733]
FVIIa (1.5 µg/mL)	4.1 [3.4–5.5]	371 [215–462]
FIX-FIAV (100%) **+ FVIIa** (1.5 µg/mL)	3.0 [1.9–4.9]	363 [203–462]

Mild HA (*n* = 3)

	Lag time (min)	Thrombin peak (nM)
Plasma only	3.6 [2.6–5.8]	476 [312–484]
FIX-FIAV (100%)	2.5 [1.8–5.0]	465 [284–468]
aPCC (1 U/mL)	4.0* [2.5–5.5]	647.0* [584–711]
FIX-FIAV (100%) **+ aPCC** (1 U/mL)	2.9 [2.0–5.3]	695 [527–703]
FVIIa (1.5 µg/mL)	3.1 [2.3–5.3]	471 [326–488]
FIX-FIAV (100%) **+ FVIIa** (1.5 µg/mL)	2.5 [1.8–4.9]	463 [265–475]

*One patient had missing data.

ETP (nM*min)	Velocity index (nM/min)	Time to peak (min)	APTT (sec)
750 [266–1573]	7.9 [1.9–30.1]	17.1 [16.3–18.5]	188 [160–196]
1501 [1472–1553]	21.8 [20.4–26.3]	13 [12.4–13.8]	145 [128–151]
1966 [850–2749]	29.3 [12.9–41.9]	13.9 [13.8–14.1]	62 [55–64]
2668 [2257–2745]	38.6 [33.0–43.7]	12.8 [12.7–13.4]	62 [50–63]
869 [367–1520]	9.0 [2.7–30.9]	16.4 [14.6–17.5]	71 [58–73]
1570 [1419–1617]	22.5 [22.5–23.9]	12.9 [12.5–13.4]	73 [53–74]

ETP (nM*min)	Velocity index (nM/min)	Time to peak (min)	APTT (sec)
1675 [1560–1934]	284.6 [72.2–353.2]	6.2 [5.3–9.1]	116 [109–118]
1529 [1501–1850]	245.3 [74.7–343.5]	4.8 [3.8–7.9]	96 [95–105]
3500 [2431–3583]	447.0 [169.7–496.9]	5.8 [4.8–8.1]	52 [48–105]
3309 [3020–3600]	452.1 [153.5–453.0]	5.4 [4.3–7.9]	52 [51–56]
1621 [1513–1805]	247.0 [72.3–312.7]	5.6 [4.9–8.5]	57 [54–57]
1521 [1467–1781]	242.3 [67.7–306.8]	4.5 [3.4–7.9]	57 [55–58]

ETP (nM*min)	Velocity index (nM/min)	Time to peak (min)	APTT (sec)
2171 [2163–2205]	322.3 [132.0–347.9]	5.0 [4.1–8.1]	101 [95–109]
2036 [2015–2045]	310.0 [108.2–311.1]	4.0 [3.3–7.6]	88 [86–98]
2219* [1798–2640]	450.9 [333.5–568.4]*	5.5* [3.8–7.3]	51 [49–51]
2401 [1849–2927]	468.5 [249.8–553.7]	4.1 [3.5–7.4]	49 [48–51]
2178 [2139–2293]	312.6 [144.9–325.4]	4.6 [3.8–7.5]	59 [56–59]
2120 [1978–2179]	316.7 [96.5–338.1]	3.9 [3.3–7.6]	59 [54–59]

The data are presented as median [minimum–maximum values]. 100% equals 5 μg/mL FIX-FIAV.

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Supplementary Table S4: Coagulation and thrombin generation parameters of HA patient plasma in the presence of FIX-FIAV and therapeutic agents FVIII or emicizumab.

Severe HA (*n* = 3)

	Lag time (min)	Thrombin peak (nM)
Plasma only	8.9 [7.8–10.5]	62 [25–279]
FIX-FIAV (200%)	4.8 [4.3–3.5]	188 [127–262]
FVIII (100%)	1.8 [1.6–2.0]	469 [418–535]
FIX-FIAV (100%) **+ FVIII** (100%)	1.6 [1.5–1.6]	459 [409–516]
Emicizumab (7 µg/mL)	2.5 [2.4–3.1]	378 [356–450]
FIX-FIAV (100%) **+ emicizumab** (7 µg/mL)	2.3 [2.0–2.5]	379 [370–456]
Emicizumab (26 µg/mL)	1.8 [1.6–2.0]	411 [391–485]
FIX-FIAV (100%) **+ emicizumab** (26µg/mL)	1.6 [1.5–1.8]	420 [386–471]
Emicizumab (55 µg/mL)	1.4 [1.3–1.8]	416 [402–502]
FIX-FIAV (100%) **+ emicizumab** (55 µg/mL)	1.3 [1.3–1.5]	416 [390–503]

Moderate HA (*n* = 3)

	Lag time (min)	Thrombin peak (nM)
Plasma only	4.8 [4.6–5.1]	476.8 [390–506]
FIX-FIAV (200%)	2.8 [2.8–3.4]	444.6 [379–469]
FVIII (100%)	2.8 [2.8–3.4]	481 [427–523]
FIX-FIAV (100%) **+ FVIII** (100%)	1.5 [1.5–1.5]	464 [437–482]
Emicizumab (7 µg/mL)	2.3 [2.1–2.4]	478 [410–493]
FIX-FIAV (100%) **+ emicizumab** (7 µg/mL)	1.8 [1.8–2.0]	451 [399–472]
Emicizumab (26 µg/mL)	1.6 [1.5–1.8]	464.8 [407–482]
FIX-FIAV (100%) **+ emicizumab** (26µg/mL)	1.4 [1.3–1.5]	450.0 [403–477]
Emicizumab (55 µg/mL)	1.4 [1.3–1.5]	470 [400–487]
FIX-FIAV (100%) **+ emicizumab** (55 µg/mL)	1.1 [1.0–1.3]	455 [392–477]

ETP (nM*min)	Velocity index (nM/min)	Time to Peak (min)
872 [370–1474]	8.0 [3.3–93.0]	16.6 [10.8–18.0]
1348 [1321–1506]	38.6 [22.1–91.1]	9.3 [47.8–10.5]
1805 [1769–2058]	344.3 [307.6–428]	9.3 [7.8–10.5]
1715 [1612–1863]	367.0 [300.3–412.9]	2.9 [2.8–3.0]
1637 [1622–1879]	234.3 [142.6–278.9]	4.1 [4.0–5.6]
1567 [1493–1777]	246.8 [189.3-260.9]	3.8 [3.8–4.5]
1723 [1629–1951]	260.7 [34.9–356.1]	3.3 [3.0–3.8]
1613 [1536–1822]	282.6 [279.7–346.0]	3.0 [2.9–3.3]
1785 [1637–1984]	304.0 [267.6–334.9]	2.9 [2.8–3.1]
1660 [1519–1839]	277.4 [259.7–402.0]	2.8 [2.5–3.0]

ETP (nM*min)	Velocity index (nM/min)	Time to Peak (min)
1675 [1560–1936]	317.9 [259.9–371.8]	6.1 [6.1–6.6]
1745.9 [1564–1815]	296.4 [252.9–343.8]	4.3 [4.3–4.9]
2116 [1919–2205]	352.6 [313.7–418.5]	3.4 [3.0–3.4]
1945 [1788–1965]	309.6 [272.6–399.1]	3.0 [2.8–3.0]
2116 [1884–2143]	350.8 [300.6–361.8]	3.6 [3.5–3.8]
1835 [1713–1911]	314.8 [301.0–319.0]	3.3 [3.0–3.5]
2102.3 [1917–2121]	321.2 [271.1–340.7]	3.1 [3.0–3.1]
1916.7 [1765–1940]	322.0 [300.0–350.2]	2.8 [2.5–3.0]
2071 [1824–2071]	344.5 [320.3–357.3]	2.8 [2.6–2.9]
1857 [1660–1869]	313.8 [303.2–350.2]	2.5 [2.3–2.8]

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Supplementary Table S4: Continued

Mild HA (*n* = 3)

	Lag time (min)	Thrombin peak (nM)
Plasma only	3.5 [2.5–4.5]	496 [494–554]
FIX-FIAV (200%)	2.5 [1.9–3]	470.3.0 [465.8–518]
FVIII (100%)	1.9 [1.6–2.0]	496 [487.5–554.7]
FIX-FIAV (100%) **+ FVIII** (100%)	1.6 [1.4–1.8]	473 [469.5–537.8]
Emicizumab (7 µg/mL)	2.0 [1.5–2.3]	479 [475–522]
FIX-FIAV (100%) **+ emicizumab** (7 µg/mL)	1.8 [1.3–2.0]	452 [447–518]
Emicizumab (26 µg/mL)	1.3 [1 - 1.5]	479.0 [459.7–516.1]
FIX-FIAV (100%) **+ emicizumab** (26 µg/mL)	1.5 [1.3–1.75]	447.4 [443.2–507.8]
Emicizumab (55 µg/mL)	1.3 [0.9–1.5]	488 [478–523]
FIX-FIAV (100%) **+ emicizumab** (55 µg/mL)	1.0 [0.9–1.3]	457 [450–506]

The data are presented as median [minimum–maximum values].

ETP (nM*min)	Velocity index (nM/min)	Time to Peak (min)
2089 [2076–2453]	396.9 [362.8–406.7]	4.9 [3.8–5.9]
1936 [1896–2258]	345.2 [341.6–345.3]	3.9 [3.3–4.5]
2019 [1956–2426]	364.0 [358.1–369.8]	3.4 [3.0–3.4]
2019 [1956–2426]	378.6 [344.6–394.7]	3.0 [2.8–3.0]
2159 [2127–2573]	351.7 [316.9–383.1]	3.4 [3.0–3.6]
1979 [1850–2303]	301.0 [297.9–345.2]	3.3 [2.8-3.5]
2172 [2111–2546]	352.0 [307.0–379.0]	3 [2.6–3.1]
2019 [1956–2426]	298.0 [295.0–339.0]	2.8 [2.5–3]
2196 [2128–2567]	359.0 [319.0–383.0]	2.6 [2.5–2.8]
1938 [1920–2225]	337.0 [300.0–365.0]	2.5 [2.3–2.6]

100% equals 5 µg/mL FIX-FIAV.

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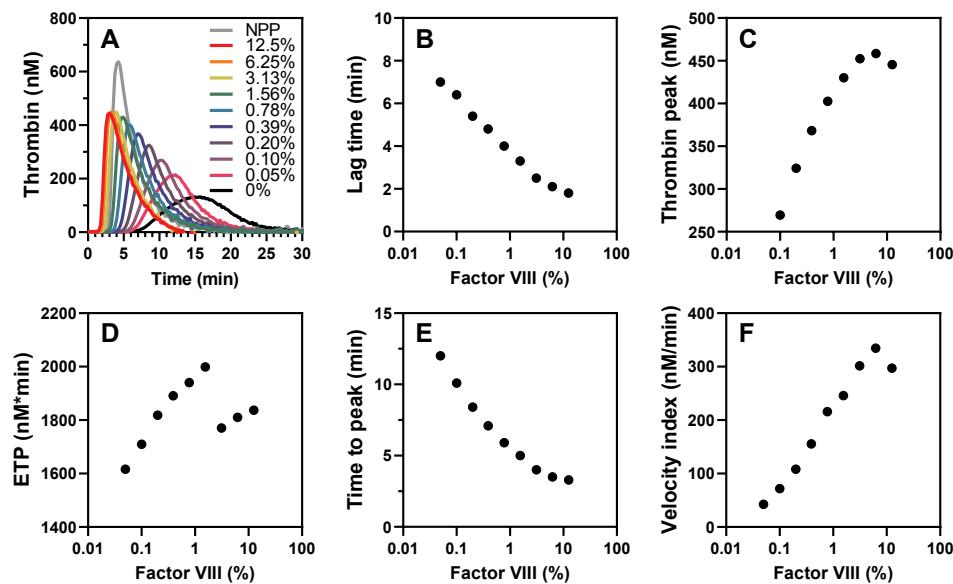
Supplementary Figure S3

FXIa-triggered thrombin generation in FVIII-deficient plasma supplemented with FVIII. Thrombin generation was initiated with 5 nM FXIa in FVIII-deficient plasma supplemented with 0% to 12.5% recombinant FVIII (NovoEight). A: Representative curves are shown. B-F: The FVIII concentration was plotted versus the thrombin generation lag time (B), thrombin peak (C), ETP (D), time to peak (E), or velocity index (F). The data are representative of at least two independent experiments. The lag time demonstrated a correlation with FVIII concentration, while this was less apparent for the ETP and thrombin peak, corroborating previous observations[1]. The data represent two independent experiments.

Supplementary Figure S4

Thrombin generation in FVIII-deficient plasma supplemented with FIX-FIAV or FIX-WT. Thrombin generation was initiated with 5 nM FXIa in FVIII-deficient plasma in the absence ('control'; dashed line, black columns) or presence of 100% (5 µg/mL) FIX-FIAV (red line/columns) or FIX-WT (blue line/columns). A: Representative curves are shown. B-F: The thrombin generation lag time (B), thrombin peak (C), ETP (D), time to peak (E), or velocity index (F) are shown. Values represent averages of two independent experiments ± 1 S.D.

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Supplementary Figure S5

Augmenting role of residual FVIII levels on the procoagulant effect of FIX-FIAV. The plasma FVIII:C levels at time of inclusion (one-stage assay (U/mL)) of each individual patient were plotted against their individual increase in FVIII-equivalent activity by FIX-FIAV, as exemplified in Figure 2D-F. The data were fitted using linear regression (r^2 = 0.8798, p < 0.0001) and are displayed as mean ± 95% confidence interval. Analyses in plasma from one moderate and one mild HA patient were excluded as a FVIII inhibitor titer ≥2.8 BU did not allow for quantification due to interference with the individual FVIII reference curves.

References

1.   Desborough MJR, Naeem H, Baker P, Curry N. Factor XIa-triggered thrombin generation in severe haemophilia A. *British Journal of Haematology*. Feb 2019;184(4):637-640.

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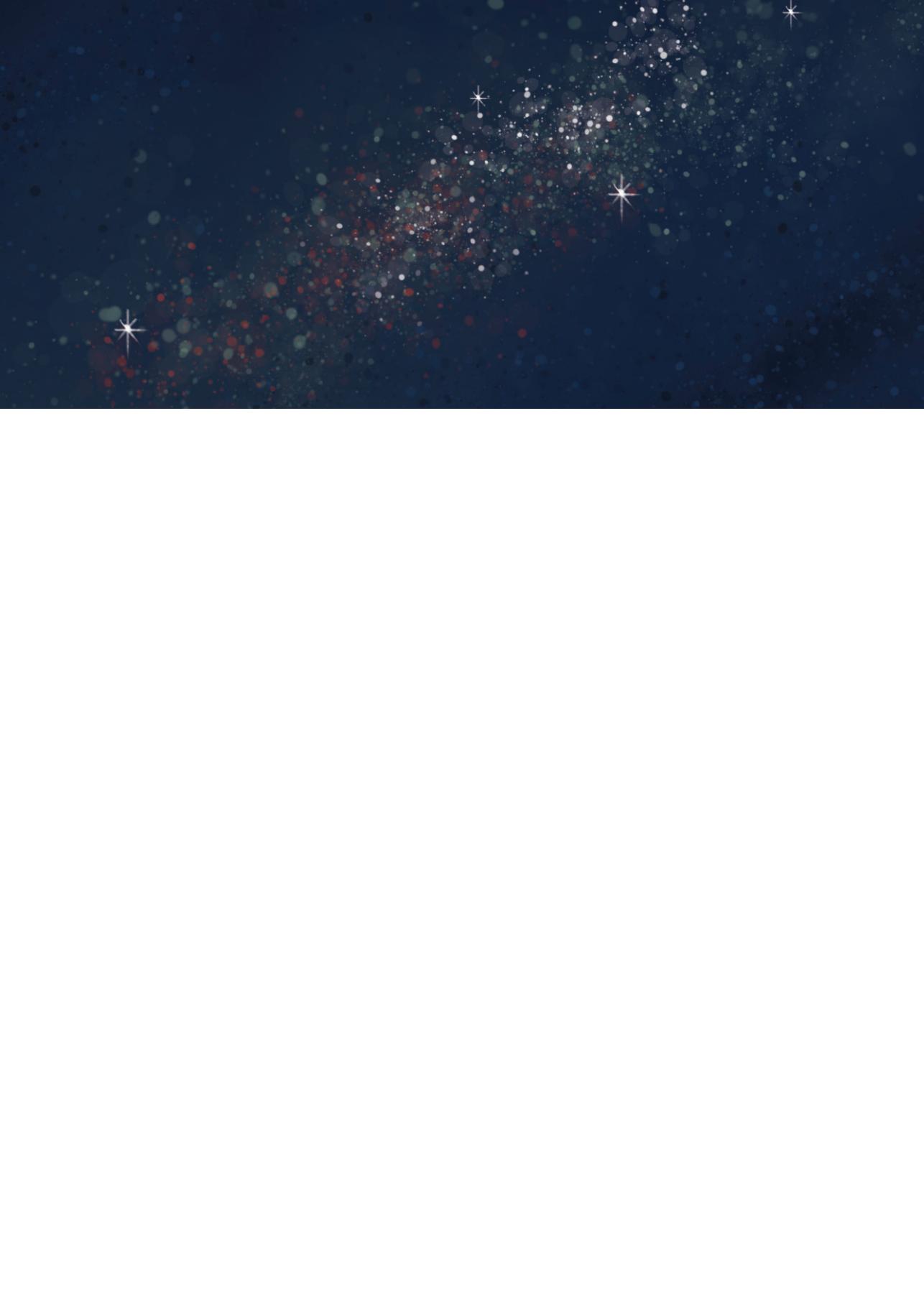
CHAPTER 9

GENERAL DISCUSSION

General discussion

The objective of the studies described in this thesis was to personalize the management of hemophilia A.   The main aim was to study the use of PK-guided dosing using Bayesian forecasting of factor VIII concentrate in combination with desmopressin in patients with non-severe hemophilia A. Desmopressin is a treatment option for many patients with mild and moderate hemophilia, but its use is still limited[1]. Therefore, we performed additional studies on the responsiveness of patients to desmopressin in the peri-operative setting. Patient perspectives on the use of desmopressin were surveyed in order to identify possible barriers and facilitators to the use of desmopressin in non-severe hemophilia A[2].

Management of hemophilia A

In hemophilia A patients, novel treatment modalities are being developed to overcome existing drawbacks of current treatment, such as they exist for the use of FVIII concentrate. In 7%–15% of patients with mild or moderate hemophilia A and up to 30% of the severe hemophilia A patients factor VIII concentrate treatment results in the formation of inhibiting FVIII antibodies, which is associated with a higher mortality and morbidity[3-5]. The risk of the development of inhibitors to factor VIII is dependent upon several factors, including peak treatment moments such as surgery. Our hypothesis was that by combining treatment of desmopressin and FVIII concentrate, the FVIII concentrate dose can be reduced, which may lower the risk of FVIII inhibitor formation[6]. In addition, this might reduce the costs of treatment, without increasing the risk of bleeding during a surgical procedure. In this thesis we reported the DAVID studies, which investigated the feasibility, safety and efficacy of PK-guided combination treatment. The patient reported side effects, mostly flushing, were mild and transient. Peri-operative bleeds occurred in two patients, but this was not related to inadequate FVIII levels. These levels were >1.00 IU/mL in both patients at the time of bleeding. Importantly, patients experienced the quality of care of combination treatment as high, with a median score of more than 9 out of 10. These results confirm the statement in the most recent WFH guidelines, that combination treatment can overcome the drawbacks of desmopressin or FVIII concentrate monotherapy[7].

In our study we used PK guidance of treatment with FVIII concentrate and desmopressin. This requires additional blood sampling and dose calculations that had to be performed by a pharmacologist based on achieved FVIII levels. These additional requirements in the management of these patients may hamper the implementation of this combination treatment approach. In the future, facilitating the application of PK-guided combination treatment by using a web portal may overcome this issue. A web portal has recently been developed by the SYMPHONY Consortium and currently available (URL: http://opticlot.nl)[8].

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Although promising, the results of our studies have yet to be validated in larger cohorts of patients. It may also be used in females with hemophilia A (carriers with FVIII levels <40%) and symptomatic carriers of hemophilia A. For hemophilia centers that do not have the ability to use PK guidance, combination treatment could also be applied in the peri-operative setting, but in that case FVIII:C should be monitored frequently in order to adjust the subsequent doses of FVIII concentrate. Our studies have shown that achieved FVIII:C levels after desmopressin treatment may be different from earlier performed DDAVP test dose results and tachyphylaxis may occur to a higher extent than previously reported. The latter was observed after inclusion of the first patients in the DAVID study. The second and third FVIII:C rise after consecutive daily desmopressin administrations were only 36%–43% of the initial FVIII:C response. Previous studies in a desmopressin test setting showed a remaining FVIII:C response of about 50%–70% in healthy individuals or hemophilia A patients receiving desmopressin dosed every 12 hours[9,10]. Both the different timing of dosing in our study, namely every 24 hours, and the assessment of tachyphylaxis in peri-operative hemophilia A patients instead of in a test setting, could have contributed to the difference in the extent of tachyphylaxis. However, we were not able to assess the determinants of tachyphylaxis with the limited number of included patients in the DAVID studies. Recently, Kumar at al. found that moderate-intense exercise increases FVIII:C in non-severe hemophilia A patients to the same magnitude as desmopressin, but with a shorter duration of FVIII:C[11]. Moderate-intense exercise may therefore be an alternative for desmopressin to temporarily increase FVIII:C for interventions and serves as an example of personalized medicine. This might especially be used in low income countries or in case desmopressin is not available.

As FVIII concentrate availability worldwide is limited and its use is costly, with yearly patient costs up to €224,712 for patients on prophylaxis[12], cost-efficient use of FVIII concentrate in general is warranted. In the Little DAVID study, a significant pre-operative FVIII concentrate dose reduction of 47% for the desmopressin-PK-guided FVIII combination treatment compared to PK-guided FVIII concentrate monotherapy was achieved[13]. In the DAVID study, 39% less pre-operative FVIII concentrate was used compared to hypothetical body weight based dosing[13]. This was further evaluated in a cost-minimization study to assess the total costs of combination treatment compared to body weight based dosing in the DAVID study, leading to a reduction of approximately €1000 (10% of the total costs) per procedure. The time spent by nursing staff for administration of desmopressin can also be reduced by 107 to 183 minutes (50% of the total time) by subcutaneous desmopressin administration and by 12 minutes (about 4% of the total time) by limiting the number of FVIII:C measurements to only a peak FVIII:C level. The time saved for health care personnel for these administrations can then be applied more efficiently for other (hemophilia) care.

The merits of desmopressin have been reported in many studies, such as its use in moderate hemophilia A patients[14], no risk of FVIII inhibitor formation, and only limited side effects[15]. Despite these merits, the use of desmopressin in non-severe hemophilia A patients has been reported to be suboptimal: a national cohort study assessing desmopressin use showed that in 54% of patients who had received a single dose of FVIII concentrate, the desmopressin response would have exceeded the level targeted with that dose of FVIII concentrate[1]. Additionally, 60% of these patients and 81% of patients with a high inhibitor risk *F8* mutation, achieved a FVIII:C peak level ≥0.3 IU/mL after desmopressin. Patients' perspectives about desmopressin use were not yet been reported in literature and could provide useful information in order to increase desmopressin use for these patients. We assessed patient perspectives on desmopressin use in non-severe hemophilia A patients in The Netherlands[2].

Only about 50% of the included non-severe hemophilia A adults and 28% of children had been treated with desmopressin, most often used intranasally. Ninety percent of adults and children reported at least moderate effectiveness. Unfortunately intranasal desmopressin is currently not widely available. Therefore desmopressin is now mainly administered intravenously or subcutaneously. The possibility of home treatment with desmopressin is therefore limited: only subcutaneous administration can be self-administered and requires patient training. Despite side effects being reported as one of the most frequent disadvantages, 42% of the adult and 32% of the pediatric patients reported no side effects of desmopressin, in contrast to other studies reporting a higher incidence of side effects. These studies revealed that up to 97% experienced side effects one hour after infusion[15,16]. Therefore, it seems that even though many patients report side effects when asked immediately after administration, these side effects seem to be minor and do not have a memorable impact as they are not reported in the retrospective HiN6 questionnaire study[2]. For patients reporting side effects after intravenous administration, subcutaneous or intranasal administration could be possible alternatives. The vasoactive effects, including flushing and headache, the most reported side effects of desmopressin occur less frequently after subcutaneous or intranasal administration[16]. A quarter of the patients reported not to be adequately informed about the efficacy and side effects of desmopressin. Several studies have shown that health care education of hemophilia A patients, severe and non-severe, increases quality of life for these patients[17-19]. Patient education has shown to improve the quality of life in other chronic diseases as well, such as diabetes mellitus[20]. Therefore it is important to (repeatedly) inform patients about the potential use and (dis)advantages of desmopressin, such as its convenient use in home setting and worldwide availability.

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In pregnancy, desmopressin use is debated due to safety concerns, related to desmopressin's vasomotor and antidiuretic effects, leading to a hypothetical intra-uterine growth restriction or hyponatremia. We performed a systematic review reported on the safety and efficacy of desmopressin for bleeding disorders in pregnancy an during delivery[21]. Safety outcome was reported in 245 pregnancies with two adverse events in pregnancy, namely hyponatremia with neurologic symptoms. In sixty pregnancies reporting on safety in neonates, one preterm delivery (n = 1) and one neonate with a fetal growth restriction were reported. In two of 114 neonates (2%), a moderate adverse event was reported, namely a low Apgar score (n = 1) and a transient hyperbilirubinemia, which was not attributed to desmopressin use (n = 1). We conclude that desmopressin use during pregnancy and delivery seems safe, also taking into consideration the very low occurrence of hyponatremia. However, included studies had a poor design and a relatively small number of outcomes were documented. More international, high quality registries are needed to validate these results for desmopressin use in pregnancy, but its use seems plausible peri-partum after clamping of the umbilical cord, especially considering the high risk risk of post-partum hemorrhage[22].

Bleeding in hemophilia patients
Joint bleeding and muscle bleeds are the most common bleeds in severe and moderate hemophilia patients, however other bleedings may also occur. Hematuria is a less frequently experienced bleeding episode but may lead to painful and severe complications, caused by obstructive blood clots. In many hemophilia patients with hematuria, no cause is found and it remains unknown whether it is necessary to perform additional diagnostic tests for the cause of bleeding: on the one hand it is considered as a benign affliction, but may in hemophilia patients, by ignoring the potential of an underlying treatable etiology, lead to recurrent bleeding and additional use of factor concentrate. Therefore, we investigated the occurrence, diagnostic management and treatment of macroscopic hematuria in 35 patients, in whom in a quarter of patients a cause was found after diagnostic evaluation[23]. We therefore suggest to perform diagnostic evaluation in case of hematuria as is also recommended in the Dutch guidelines for the general population[23]. This is especially important for those individuals with a high risk for a malignancy, such as smokers. This recommendation is in accordance with the current WFH guidelines for patients with hemophilia[7]. Based on our findings we suggest to use ultrasound and urinalysis as first line diagnostic tools to improve the diagnostic strategy, with the consideration to refer to a urologist in case of a high risk for malignancy. Additionally, by identifying a cause of hematuria, this may prevent rebleeding and thereby prevent unnecessary prolonged and recurrent use of hemostatic treatment. However, cost-effectiveness studies of this strategy for hemophilia patients are lacking, but for the general population, the Dutch strategy of evaluating hematuria has been shown to be one of the most cost-effective out of all available international guidelines[24]. Additionally, the

costs of urinalysis and ultrasonography in general are lower than the costs for multiple doses of FVIII concentrate. Therefore, the Dutch guideline can also be considered an effective guideline for hemophilia patients with macroscopic hematuria, but further studies are needed to assess the economic outcomes.

Novel hemophilia treatment modalities

Despite the fact that recombinant FVIII concentrate is available for many hemophilia A patients, the regular intravenous injections with FVIII remain a large burden. Therefore, novel hemophilia A treatments are currently being investigated or have recently been introduced. Emicizumab, a bispecific monoclonal antibody that mimics FVIII activity by bridging FIXa and FX can be administered subcutaneously once every two to four weeks and is approved for prophylaxis in severe hemophilia A patients and is at least as effective as prophylaxis with FVIII concentrate[25]. Emicizumab is also approved for prophylaxis in hemophilia A patients with inhibitors and is effective in preventing bleeds. In combination with aPCC, which was administered in case of breakthrough bleed, emicizumab was associated with thrombotic microangiopathy[26]. Despite the introduction of emicizumab, the need for additional hemophilia treatments remains. One of these potential new treatment modalities is FIX-FIAV, a variant of factor IX that can activate FX independently of FVIII. It can be used both in patients with and without inhibitors to FVIII. In an vitro study using plasma of hemophilia patients of different severities, we showed that thrombin generation was increased after addition of FIX-FIAV[27]. The combination of FIX-FIAV with other bypassing agents such as emicizumab, aPCC or recombinant factor VIIa (rFVIIa), did not show any significant procoagulant changes in the studied patient samples using thrombin generation assays. The fact that FIX-FIAV can act independently of FVIII, confirmed in the study with inhibitor patient plasma, makes it a potential treatment modality for patients with FVIII inhibiting antibodies[28].

Recently, AAV-based gene therapy has been approved for the treatment of haemophilia A. One single injection of valoctocogene roxaparvovec, the first AAV-based FVIII gene therapy, increased factor VIII activity to levels within the normal range, enabling patients to stop FVIII prophylaxis, without any bleeding episode[29]. Unfortunately the FVIII:C activity declines after a peak at six months and around ten percent of patients have to resume FVIII prophylaxis after three years following gene therapy[30,31]. In addition many patients developed long-term liver function abnormalities, which required treatment with corticosteroids. These long-term liver function abnormalities, namely increased hepatic transaminase (ALT) levels, may be caused by the different folding of FVIII protein in hepatocytes leading to an increase in oxidative stress, as well as a different site of production than physiological FVIII - namely the endothelial cell - also leading to oxidative stress, eventually leading to cell apoptosis and loss of the gene therapy[32-34].

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This was not observed after gene therapy for hemophilia B, which has shown long-term lasting FIX expression up to 8 years[35,36]. This may be due to the fact that FIX is normally also produced in hepatocytes, the main target of current AAV gene therapy. Therefore, gene therapy targeting hepatocytes with FIX-FIAV, as a variant of FIX that can activate FX independently of FVIII, could potentially be used as gene therapy for patients with hemophilia A. However, further in vivo studies of FIX-FIAV in non-human primates still have to be performed. Another potential treatment for patients with hemophilia A is rondoraptivon pegol. This novel RNA aptamer decreases the clearance of VWF and was studied in 19 severe and non-severe hemophilia A patients[37]. It was used as a monotherapy or add-on in combination with short- or long-acting FVIII concentrate. In this small preliminary study, it extended the half-life of FVIII concentrate approximately threefold, and increased endogenous FVIII:C approximately twofold. Future studies are necessary to further assess its properties and clinical efficacy.

All these novel therapeutics, despite being promising, still remain costly and their worldwide availability is limited, warranting another approach for current, affordable treatment options such as desmopressin in the form of combination treatment with pharmacokinetic guided dosing of desmopressin and FVIII concentrate.

Population pharmacokinetics in coagulation disorders

Pharmacokinetic guided dosing of drugs by Bayesian forecasting can be applied to individualize patient care as individualized pharmacokinetic parameters of patients can be estimated on basis of a limited number of blood samples. In the aforementioned paragraph the decrease in FVIII concentrate use with combination treatment in the DAVID studies was discussed.

Population PK modeling can also be applied to use novel medicines in hemophilia patients more efficiently, for instance in treatment with emicizumab. The current drug labels advise a body weight based dosing approach leading to a mean trough level of emicizumab of 55 μg/mL. Studies using Monte Carlo simulations with a PK-guided dosing of emicizumab suggests that an alternative dosing scheme aiming at trough levels between 40 and 60 μg/mL could already lead to a theoretical yearly cost reduction of approximately €29,529 for patients weighing between 76 and 85 kg. Several studies suggest that the number of bleeds do not increase if trough levels are ≥30 μg/mL[38]. The ongoing DoSeMi trial will assess whether this trough is adequate enough to prevent bleeding, by assessing the annual bleeding rate and other important clinical outcomes such as major bleeds, in patients receiving PK-guided emicizumab versus body weight dosing[39].

Future research

As the landscape of treatment for male hemophilia A patients seems to expand with novel therapeutics such as emicizumab and gene therapy, it is important to realize that the outcomes of new treatments have not yet been assessed in females with hemophilia A and symptomatic carriers. Despite the X-linked inheritance pattern of hemophilia A, females who carry one afflicted X chromosome can still be symptomatic and/or have low FVIII:C. A Dutch study in females (persons with genotype XX) and a known *F8* gene mutation reports that 28% of these females classify as females with hemophilia A (62/225), with a FVIII:C <0.40 IU/ml[40]. Despite achieving FVIII:C levels of >0.40 IU/ml after desmopressin administration, the relative rises of FVIII in females with hemophilia and carriers are lower than in healthy controls. In addition, carriers of hemophilia A with a higher ISTH-BAT bleeding score tend to have lower desmopressin FVIII responses, possibly signifying a diminished response of these carriers to stressors, leading to a higher bleeding risk[41-43]. This indicates that more studies have to be performed in females with hemophilia, including during pregnancy and delivery, in order to improve their management.

Besides current formulations of desmopressin, such as subcutaneous or intravenous administration, other forms with a high and stable bioavailability such as transdermal[44] or sublingual[45] dosages have been developed and their application should be studied further. Additional studies should be performed to assess and compare the pharmacokinetics, pharmacodynamics and experienced side effects of these desmopressin formulations in all hemophilia A patients, including females with hemophilia A. Practical application of combination treatment for all hemophilia A patients and carriers using the aforementioned and easily available web portal utilizing PK profiles of these hemophilia A patients, will provide individualized treatment for hemophilia A patients worldwide. Modeling of novel treatment modalities in patients with hemophilia A, will individualize and improve treatment with better outcomes and lower costs.

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CHAPTER 10

ENGLISH SUMMARY &
NEDERLANDSE SAMENVATTING

English summary

In this thesis studies on personalized management of hemophilia A are presented, in order to improve current clinical care. **Chapter 1** provides an introduction of the thesis including the aims of the studies reported. A disturbance of hemostasis can cause bleeding or thrombosis. In hemophilia A, desmopressin or FVIII concentrate can be used to treat spontaneous or traumatic bleeding or to prevent bleeding during and after a medical procedure. Both treatment modalities have their limitations. Despite its widespread availability and low costs, desmopressin is currently used suboptimally in eligible hemophilia A patients, because these patients are more often treated with FVIII concentrate than desmopressin. Desmopressin results in a high inter-individual variability of FVIII:C response. On the other hand the limitations of FVIII concentrate use are the potential formation of FVIII inhibitors, high costs and limited availability worldwide. Novel treatment options, such as emicizumab and gene therapy, are promising, but more studies are needed to optimize the use of these costly treatment modalities. Also the use of desmopressin and factor VIII concentrate can be improved by more personalized treatment. Pharmacokinetic (PK)-guided dosing by using Bayesian forecasting can be applied to personalize these treatments for patients with hemophilia A, to increase the attainment of predefined FVIII:C targets and optimize treatment. The use of combination treatment, desmopressin directly followed by FVIII concentrate, is reported by the international World Federation of Hemophilia (WFH) guideline as a possible solution to overcome drawbacks related to treatment with only desmopressin or FVIII concentrate[1].

In chapter 2, the results of the DAVID and Little DAVID studies were reported: the first clinical studies on peri-operative PK-guided combination treatment of desmopressin and factor VIII concentrate in non-severe hemophilia A. Depending on the planned duration of FVIII concentrate administration, patients were included in the DAVID or Little DAVID study. In both studies, intravenous desmopressin (0.3 µg/kg) once-a-day was immediately followed by infusion of a PK guided dose of FVIII concentrate for maximally three consecutive days to reach predefined target levels of FVIII:C. In total 32 patients, undergoing 33 medical procedures, were included. In the DAVID study (n = 21), on the day of the medical procedure (D0), 61.9% of the peak FVIII:C level predictions were accurate. On day 1 (D1) after the medical procedure, 73.7% (14/19) of the FVIII:C trough levels were predicted accurately and on day 2 (D2) 76.5% (13/17). In the randomized Little DAVID study (n = 12), on D0 83.3% of FVIII:C peak levels of both combination treatment and PK-guided FVIII concentrate were predicted accurately. In the Little DAVID study, combination treatment reduced pre-operative FVIII concentrate use by 47% compared to FVIII monotherapy (p = 0.009). In both studies, side effects of desmopressin were mild

and transient. Two bleeds occurred, both despite the fact that FVIII:C was above 1.00 IU/ mL. We concluded that peri-operative combination treatment with desmopressin and PK-guided FVIII concentrate dosing in non-severe hemophilia A is feasible, safe and reduces FVIII consumption for the pre-operative FVIII concentrate dose.

Repeated daily use of desmopressin is associated with tachyphylaxis, a reduced FVIII:C response compared to the previous dose administered 12 to 24 hours before[2]. **In chapter 3,** the tachyphylaxis and the reproducibility of desmopressin response was studied in 26 patients included in the DAVID studies. In contrast to an earlier study in hemophilia A patients where a response FVIII:C of approximately 70% was reported on the second day compared to response after the first dose of desmopressin, we found a response of approximately 40% after the second dose compared to the first dose. No further reduction of response was observed after the third dose. The reproducibility of the first desmopressin response in comparison to a previously performed desmopressin test (at least 48 hours earlier) dose was high (74%; *n* = 23). It is concluded that clinicians should be aware that repeated daily desmopressin dosing produces only approximately 40% of the initial response, considerably lower than previously reported.

In chapter 4, we performed a cost-minimization study with sensitivity analyses of the 22 procedures performed in the DAVID study in order to assess the total costs of combination treatment compared to FVIII body weight based dosing, the current clinical standard. Use of combination treatment leads to a reduction of €1066 to €1126 (approximately 10%) in total costs per procedure. The use of subcutaneous instead of intravenous administration of desmopressin would reduce time allocated by nurses by 95 to 171 minutes, approximately 50% of the total time per procedure. In addition, the omission of FVIII:C measurements after desmopressin would save time by 12 minutes and thereby also save 1% of the total costs per procedure. We therefore conclude that combination treatment is promising as a cost-effective method to reduce FVIII concentrate related procedure costs.

The use of desmopressin as treatment in non-severe hemophilia A patients is suboptimal. Despite the fact that patients have an adequate FVIII:C response after desmopressin and require only one treatment dose of desmopressin, most of these patients receive FVIII concentrate in case of a bleeding or intervention. **In chapter 5,** non-severe hemophilia A patients' perspectives on the use of desmopressin were assessed in order to find aspects to increase its use. Of the non-severe hemophilia A patients included in the HiN6 study, 468 completed the questionnaire on treatment. Of these 50% reported that they had been treated with desmopressin in the past. Overall, desmopressin was most often used intranasally, and was considered effective by 90%, with flushing as the most common

side effect. The most frequent reported advantage was the convenience of having the possibility of intranasal administration, which was reported by 56% of the users. The most frequent reported disadvantage was the occurrence of side effects, although this was mentioned by only 18% of the respondents. About a quarter of the respondents who had received desmopressin, considered their knowledge on desmopressin as unsatisfactory or did not know how to respond this question. We concluded that more information and education on desmopressin could resolve unmet needs in patients with current or future desmopressin treatment and therefore increase its use.

The use of desmopressin in pregnancy and delivery is debated because of safety concerns related to the side effects of desmopressin, such as fluid retention potentially leading to hyponatremia. **In chapter 6,** the safety and efficacy of desmopressin in pregnancy and delivery was assessed by a systematic review of all literature. Fifty-three studies were included, comprising 273 pregnancies, where desmopressin was administered in 73 during pregnancy and in 232 during delivery. Safety outcome was reported in 245 pregnancies, reporting two adverse events, namely hyponatremia with neurologic symptoms in the parent. In a total of sixty pregnancies, during which women were treated with desmopressin and safety of desmopressin for neonates was reported, two neonates had a severe adverse event, namely preterm delivery and fetal growth restriction. Of 232 deliveries in which desmopressin was used during delivery (pre-or post-clamping of the umbilical cord) 169 neonates were exposed to desmopressin during delivery with safety outcome reported in 114. In two of these neonates, a moderate adverse event was reported, namely a low Apgar score and a transient hyperbilirubinemia not associated with desmopressin use . We conclude that desmopressin use during pregnancy and delivery seems safe. However, included studies had a poor design and a relatively small number of outcomes were documented.

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Hemophilia patients may experience spontaneous macroscopic hematuria. The necessity of diagnostic evaluation and hemostatic treatment in these patients is debatable: the current guideline of the World Federation of Hemophilia (WFH) does not elaborate on the proposed difference in diagnostic management and treatment strategy between a urinary tract hemorrhage, renal bleeding and a mild painless hematuria episode. For the general population various international guidelines concerning evaluation of macroscopic hematuria are available. Most guidelines, including the Dutch guideline, advise to perform urinalysis followed by imaging and/or cystoscopy. In **chapter 7,** the diagnostic evaluation and treatment of spontaneous macroscopic hematuria was assessed in 35 hemophilia patients in our hemophilia treatment center of whom 31 underwent diagnostic evaluation to establish the hematuria etiology after the first episode. This revealed a cause in 26%. In about half of patients (56%) treatment consisted of multiple hemostatic therapies.

Spontaneous macroscopic hematuria in hemophilia patients can be a first sign of underlying pathology in a considerable number of patients. We conclude that diagnostic evaluation at first lifetime episode of hematuria including urinalysis and imaging is indicated as is the case for the general population. Referral to a urologist should be considered in case of a high risk for malignancy.

In most non-severe hemophilia A patients with inhibitors to FVIII, FVIII concentrate cannot be used and desmopressin may not result in the required target levels, and recombinant FVIIa and APCC are costly options, and may increase the risk of thrombotic complications. **In chapter 8,** FIX-FIAV, a novel FIX variant, which has FVIII-like activity was analyzed for its prohemostatic effect and therefore potential treatment in hemophilia A patients with or without inhibitors. By using thrombin generation and intrinsic coagulation activity analyses, we showed that FIX-FIAV is capable of improving thrombin generation and shortens the APTT in all plasma samples obtained from 21 hemophilia A (severe, moderate and mild) patients regardless of inhibitor status or FVIII:C. Furthermore, no substantial effects were observed on thrombin generation when FIX-FIAV was combined with current hemophilia A therapies, such as rFVIIa, aPCC or emicizumab. We concluded that FIX-FIAV is capable of increasing FVIII-equivalent activity and intrinsic coagulation activity in patient plasma, mitigating the hemophilia A phenotype. Therefore, FIX-FIAV could serve as a potential treatment for hemophilia A patients, regardless of inhibitor status.

Finally, **in chapter 9** in the general discussion the findings of the thesis on personalized management of hemophilia A are discussed with respect to current literature and provide suggestions for future research.

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Nederlandse samenvatting

In dit proefschrift worden studies betreffende de gepersonaliseerde behandeling van hemofilie A gepresenteerd met als doel het verbeteren van de huidige zorg. **Hoofdstuk 1** biedt een overzicht van het proefschrift samen met de doelen van de beschreven studies. Een verstoring in de hemostase kan bloeding of trombose veroorzaken. Bij hemofilie A kan desmopressine of FVIII (factor VIII)-concentraat gebruikt worden ofwel om spontane of traumatische bloedingen te behandelen, ofwel om vóór en na een medische ingreep bloedingen te voorkómen. Beide behandelmogelijkheden hebben hun beperkingen. Desondanks de grootschalige beschikbaarheid en lage kosten van desmopressine, wordt desmopressine suboptimaal toegepast in daarvoor geschikte hemofilie A-patiënten omdat deze vaker behandeld worden met FVIII-concentraat. Desmopressine zorgt voor een FVIII:C (FVIII-spiegel) met hoge interindividuele variabiliteit. Echter zijn de beperkingen van FVIII-concentraat de mogelijke vorming van FVIII-remmers, hoge kosten en beperkte wereldwijde beschikbaarheid. Nieuwe behandelmogelijkheden zoals emicizumab en gentherapie zijn veelbelovend, maar meer onderzoek is nodig om optimaal gebruik te maken van deze dure behandelmogelijkheden. Ook kan het gebruik van desmopressine en FVIII-concentraat verbeterd worden middels een meer gepersonaliseerde behandeling. Farmacokinetisch modelleren middels Bayesiaanse voorspelling kan toegepast worden om deze behandelingen voor hemofilie A-patiënten te personaliseren, om het bereiken van vooraf opgestelde FVIII:C-doelen te vergroten en om behandeling te optimaliseren. Het gebruik van combinatietherapie, desmopressine direct gevolgd door FVIII-concentraat, wordt door de internationale World Federation of Hemophilia-richtlijn (WFH) vermeld als mogelijke oplossing om de nadelen van alleen desmopressine- of alleen FVIII-concentraatbehandeling te overwinnen[1].

In hoofdstuk 2, worden de resultaten van de DAVID- en Little DAVID-studie beschreven: de eerste klinische studies over het perioperatief farmacokinetisch gestuurde combinatietherapie van desmopressine en FVIII-concentraat in niet-ernstige hemofilie A-patiënten. Afhankelijk van de geplande duur van de toediening van FVIII-concentraat, werden patiënten geïncludeerd in de DAVID- of Little DAVID-studie. In beide studies werd intraveneus desmopressine (0,3 µg/kg) eenmaal per dag toegediend, onmiddellijk opgevolgd door een infuus van farmacokinetisch gestuurde dosis van FVIII-concentraat voor maximaal drie opeenvolgende dagen, om zo vooraf opgestelde FVIII:C-spiegels te bereiken. In totaal werden 32 patiënten, die 33 medische ingrepen ondergingen, geïncludeerd. In de DAVID-studie (n = 21) waren op de dag van de medische ingreep (D0) 61,9% van de piek-FVIII:C-spiegelvoorspellingen nauwkeurig. Op dag 1 (D1) na de medische ingreep waren 73,7% (14/19) van de FVIII:C-dalspiegels nauwkeurig voorspeld en op dag 2 (D2) na de ingreep waren 76.5% (13/17) nauwkeurig voorspeld. In de gerandomiseerde Little DAVID-studie (n = 12) waren op de dag van de medische ingreep (D0) 83.3% van de FVIII:C-piekspiegels in zowel de combinatietherapiearm als

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FVIII-concentraatmonotherapiearm nauwkeurig voorspeld. In de Little DAVID-studie, verminderde combinatietherapie het gebruik van pre-operatief FVIII-concentraat met 47% in vergelijking met FVIII-concentraatmonotherapie (p = 0.009). Twee bloedingen traden op, allebei desondanks dat de gemeten FVIII:C groter was dan 1.00 IU/mL. We concludeerden dat perioperatieve combinatietherapie met desmopressine en farmacokinetisch gestuurde FVIII-concentraatdosering in niet-ernstige hemofilie A uitvoerbaar en veilig is en FVIII-verbruik vermindert voor de dosis van pre-operatieve FVIII-concentraat.

Herhaald dagelijks gebruik van desmopressine is geassocieerd met tachyfylaxie, een verminderde FVIII:C-respons in vergelijking met de eerdere dosis, die 12 tot 24 uur eerder toegediend is[2]. **In hoofdstuk 3,** werd de tachyfylaxie en reproduceerbaarheid van de desmopressinerespons bestudeerd in 26 patiënten die geïncludeerd waren in de DAVID-studies. In tegenstelling tot een eerdere studie in hemofilie A-patënten waar een FVIII:C-respons van ongeveer 70% werd gerapporteerd op de tweede dag in vergelijking met de respons na de eerste gift van desmopressine, vonden wij een respons van ongeveer 40% na de tweede gift in vergelijking met de eerste. Geen verdere responsafname werd gezien na de derde dosis. De reproduceerbaarheid van de eerste desmopressinerespons in vergelijking met een eerdere desmopressinetestgift (ten minste 48 uur geleden) was hoog (74%; *n* = 23). We concludeerden dat clinici zich bewust moeten zijn dat herhaalde doses van desmopressine slechts ongeveer 40% van de oorspronkelijke respons oplevert, aanzienlijk lager dan eerder in literatuur beschreven.

In hoofdstuk 4, verrichten we een kostenminimalisatiestudie met sensitiviteitsanalyses van de 22 ingrepen verricht in de DAVID-studie om de totale kosten van combinatietherapie te vergelijken met die van FVIII-concentraatdosering gebaseerd op lichaamsgewicht, de huidige klinische standaard. Het gebruik van combinatietherapie leidt tot een afname van €1066 tot €1126 (ongeveer 10%) van de totale kosten per procedure. Het gebruik van subcutaan in plaats van intraveneus desmopressine zou de tijd die verpleegkundigen kwijt zijn 95 tot 171 minuten verminderen, ongeveer 50% van de totale tijd per verpleegkundige procedure. Daarbij zou het weglaten van FVIII:C-metingen na desmopressine 12 minuten besparen en 1% van de totale kosten per procedure besparen. We concluderen daarom dat combinatietherapie veelbelovend is als kosteneffectieve methode om FVIII-concentraatgerelateerde kosten per ingreep te verminderen.

Het gebruik van desmopressine als behandeling in niet-ernstige hemofilie A-patiënten is suboptimaal. Desondanks het feit dat patiënten een adequate FVIII:C-respons hebben na desmopressine en maar één gift van desmopressine nodig hebben, krijgen de meeste van deze patiënten FVIII-concentraat in het geval van een bloeding of een ingreep. **In hoofdstuk 5** worden de perspectieven van niet-ernstige hemofilie A-patiënten bestudeerd

om aspecten te vinden die het gebruik van desmopressine kunnen doen toenemen. Van de niet-ernstige hemofilie A-patiënten geïncludeerd in de HiN6-studie, hadden 468 de vragenlijst over behandeling ingevuld. Van deze rapporteerden 50% dat ze in het verleden met desmopressine waren behandeld. Over het algemeen werd desmopressine het vaakst intranasaal gebruikt en werd het als effectief geacht door 90% van de patiënten, met opvliegers als vaakst voorkomende bijwerking. Het vaakst benoemde voordeel was het gemak van het gebruiken van de intranasale toediening, gerapporteerd door 56% van de gebruikers, ook al werd dit voordeel maar door 18% van alle respondenten benoemd. Ongeveer een kwart van de respondenten die desmopressine hadden gehad, vonden hun kennis over desmopressine onvoldoende of wisten niet hoe ze deze vraag moesten beantwoorden. We concludeerden dat meer informatie en educatie over desmopressine onbeantwoorde behoeftes bij deze patiënten zou kunnen vervullen en daarmee het gebruik van desmopressine zou kunnen doen toenemen.

Het gebruik van desmopressine in de zwangerschap en rondom de partus is discutabel wegens veiligheidsbezwaren gerelateerd aan de bijwerkingen van desmopressine, zoals vochtretentie mogelijk leidend tot hyponatriëmie. **In hoofdstuk 6** werd de veiligheid en effectiviteit van desmopressine in de zwangerschap en rondom de partus geanalyseerd middels een systematische review van alle beschikbare literatuur. Drieënvijftig studies werden geïncludeerd, waarin 273 zwangerschappen werden beschreven, waarbij desmopressine was toegediend gedurende 73 zwangerschappen en 232 bevallingen. Veiligheidsuitkomsten werden beschreven in 245 zwangerschappen waarbij twee ongewenste voorvallen werden beschreven, namelijk hyponatriëmie met neurologische symptomen in de ouder. In totaal 60 zwangerschappen, waarbij gedurende de vrouw behandeld werd met desmopressine en de veiligheid van desmopressine voor neonaten werd beschreven, hadden twee neonaten een ongewenst vooral, namelijk vroeggeboorte en foetale groeirestrictie. In 232 bevalling waarbij desmopressine werd gebruikt rondom de bevalling (voor of na het afklemmen van de navelstreng), waren 169 neonaten blootgesteld aan desmopressine bij de bevalling met veiligheidsuitkomsten gerapporteerd in 114 van deze gevallen. In twee van deze neonaten, werd een niet-ernstig ongewenst voorval gerapporteerd, namelijk een lage Apgarscore en een passagère hyperbilirubinemie die niet geassocieerd was met desmopressinegebruik. We concluderen dat desmopressinegebruik tijdens de zwangerschap en rondom de bevalling veilig lijkt. Echter, hebben de geïncludeerde studies een slechte studie-opzet en relatief klein aantal aan uitkomsten werden gedocumenteerd.

Hemofiliepatiënten kunnen soms spontane macroscopische hematurie krijgen. Het nut van diagnostiek en hemostatische behandeling bij deze patiënten staat ter discussie: de huidige richtlijn van de World Federation of Hemophilia (WFH) weidt niet uit over de verschillen in diagnose en behandeling van een bloeding in de tractus urogenitalis, een

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nierbloeding en een milde, pijnloze hematurie-episode. Voor de algemene bevolking zijn er meerdere internationale richtlijnen over de evaluatie van macroscopische hematurie beschikbaar. De meeste richtlijnen, inclusief de Nederlandse richtlijn, adviseren urine-onderzoek gevolgd door beeldvorming en/of een cystoscopie. **In hoofdstuk 7,** wordt de diagnostiek en behandeling van spontane macroscopische hematurie beschreven in 35 hemofiliepatiënten van ons hemofiliebehandelcentrum van wie 31 diagnostiek ondergingen om de oorzaak van hematurie na de eerste episode te achterhalen. Dit leidde tot een diagnose in 26% van de gevallen. In ongeveer de helft van de patiënten (56%) waren meerdere giften van hemostatische behandelingen nodig om het bloeden te stoppen. Spontane macroscopische hematurie in hemofiliepatiënten kan een eerste teken zijn van onderliggende pathologie in een aanzienlijk aantal patiënten. We concluderen dat diagnostiek geïndiceerd is bij de allereerste episode van hematurie ooit, inclusief urine-onderzoek en beeldvorming, net zoals bij de richtlijn in de algemene bevolking. Verwijzing naar een uroloog zou overwogen moeten worden in het geval van een hoog risico op een maligniteit.

Bij de meeste niet-ernstige hemofilie A-patiënten met FVIII-remmers, kan FVIII-concentraat niet gebruikt worden en kan desmopressine niet zorgen voor de vereiste, gewenste FVIII-spiegels. Daarnaast zijn recombinant FVIIa en aPCC dure keuzes en kunnen ze het risico op trombotische complicaties vergroten. **In hoofdstuk 8** werd FIX-FIAV, een nieuwe FIX-variant die FVIII-achtige activiteit vertoont, geanalyseerd voor diens prohemostatische activiteit en derhalve behandeloptie in hemofilie A patiënten met én zonder FVIII-remmers. Middels trombinegeneratieassays en intrinsieke stollingsassays hebben we aangetoond dat FIX-FIAV de trombinegeneratie kan verbeteren en APTT verkort in alle plasmamonsters verkregen van 21 hemofilie A-patiënten (ernstig, matig-ernstig en mild), ongeacht FVIII:C en ongeacht de aanwezigheid van remmers. Daarenboven waren er geen wezenlijke effecten gezien op trombinegeneratie wanneer FIX-FIAV gecombineerd werd met de huidige hemofilie A-behandelingen, zoals rFVIIa, aPCC of emicizumab. We concluderen dat FIX-FIAV FVIII-vergelijkbare activiteit en intrinsieke stollingsactiviteit kan doen toenemen in patiëntplasma, waarmee het het hemofilie A-fenotype verbetert. Daarom zou FIX-FIAV kunnen dienen als mogelijke behandeling voor hemofilie A-patiënten, ongeacht de aanwezigheid van FVIII-remmers.

Tenslotte wordt **in hoofdstuk 9** de bevindingen van dit proefschrift op de gepersonaliseerde behandeling van hemofilie A bediscussieerd in verhouding tot de huidige literatuur, waarbij voorstellen worden gedaan voor toekomstig onderzoek.

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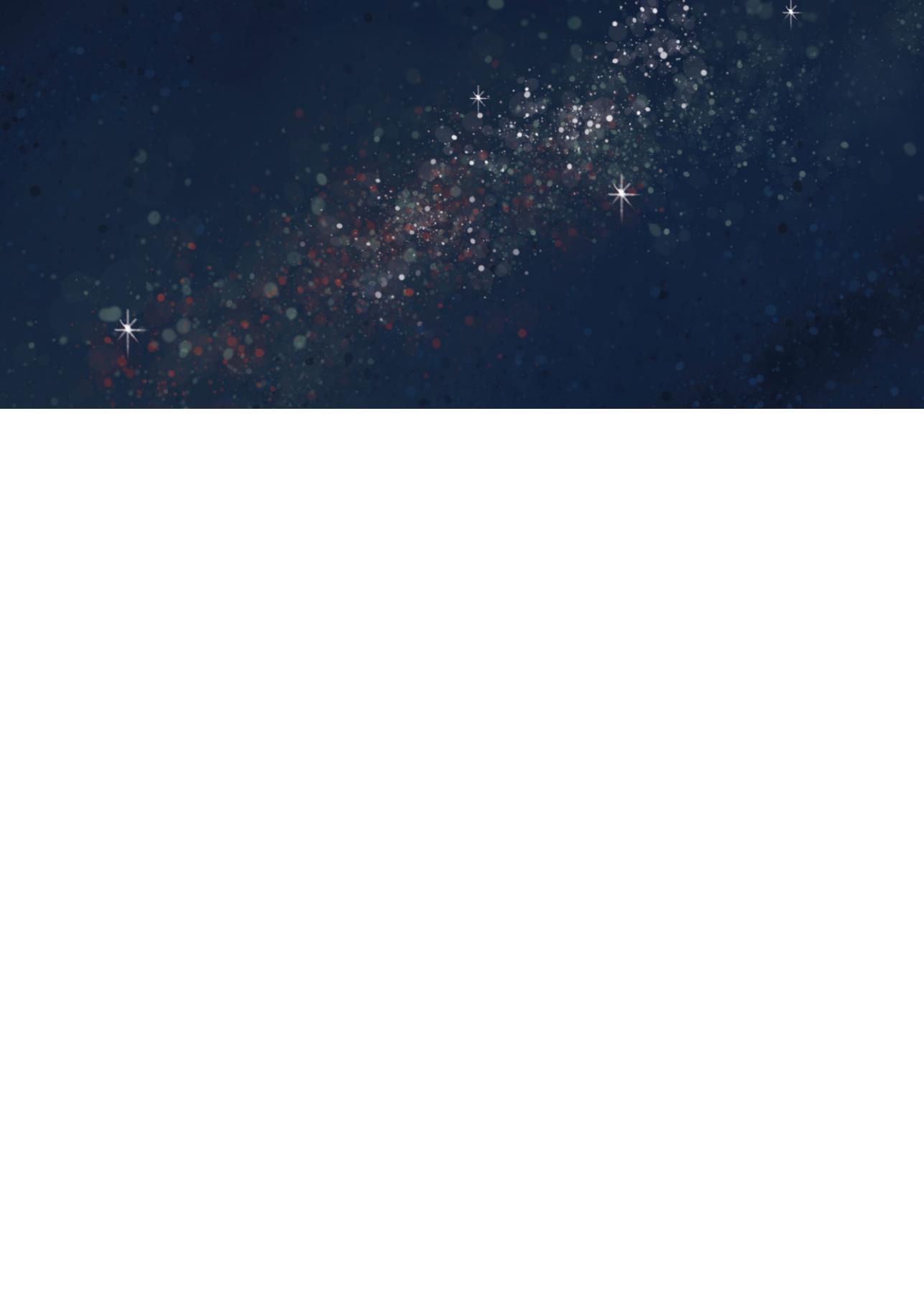
APPENDIX

ABOUT THE AUTHOR

About the author

Lorenzo G.R. Romano was born on the 27th November 1993 in Maastricht and grew up in Lanaken and Maasmechelen, near the Dutch-Belgian border. After graduating from secondary school in 2011 at K.T.A. Alicebourg (now Linc Parc Lanaken) in Lanaken, he studied Medicine at Maastricht University in the same year.

During his Bachelor, he followed the Honours Programme Research, focused on inflammation in neonates and COPD. Additionally, he was volunteer for the Red Cross in Valkenburg.

After receiving his medical degree in 2017, he started as a resident (not in training) in October 2017 at the Amphia Hospital in Breda under supervision of hematologist dr. Joost van Esser. Interested in clinical research after working in patient care, he applied for a PhD position. In March 2019, he was admitted as PhD student at the department of Hematology of the Erasmus Medical Center under the supervision of professor dr. Leebeek, professor dr. Mathôt, dr. Kruip and dr. van Hest. Because of COVID-19, part of his research was temporarily halted and he briefly assisted as a resident (not in training) at the hematology ward of the Erasmus Medical Center. At March 2023, he returned to his earlier workplace at the Amphia Hospital as a resident internal medicine (not in training).

Currently, he is working as a resident internal medicine at the Amphia Hospital under supervision of dr. Joost van Esser for the first part of his residency, followed by supervision by dr. Mariëtte Kappers.

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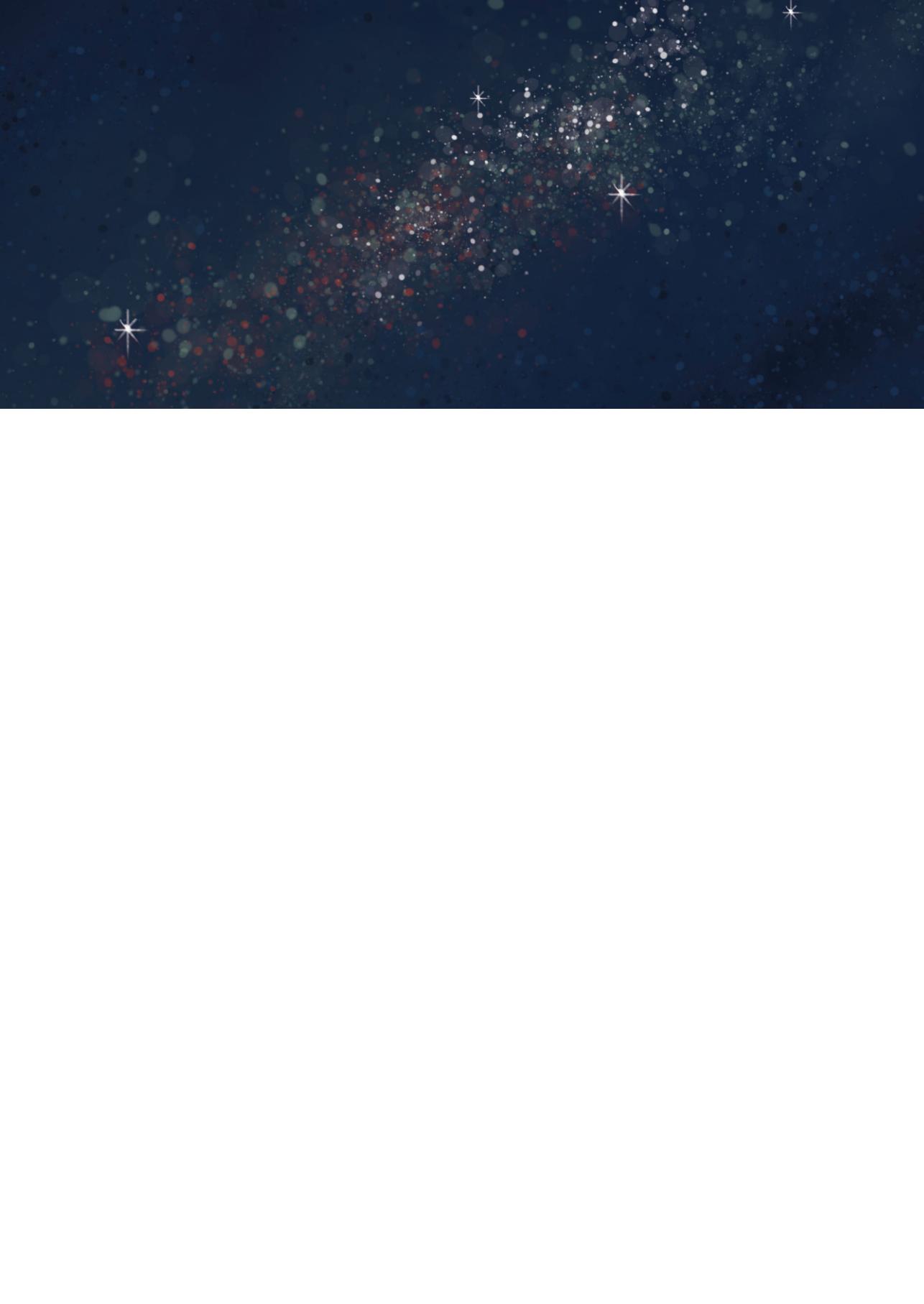
APPENDIX

LIST OF PUBLICATIONS

List of publications

L.G.R. Romano, L.M. Schütte, R.M. van Hest, K. Meijer, B.A.P. Laros-van Gorkom, L. Nieuwenhuizen, J. Eikenboom, F.C.J.I. Heubel-Moenen, N. Uitslager, M. Coppens, K. Fijnvandraat, M.H.E. Driessens, S. Polinder, M.H. Cnossen, F.W.G. Leebeek, R.A.A. Mathôt, M.J.H.A. Kruip, on behalve of the DAVID and SYMPHONY consortium. Tachyphylaxis and reproducibility of desmopressin response in perioperative persons with nonsevere hemophilia A: implications for clinical practice. *Research and Practice in Thrombosis and Haemostasis.* Volume 8, Issue 3, March 2024, 102367.

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*Both authors contributed equally to this study.

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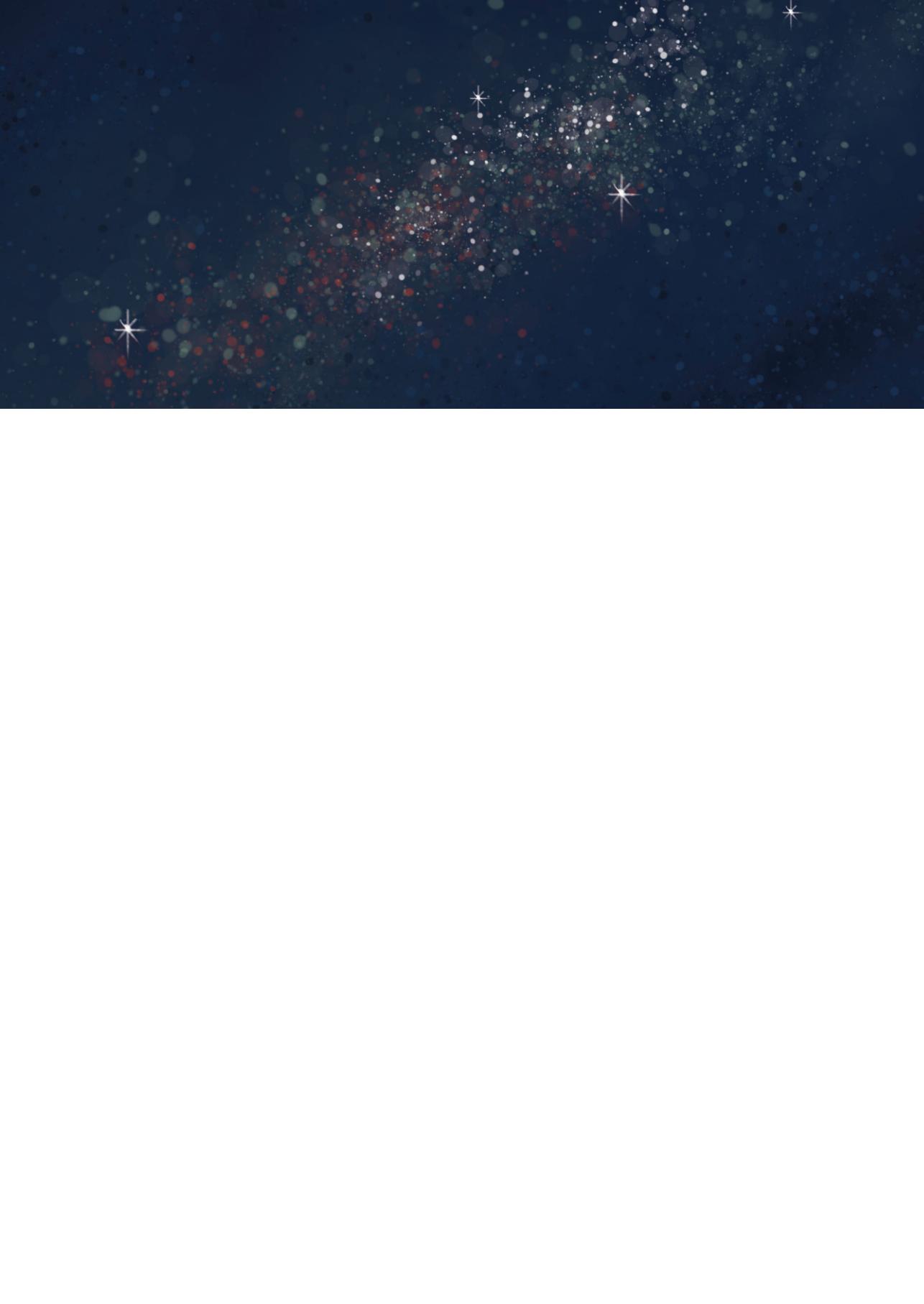
APPENDIX

PHD PORTFOLIO

PhD portfolio

Summary of PhD training and teaching

Name: **Lorenzo G.R. Romano**

Erasmus MC department: **Hematology**

Research school: **COEUR**

PhD period: **March 2019 - February 2023**

Promotors: **prof. dr. F.W.G. Leebeek,**

prof. dr. R.A.A. Mahtôt

Supervisors: **dr. M.J.H.A. Kruip, dr. R.M. van Hest**

PhD Training	Year	Workload (ECTS)*
General courses		
Biomedical English Writing	2021	**2.5**
Scientific Integrity	2019	**0.3**
CPO Course (Patient Oriented Research)	2019	**0.3**
Embase, Pubmed, EndNote and Project Management Workshop	2019	**1.0**
SPSS workshop	2019	**1.0**
BROK course	2019	**1.5**
Specific courses		
NVTH annual PhD course on hemostasis and thrombosis (3x)	2019, 2021, 2022	**3.0**
Biostatistical Methods II: Classical Regression Models	2020	**4.3**
NVTH alternative digital course and symposium	2020	**0.6**
COEUR PhD Day	2019	**0.3**
Sex and Gender in Cardiovascular Research (COEUR)	2019	**0.5**
Biostatistical Methods I: Basic Principles	2019	**5.7**
Presentations and Conferences		
Oral presentations		
NVTH Symposium	2021 - 2022	**1.0**
Dutch Hematology Congress	2022	**0.5**
International Society on Thrombosis and Hemostasis Congress	2021	**0.5**
Poster pitch and presentation		
NVTH symposium	2021	**0.3**

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PhD Training	Year	Workload (ECTS)*
Poster presentations		
European Association of Haemophilia and Allied Disorders	2023	**0.3**
International Society on Thrombosis and Hemostasis Congress (7x)	2020 - 2022	**2.1**
European Hematology Association	2021	**0.3**
International Symposium on Intensive Care and Emergency Medicine	2021	**0.3**
International and national conferences		
International Society on Thrombosis and Hemostasis Congress	2020 - 2022	**3.9**
International Symposium on Intensive Care and Emergency Medicine	2021	**1.2**
European Association of Haemophlia and Allied Disorders (2x)	2020, 2022	**1.8**
NVTH Symposium (3x)	2019, 2021, 2022	**1.95**
Bari in Conference	2019	**0.75**
Research meeting Hemostasis group and Friday floor	2019-2023	**8.0**
Teaching activities		
Presentation "Thrombosis and Hemostasis": GP's in training	2022	**0.3**
Lecturer "Thrombosis and Hemostasis": nurses and obstetricians in training (12x)	2019 - 2023	**2.4**
Bachelor of Medicine: student coaching	2020 - 2023	**1.5**
Supervision of students		
Tirza den Hertog, medical student	2021 - 2022	**3.5**
Total		**51.6**

*ECTS - European Credit Transfer and Accumulation System (1 ECTS represents 28 hours)

APPENDIX

DANKWOORD

Dankwoord

Allereerst wil ik alle patiënten die deel hebben genomen aan deze onderzoeken bedanken voor hun inzet: zonder hen was dit proefschrift er niet geweest.

Beste **Frank**, jouw scherpe blik ziet altijd een oplossing in het geval van lastige zaken tijdens mijn onderzoekstijd. Hierdoor heb je mij ook zelf het inzicht gegeven hoe om te gaan met dergelijke situaties in de toekomst. Dank voor al je begeleiding met mijn proefschrift.

Beste **Marieke**, bij onze overleggen had je altijd een goed overzicht klaar. Ook heb je me de kans gegeven om een eigen project binnen mijn PhD aan te gaan waarbij ik, ook in het algemeen, veel ruimte heb gekregen. Dank voor al je vertrouwen en begeleiding.

Beste **Reinier**, onze samenwerking is altijd vlekkeloos gelopen. Ik wist je altijd te enthousiasmeren voor een inclusie en je was altijd bereid om mijn vragen te beantwoorden. Dank voor de leerzame en ook gezellige tijd.

Beste **Ron**, bij het bespreken van onze stukken begrijp je snel waar ik tegen aan loop. Ook buiten het werk om heb ik altijd een leuke tijd samen gehad, dank daarvoor.

Beste **Joost**, zonder jouw hulp was ik nu niet in het Amphia in opleiding geweest. Je hebt namelijk de gave om als opleider mensen in hun waarde te laten en ernaast toch te laten groeien, zonder veroordelend te zijn. Dat is een gave die niet veel mensen zomaar is weggelegd. Dank voor al je hulp, ik zal het niet vergeten. Jou als deel van mijn commissie hebben is dan ook een groot feest!

Beste **Moniek**, al de labbesprekingen en alle gezellige momenten op de promovendikamer zijn goud waard. Dank voor het luisterend oor en je deelname aan mijn commissie.

Beste **Karina**, bij al onze ontmoetingen heb je altijd een visie klaar en voor de DAVID-studies is jouw steun van grote waarde geweest. Ik ben blij dat je aan mijn commissie deelneemt.

Beste **Gerard**, jouw humor en ervaring komen zeker van pas bij mijn verdediging. Dank voor jouw deelname aan mijn commissie.

Professor **Koch** en professor **Schutgens**, dank voor jullie deelname in mijn commissie. Ik ben benieuwd naar jullie inbreng bij mijn verdediging.

D

Beste **Niels**, ook jouw hulp bij mijn sollicitatie heeft mij gebracht tot waar ik nu ben. Zowel binnen als buiten het ziekenhuis heb je me dingen geleerd en bijgebracht waar ik je tot de dag van vandaag erg dankbaar voor ben. Ik hoop dat we elkaar nog lang kunnen zien!

Beste **Marjon**, dank voor al je hulp bij het meedenken met de artikelen en dank voor jouw inzet met SYMPHONY. Jouw enthousiasme zorgt ervoor dat iedereen weet waar SYMPHONY voor staat.

Britta, **Laurens**, **Jeroen**, **Floor**, **Nanda**, **Michiel**, **Karin**, **Mariëtte**, **Suzanne** en **alle andere medewerkers van de hemofiliebehandelcentra in Nederland**, mede dankzij jullie inzet als deel van het DAVID-team is dit proefschrift tot stand gekomen. Veel dank hiervoor!

Lieve **Greta**, al die jaren heb ik altijd veel met je kunnen lachen. Jouw zorgzame, warme persoonlijkheid laat iedereen zich meteen welkom voelen. Dank je voor alle wijze levenslessen!

Beste **Tobias**, **Toto**, van Ibiza tot Sri Lanka: je weet me altijd uit mijn comfortzone te trekken sinds onze ontmoeting in het Amphia. Ook weet je altijd de spijker op z'n kop te slaan als er iets aan de hand is, wat ik erg waardeer. Ik wens je alle geluk samen met **Manon**. Op naar de volgende reis!

Beste **Kevin** en **Vladimir**, na Barcelona is het allemaal begonnen en sinds Californië zijn de 4x4x4 tripjes niet meer weg te denken uit het arsenaal. Dank voor jullie hilarische en ontspannende tijden, van bordspellen tot Route 66.

Beste **Rik**, lieve **Nishi**, al sinds het oudste coschap (ook wel semiartsstage) interne hebben we een geweldige tijd gehad. Nishi's vision is never wrong en Rik, je kan dat beamen. Ik kijk dan ook uit naar onze toekomstige diners.

Beste **Rob** en **Michel**, hoe "kansloos" het ook eruit ziet: het is niet belangrijk wat men van ons maakt, maar wat wij zelf maken van wat ze van ons gemaakt hebben. Dat weten jullie als geen ander.

Beste **Max** en **Pepijn**, jullie afleiding tijdens de drukke momenten van mijn proefschrift ben ik nog altijd heel erg dankbaar, samen met Fjorm! **Tom** en **Iris**, de Limburgse gezelligheid is altijd present als we elkaar tegenkomen en maakt mijn dag altijd weer goed. **Marco** en **Vasil**, onze momenten samen waren kort maar krachtig, en het goed lachen heeft zeker bijgedragen aan het afronden van mijn proefschrift.

Beste **Vince**, dank voor alle broodnodige momenten om bij te praten de afgelopen jaren, samen is het altijd een groot feest. Sinds het middelbaar zijn we toch niet heel veel veranderd!

Beste **Stephan**, ook al zit je nu aan de andere kant van de wereld, bij ontmoetingen is het weer als vroeger. Ik hoop je nog iets vaker in Nederland te zien.

Dear **AJ**, **Andrew**, **Jon** and **Joe**, times have always been a blast with you guys. Even from across the pond, we'll still have fun times. Meeting up is always a pleasure and I hope to see all of you soon.

Beste **Chris**, **Indra** en **Stephan Segers**, ook jullie oudgedienden laat ik liever niet los, met raad en daad stonden jullie me bij. Dank voor de bootjestijd en de boulderbaan, volgende keer stem ik voor de activiteit!

Beste **Mark**, **Robin**, **Luuk** en **Clint**, de jaren zijn voorbijgevlogen, de tijden van 2,50 zijn voorbij. Iedereen is zijn eigen pad opgegaan, maar soms komen we elkaar toch nog tegen en komen de verhalen weer naar boven. Op naar de volgende!

Beste **Lisette**, dankzij jouw werk is dit proefschrift tot stand kunnen komen. Ik dank je voor alle wijze woorden en adviezen die je me hebt gegeven.

Beste **Viola** en **Mettine**, dank voor jullie steun in onze samenwerking. Ik heb dankzij jullie veel meer labkennis kunnen vergaren.

Beste **Tim**, jouw steun in de samenwerking die we hebben gehad tijdens coronatijd heeft me doen beseffen hoe leuk de klinische farmacologie kan zijn. Je bent daarbij zeker de grondlegger geweest voor mijn interesse. **Nicole**, en **Rik Endeman**, ook jullie hebben daar zeker aan bijgedragen, waarvoor dank.

Beste **Samantha Gouw** en **alle andere leden van het HiN6-team**, dank voor jullie begeleiding en inzet voor de HiN6-studie.

Beste **Tirza**, dank je voor je hulp bij het verzamelen en schrijven van het stuk over hemofiliedraagsters. Je was altijd erg enthousiast en bracht ook je eigen visie op het onderzoek.

Dear **Leon** and **Evelyn**, thank you for all the good times. I wish you all the best in Florida and I hope I can visit you soon.

D

Beste **Ron** en **Marissa**, dank voor jullie gezelligheid en betrokkenheid over alle jaren. Het is altijd een feest om bij jullie op bezoek te komen.

Erasmuscollega's, jullie hebben individueel allemaal me iets geleerd of bijgebracht wat ik meeneem. Lieve **Chantal**, naast het belang van hard werken, leerde je me ook dat ontspanning ernaast met **Bianca** een enorme steun kan zijn. Ik ben trots op wat je hebt bereikt en je zal later een goede collega zijn. Lieve **Tine**, soms is mijn creatief geordend bureau ook een manier tot het vormen van succesvol onderzoek. Desalniettemin ben ik het eens dat jouw geordende methode wel diens vruchten heeft afgeworpen! Ik wens **Joppe** en jullie kleine **Joep** het allerbeste in de toekomst. **Ferdows**, jouw focus en drive om tot het beste resultaat te komen motiveert me om wat er ook gebeurt het beste ervan te maken, hou die kracht vast! **Wala**, naast onze samenwerking is er met jou ook tijd om de diepgang te zoeken over onderwerpen waar niet iedereen voor open staat. **Ryanne**, jouw harde werk, creativiteit en bereidheid om te helpen waar nodig inspireerde me tijdens mijn promotietraject. Lieve **Maite**, je hebt me geleerd dat je passie achterna gaan altijd de optie is, dat dat soms ook vereist dat je met je figuurlijke vuist op tafel moet slaan en vooral dat dat ook gewoon mag! Ik waardeer je vastberadenheid om te vechten voor waar je in gelooft. **Judith**, je inzet en doorzettingsvermogen vormde een inspiratie voor iedereen om je heen. **Aarazo**, ik heb bewondering voor hoe je alles voor elkaar hebt gekregen en hoe ver je bent gekomen. Ik waardeer onze tijd samen en de gedeelde passie voor muziek in het lab. **Amica**, je hebt een sterk analytisch vermogen en kan daarmee goed problemen doorgronden. **Calvin**, je hebt me geleerd dat met hard werken er meer tijd vrij is voor andere zaken in het leven. **Iris**, het is altijd leuk om sarcasme met je te delen, hou dat vast. **Samantha** en **Jessica**, lunchpauze is belangrijk en de bezinning daarbij net zo zeer. **Sophie**, het was altijd gezellig op de dansvloer, met extra karaoke: zingen is essentieel! **Isabel**, I enjoyed sharing our knowledge on German music, I hope you have some new songs at hand. **Aida**, het is belangrijk om gericht bepaalde gewoontes binnen de geneeskunde in vraag te stellen, ik ben benieuwd naar je inzichten. **Hande**, even though our shared moments were limited, the banter was a lot of fun.

Beste **collegapromovendi van alle centra**, jullie aanwezigheid op congressen was altijd een gezellige ontmoeting. Ik mis daarbij dan ook de kleine momenten zoals diners of cursusdagen!

Alle **Amphianen**, zowel de overige oudgedienden als de nieuwe collega's: ik dank jullie voor jullie motivatie en goede sfeer. Het Erasmus MC is toch nóg gezelliger bij het ROIG-onderwijs zodra we er in grote getale verschijnen.

Lieve **mam**, **pap**, **Aurora**, **Jaxx**, **Stephanie** en **Danny**, dank voor jullie onvoorwaardelijke steun in dit traject. Ook aan mijn overige familie in Limburg en Sicilië: dank voor jullie steun, ook op afstand.

Lieve **grootouders**, helaas zijn jullie niet meer onder ons, maar ik voel alsnog dat jullie mij hebben geholpen. Het was niet vanzelfsprekend om te komen waar ik nu ben en daar hebben jullie met hard werk de basis voor kunnen leggen. Derhalve draag ik trots de nagedachtenis van jullie mee in mijn hart.

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