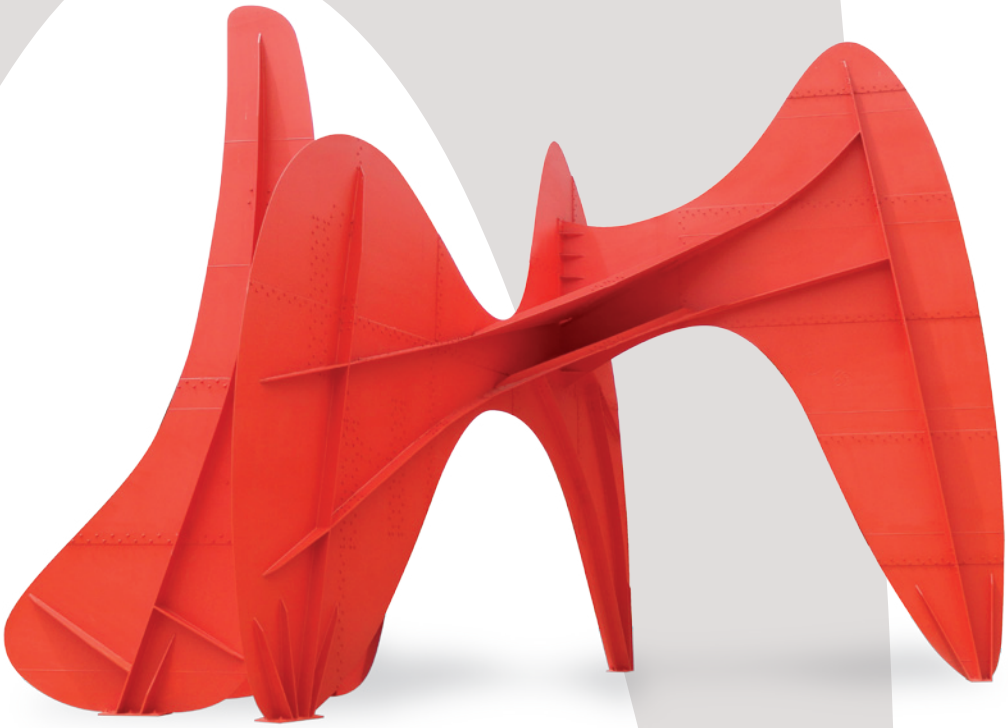
Effects and outcome of hemostatic treatment in bleeding disorders

S. Carina M. Stoof



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EFFECTS AND OUTCOME OF HEMOSTATIC TREATMENT IN BLEEDING DISORDERS

Effecten en gevolgen van de behandeling van bloedingsziekten

Proefschrift

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'Nil sine magno labore'
Horatius

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CHAPTER 1

General introduction and outline of the thesis

GENERAL INTRODUCTION

Bleeding disorders

Hemostasis is a process driven by multiple factors resulting in the formation of a blood clot to stop bleeding in case of injury.[1] When hemostasis is impaired, it can lead to excessive bleeding. A bleeding diathesis can either be inherited, for example in case of hemophilia or Von Willebrand disease (VWD) or can be acquired as is the case with medication, uremia or cirrhosis.[1]

Hemophilia A and B are X-linked inherited bleeding disorders characterized by insufficient or absent coagulation factor VIII (FVIII) or factor IX (FIX), respectively. Hemophilia A affects 1 in 5000 live male births[2], hemophilia B 1 in 30.000[3] and around 1600 patients are known with hemophilia in the Netherlands.[4] Hemophilia is classified as severe when the coagulation factor level is <0.01 IU/ml, resulting in spontaneous joints and muscle bleeds. When coagulation factors levels are 0.01-0.05 IU/ml this is called moderate hemophilia and these patients bleed mostly only after minor trauma or surgery. In case of mild hemophilia, defined as coagulation factor levels between 0.05-0.40 IU/ml, patients mainly bleed after major trauma or surgery.[5] Hemophilia A can be caused by a variety of mutations in the *F8* gene, which is situated at the distal end of the long arm of the X-chromosome (Xq28). Hemophilia B is caused by mutations in the *F9* gene, located at Xq27.1.[6] Mild and moderate hemophilia A are most often caused by missense mutations[7], and point mutations are the most frequent cause of hemophilia B.[8] Due to the inheritance pattern, males are affected and females are carriers of the disease. Approximately 80% of hemophilia carriers have coagulation factor levels that enable adequate hemostasis, but the range of coagulation factor levels is wide and an increased bleeding tendency has been observed in carriers, even with normal FVIII or FIX levels.[9]

VWD is the most common inherited bleeding disorder and is present in 1% of the population.[10] However, only 1 in 10.000 individuals has clinically relevant bleeding that requires treatment.[11] VWD occurs both in men and women and is caused by quantitative (type 1 and 3) or qualitative (type 2) abnormalities of von Willebrand factor (VWF).[12] Type 1 VWD is the most common subtype and accounts for nearly 80%. VWF plays an important role in primary hemostasis via its involvement in platelet adhesion and aggregation, thus enabling platelet plug formation. Also, it acts as a carrier protein for FVIII, hereby preventing its premature clearance.[13] VWD is mainly characterized by mucocutaneous bleeding, including epistaxis, menorrhagia, gastrointestinal bleeds and postpartum bleeding.[11]

The liver is important in hemostasis as it synthesizes the majority of coagulation factors. In addition, it produces thrombopoietin which is responsible for platelet production from megakaryocytes. Consequently, in case of liver disease such as cirrhosis,

various aspects of hemostasis can become impaired.[14] Furthermore, due to chronic endothelial cell activation there is a continuous release of several hemostatic proteins resulting in elevated levels, including VWF.[15] Cirrhosis has long been considered a bleeding disorder, however the concept of a precarious hemostatic balance that may result both in bleeding and thrombotic complications, was recently accepted.[16]

Hemostatic treatment

The mainstay of treatment of bleeding disorders is the infusion of coagulation factor concentrates to prevent or treat a bleeding episode. Current treatment with hemostatic agents aims to temporarily improve hemostasis. To prevent bleeding in case of surgical interventions or to treat bleeds after trauma, desmopressin (1-deamino-8-D-arginine vasopressin, DDAVP) can be used in selected patients with bleeding disorders.

Desmopressin is a synthetic analogue of the antidiuretic hormone that selectively stimulates the vasopressin-2 receptors, hence causing a transient decrease in blood pressure and an antidiuretic effect.[17] In a high dose it results in a release of VWF from the Weibel-Palade bodies in the vascular endothelium and a simultaneous rise in FVIII that lasts for approximately 12-24 hours.[17,18] As a consequence, it is the treatment of choice in patients with non-severe hemophilia A or type 1 VWD who lack these coagulation factors. However, the increase in VWF and FVIII is approximately 3-5 fold and it is therefore of no use in severe hemophilia A and type 3 VWD. It is generally advised to use desmopressin in hemophilia patients with FVIII levels $\geq$0.05-0.10 IU/ml.[19-23] Patients with type 2B VWD should not use desmopressin as they have a 'gain of function' defect that results in enhanced binding to glycoprotein Ib, leading to spontaneous binding to and aggregation of platelets resulting in thrombocytopenia.[24] Desmopressin has also been shown to enhance platelet activation by the transient increase of high-molecular weight VWF multimers and may therefore be used in patients with platelet function defects.[25,26] In patients with cirrhosis, desmopressin is often used prophylactically to prevent bleeding during invasive procedures, although the mechanism of action is unclear. A shortened bleeding time after desmopressin administration in patients with cirrhosis has been reported.[27-29] On the contrary, other studies report a lack of clinical efficacy.[30-32]

Desmopressin can be administered intravenously or subcutaneously in a dose of 0.3 µg/kg body weight, and intranasally in a dose of 300 µg in patients weighing over 50 kg (150µg per nostril).[23] In the Netherlands only the intravenous and intranasal administration route are registered and consequently used. Over the years, several side effects of desmopressin have been identified, predominantly based on small studies and case reports.[33,34] Most side effects are mild and correspond with the antidiuretic and vasomotor effects of the drug, such as transient hypotension, hyponatremia, flushing, nausea and headache.[35] Life-threatening side effects such

as thrombotic complications are incidentally reported[36-38] but it can be assumed that patients with cardiovascular risk factors are more prone to develop such an event. Consequently, cardiovascular disease is considered a contraindication for desmopressin use. In general, a fluid restriction after desmopressin administration is advised to prevent hyponatremia.[18] As desmopressin causes an increase of endogenous coagulation factors, there is no risk of development of inhibitory antibodies.[39] It is a synthetic drug, thereby has no risk of viral transmission. In addition, it is less expensive than replacement therapy with coagulation factor concentrates, which makes it attractive especially for countries with limited resources. In situations where desmopressin cannot be used (such as in pregnancy or in patients with cardiovascular disease) or does not provide adequate hemostasis, coagulation factor concentrates have to be used.

The increase of FVIII and VWF after desmopressin, i.e. desmopressin response, shows a great inter-individual variation. Several factors are reported to be associated with this variation in hemophilia patients, such as age, baseline FVIII or VWF, familial response and *F8* gene mutation.[34,40-42] Because of the inter-individual variation, the response to desmopressin is tested in an elective setting in each patient prior to treatment, a so called desmopressin test-dose. There is no agreed definition of an adequate desmopressin response in hemophilia patients, nor in VWD patients. Some studies use clinical cut-offs, i.e. FVIII ≥0.30 IU/ml for minor and ≥0.50 IU/ml for major bleeds or surgery[34,42,43], whereas others also incorporate a FVIII fold increase over baseline.[41,44,45] In addition, there is no standard blood sampling protocol for a desmopressin test-dose and it varies from limited sampling, prior to and immediately (up to 1 hour) after infusion, to more extended sampling.[22,40]

Outcome of hemostatic treatment

It is internationally accepted that coagulation factors, such as FVIII and VWF, should be ≥0.50 IU/ml (just above the hemostatic value of 0.40 U/ml) for adequate hemostasis in order to prevent bleeding in case of major trauma or surgery, including delivery.[23,46,47] In women, the most challenging situation with regard to hemostasis is childbirth. During pregnancy, VWF and FVIII show a physiological increase of 150-200% leading to levels of 2.00-3.00 IU/ml, whereas factor IX only shows a minimal increase.[48,49] In women with VWD or carriership of hemophilia, the absolute increase of these coagulation factors is usually not to the same extent as in the general population.[50,51] Nonetheless, the 0.50 IU/ml cut-off for FVIII, VWF and FIX is applied at time of delivery to indicate who needs prophylactic treatment prior to delivery to prevent bleeding. This prophylactic treatment consists of administration of coagulation factor concentrates with target levels of 1.00 IU/ml at time of delivery. In addition, tranexamic acid can be used.[23] Despite these measures, deliveries can be complicated by excessive bleeding, i.e. postpartum hemorrhage (PPH) (blood loss ≥500 ml) which is a leading

cause of maternal deaths worldwide.[52] Varying PPH incidences in VWD and carriers of hemophilia have been described, some suggesting an increased risk of PPH in women with these bleeding disorders.[53-55]

In hemophilia patients, the most frequently occurring and invalidating bleeds are located in the joints (hemarthrosis) and muscles. These bleeds are mainly seen in patients with severe hemophilia.[23] Long-term consequences of hemarthrosis are chronic synovitis, cartilage damage and eventually irreversible arthropathy.[56,57] Prophylactic treatment with coagulation factor concentrates once to thrice weekly, aims to prevent joint bleeds and consequently the development of this hemophilic arthropathy. However, once joint damage has occurred, it will progress over time even if no additional bleeds occur in the affected joints.[58] Early diagnosis of joint damage is difficult, especially as joint arthropathy in patients on prophylactic treatment develops slowly.[59] Hence, prophylactic treatment often seems unable to prevent hemophilic arthropathy and only able to postpone the onset. Because of this, it is useful to monitor the musculoskeletal status in hemophilia patients as this can lead to treatment alterations, such as dosage adjustments for prophylactic treatment, physical therapy exercises or referral to a rehabilitation specialist or orthopedic surgeon.[59] The overall aim of monitoring joint function is to evaluate prophylactic treatment that intends to prevent loss of quality of life and maintain a patient's ability to work and perform sports. Musculoskeletal assessment can be performed with static imaging evaluation, passively active clinical examination by using the Hemophilia Joint Health Score or with dynamic evaluation such as gait analysis.[60] The latter is especially useful as functional limitations then become clear. Gait analysis has been studied in hemophilia patients, however in general is laborious and consequently expensive and often requires extensive equipment.[61,62] Inexpensive, portable and easy-to-use gait analysis techniques can provide objective information on a patients' musculoskeletal status at the outpatient clinic, although this has not been studied in hemophilia patients.[63]

AIM AND OUTLINE OF THE THESIS

This thesis focuses on the effects and outcome of hemostatic treatment in patients with inherited and acquired bleeding disorders for which purpose several studies are performed. Desmopressin is only administered in hemophilia A patients with baseline FVIII levels ≥0.05-0.10 IU/ml. In **chapter 2**, the response to desmopressin in patients with FVIII levels <0.10 IU/ml will be evaluated. In **chapter 3**, we try to elucidate the role of the type of *F8* gene mutation and the inter-individual variation in desmopressin response in patients with hemophilia A. In **chapter 4**, the side effects of desmopressin will be assessed in a large, unselected group of patients with inherited bleeding

disorders to substantiate the general opinion regarding the safety of desmopressin. In chapter 5, we evaluate the different desmopressin response definitions used in hemophilia A and try to develop a uniform response definition. In addition, a more individualized test-dose regimen will be discussed.

Despite its widespread use, the mechanism by which desmopressin improves hemostasis in cirrhosis is unclear. In chapter 6, a study on the potential pro-hemostatic effects of desmopressin in patients with cirrhosis will be performed to provide a rationale for its use in this population.

PPH is a life-threatening complication of delivery and may occur more frequently in women with inherited bleeding disorders. In chapter 7, this will be investigated by means of a retrospective study. In chapter 8, a study on gait analysis in hemophilia patients will be performed with a sensor-based device to evaluate its use with regard to musculoskeletal assessment in hemophilia care. Finally, in chapter 9 and 10, the findings of our studies will be summarized and discussed.

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CHAPTER 2

Desmopressin response in hemophilia A patients with FVIII:C <0.10 IU/ml

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SUMMARY

Background: Desmopressin is widely used to temporarily improve hemostasis in hemophilia A patients. Most guidelines recommend use of desmopressin only in hemophilia A patients with FVIII:C levels above 0.05-0.10 IU/ml as individuals with lower levels may not respond adequately. However, these recommendations are based upon a limited number of studies.

Objective: To determine the desmopressin response in hemophilia A patients with historically lowest FVIII:C levels <0.10 IU/ml.

Methods: We included non-severe hemophilia A patients from our center with a FVIII:C level below 0.10 IU/ml on at least 2 occasions who received desmopressin with measurement of FVIII:C levels (0, 1, 3 and 6 hours). A response was defined as FVIII:C ≥0.30 IU/ml.

Results: Forty-eight patients had historically lowest FVIII:C levels <0.10 IU/ml, 37 of whom (77%) reached FVIII:C levels ≥0.30 IU/ml one hour after desmopressin. This response was maintained in 16/32 patients (50%) and in 5/30 patients (17%) after three and six hours, respectively. Blood group and administration route did not influence the response. Patients <18 years had significantly lower baseline levels of FVIII:C than ≥18 years (0.06 IU/ml (SD 0.03 IU/ml) vs. 0.10 IU/ml (SD 0.04 IU/ml).

Conclusion: The majority of patients with historically lowest FVIII:C <0.10 IU/ml had a FVIII:C response ≥0.30 IU/ml after desmopressin. Even though the response was short, desmopressin can be considered as a treatment option for small surgery or minor injury in these patients. It is recommended to perform a desmopressin test-dose in all non-severe hemophilia A patients to evaluate its therapeutic use.

INTRODUCTION

Desmopressin (1-deamino-8-D-arginin-vasopressin, DDAVP) is a synthetic analogue of the anti-diuretic hormone vasopressin. It is inexpensive, rarely leads to adverse reactions and moreover has no risk of viral transmission or inhibitor development.[1] In a high dose it causes an increased release of von Willebrand factor (VWF) from the vascular endothelium and a subsequent 3-5 fold rise in factor VIII (FVIII).[2] Therefore it is a treatment option for patients with von Willebrand disease and mild to moderate hemophilia A. The effect is transient and may last up to 12-24 hours after administration. Desmopressin can be administered intravenously, intranasally or subcutaneously. Some guidelines recommend to use desmopressin only in patients with FVIII:C levels above 0.05 IU/ml, some even only in patients with FVIII:C levels above 0.10 IU/ml.[3-7] However, an adequate desmopressin response has been reported in some patients with moderate hemophilia A.[8] Literature that supports the current recommendations regarding desmopressin use is limited. Hence, we studied the desmopressin response in hemophilia A patients with historically lowest FVIII:C levels <0.10 IU/ml, i.e. the lowest FVIII:C level ever measured.

MATERIALS AND METHODS

Study population

Non-severe hemophilia A patients from our center with FVIII:C levels <0.10 IU/ml on at least 2 occasions, and who received desmopressin with measurement of FVIII:C levels before and after administration (0, 1, 3 and 6 hours), were included. Desmopressin was administered intravenously (0.3 µg/kg bodyweight) or intranasally (two sprays of 150 µg each; total dose 300 µg). Patients with an inhibitor at time of desmopressin administration were excluded, as were patients who received FVIII concentrate <72 hours prior to desmopressin. Patients received desmopressin as a test dose to assess its future therapeutic use or as treatment in case of mild trauma or before surgery. 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 Center, Rotterdam.

Response criteria

FVIII:C ≥0.30 IU/ml was defined as a response to desmopressin; a *complete* response if FVIII:C levels increased to ≥0.50 IU/ml after administration and a *partial* response if FVIII:C increased to ≥0.30 IU/ml but <0.50 IU/ml. FVIII:C <0.30 IU/ml was considered as no response, based on previously described definitions.[9] The response one hour after administration was defined as *initial* response, 3 hours afterwards as *sustained 3 hour* response and 6 hours after desmopressin as *sustained 6 hour* response.

Laboratory measurements

Venous whole blood was collected in 0.105 M sodium citrate tubes (Becton, Dickinson and Company (BD) Vacutainer) and centrifuged at 2000 x *g* for 10 minutes at room temperature. The plasma was centrifuged additionally at 14000 x *g* for 10 min at room temperature and stored at -80°C until analysis. FVIII activity (FVIII:C) was measured by a one-stage clotting assay, using FVIII-deficient plasma (Siemens Healthcare, Marburg/Germany) and reference plasma (Cryocheck, Precision Biologic, Kordia) on an automated coagulation analyzer (Sysmex CA1500, Siemens, Breda).

Statistical analysis

Descriptive statistics are presented as mean and standard deviation (SD) for continuous variables, except for age (median, range). Mean differences and 95% confidence intervals were calculated to estimate the influence of modifying factors on FVIII:C level at 6 hours. A $p<0.05$ was considered statistically significant. SPSS version 20.0 (IBM Corp. Armonk, NY, USA) was used.

RESULTS

Study population

In total, 48 patients were eligible for inclusion, 31 (65%) of whom had mild hemophilia A (0.06-0.40 IU/ml) and 17 (35%) moderate hemophilia A (0.01-0.05 IU/ml). We collected desmopressin responses between 1984-2012. Median age at time of administration was 28 years (range 8-67). The historically lowest FVIII:C level was 0.05 IU/ml (SD 0.02 IU/ml). In 43/48 patients (90%) desmopressin was administered intravenously and in 5/48 patients (10%) intranasally. Desmopressin was given as a test dose in 44/48 patients (92%) and as treatment prior to minor surgical interventions in 4/48 patients (8%). Twenty/48 patients (47%) had blood group 0 and 23/48 patients (53%) blood group non-0. Mean FVIII:C baseline level before desmopressin infusion was 0.09 (SD 0.04 IU/ml) and this increased to 0.42 IU/ml (SD 0.16 IU/ml) at peak level. FVIII:C levels were 0.28 IU/ml (SD 0.09 IU/ml) three hours after desmopressin and 0.22 IU/ml (SD 0.07 IU/ml) six hours after administration. Figure 1 shows the course of FVIII:C before and after desmopressin administration in moderate and mild hemophilia A patients separately.

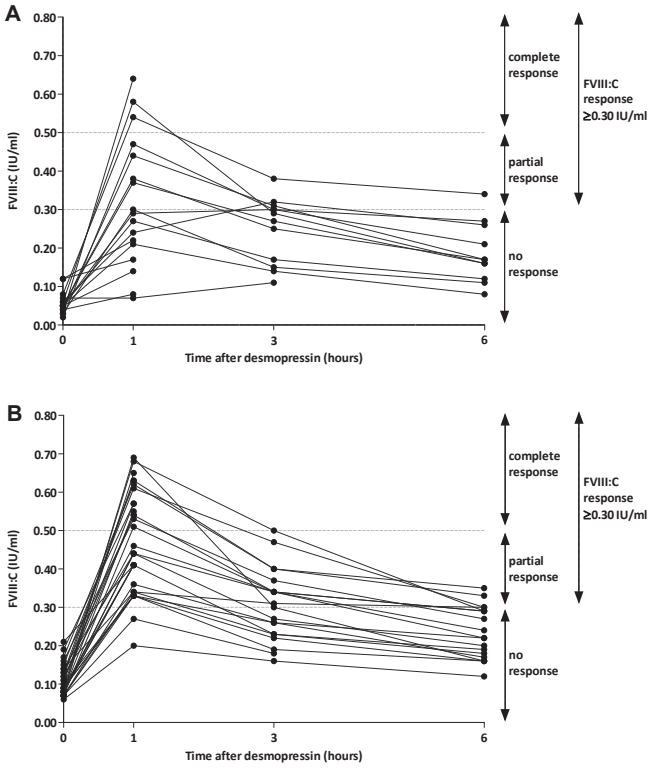

Figure 1. FVIII:C in N=17 moderate (A) and N=31 mild (B) hemophilia A patients with historically lowest FVIII:C levels below 0.10 IU/ml before and after desmopressin administration.
The dashed lines indicate the hemostatic cut-off levels that are considered sufficient for treatment of minor (0.30 IU/ml) or major (0.50 IU/ml) bleeds or surgery.

Desmopressin responses

One hour after desmopressin, 37/48 patients (77%) had an initial response, defined as FVIII:C levels ≥0.30 IU/ml; 17 patients (35%) showed a complete response, defined as FVIII:C levels ≥0.50 IU/ml. Three hours after administration, 16/32 patients (50%) had a sustained response, only one patient (3%) of whom showed a complete response. The majority of patients (83%) did not have a sustained six hour response (Table 1). Almost half of moderate hemophilia A patients (8/17) had an initial response one hour after desmopressin. Three hours after desmopressin, 5/12 patients (42%) still had a sustained response. This was hardly sustained after six hours (1/11 patients; 9%; Table 1).

Table 1: Desmopressin response in hemophilia A patients with historically lowest FVIII:C <0.10 IU/ml

	All N=48	Moderate (FVIII:C ≤0.05 IU/ml) N=17	Mild (FVIII:C 0.06-0.09 IU/ml) N=31
Initial response 1 h			
Response†	37 (77.1)	8 (47)	29 (93.5)
Complete	17 (35.4)	3 (17.6)	14 (45.2)
Partial	20 (41.7)	5 (29.4)	15 (48.3)
No response	11 (22.9)	9 (52.9)	2 (6.5)
Sustained response 3 h*	**N=32**	**N=12**	**N=20**
Response†	16 (50)	5 (41.7)	11 (55)
Complete	1 (3.1)	0 (0)	1 (5)
Partial	15 (46.9)	5 (41.7)	10 (50)
No response	16 (50)	7 (58.3)	9 (45)
6 h*	**N=30**	**N=11**	**N=19**
Response†	5 (16.7)	1 (9.1)	4 (21.1)
Complete	0 (0)	0 (0)	0 (0)
Partial	5 (16.7)	1 (9.1)	4 (21.1)
No response	25 (83.3)	10 (90.9)	15 (78.9)

FVIII:C, Factor VIII coagulation activity; h, hours.
Data are presented as n (%).
*lack of blood sampling at 3 and 6 hours is due to different desmopressin test dose protocols in the past and n=4 patients who had surgery after infusion.
†response, FVIII:C ≥0.30 IU/ml; no response, FVIII:C <0.30 IU/ml; complete response , FVIII:C ≥0.50 IU/ml; partial response, FVIII:C ≥0.30-<0.50 IU/ml.

Potentially modifying factors

We studied potentially modifying factors of desmopressin response: administration route, blood group, age and type of *F8* gene mutation. Administration route did not affect FVIII:C levels after desmopressin infusion (intravenous vs. intranasal: 0.41 IU/ml (SD 0.16 IU/ml) vs. 0.47 IU/ml (SD 0.19 IU/ml)). Nor did blood group; O vs. non-O, 0.39 IU/ml (SD 0.13 IU/ml) vs. 0.44 IU/ml (SD 0.19 IU/ml). At baseline, patients <18 years (N=8) had significantly lower FVIII:C levels than patients ≥18 years (N=40); 0.06 IU/ml (SD 0.03 IU/ml) vs. 0.10 IU/ml (SD 0.04 IU/ml). Because of the selection of patients with historically lowest FVIII:C <0.10 IU/ml there was, as expected, an enrichment of two mutations (c.6505G>A p.Arg2169His and c.446C>G p.Pro149Arg) that were previously identified as having low FVIII levels and a limited desmopressin response.[9] As a result of this enrichment and due to small numbers, no effect of these *F8* gene mutations on FVIII:C levels before and after desmopressin was found.

DISCUSSION

In general, a FVIII:C of ≥0.30 IU/ml is considered adequate for hemostasis in case of minor trauma or surgery.[10,11] Our data indicate that the majority of non-severe hemophilia A patients with historically lowest FVIII:C levels <0.10 IU/ml reaches this level at least up to three hours after desmopressin infusion, but only a few have a sustained response after six hours. It has previously been stated that a FVIII:C level of minimally 0.10 IU/ml is necessary to achieve FVIII:C levels of 0.30-0.50 IU/ml after desmopressin.[5] Our results suggest that this cut-off is too strict and that patients with FVIII:C <0.10 IU/ml can obtain FVIII:C levels after desmopressin within the target range. Even some moderate hemophilia A patients can be treated with desmopressin as 47% achieved FVIII:C levels ≥0.30 IU/ml one hour after desmopressin (Table 1). This is consistent with results reported by De La Fuente et al.[8] Their small study in n=8 moderate hemophilia A patients showed that 38% had FVIII:C peak levels ≥0.30 IU/ml. A recent study by Seary et al reported limited to no response to desmopressin in moderate hemophilia A patients.[12] However, the study was performed in children with a median age of 3.7 years. This suggests that age is a predictor of response as older children had better responses and some re-tested children at older age had improved responses. We did not find an association of age with desmopressin response. This might be because our cohort mainly consisted of adults and the median age of children was 16 years (IQR 14-17).

Many patients (16/48) had higher FVIII:C levels before desmopressin infusion than at measurement of historically lowest FVIII:C level. This might be due to an age-related FVIII increase. However, baseline FVIII:C prior to desmopressin infusion was consistently higher than historically lowest FVIII:C level in all patients, regardless of the time between these measurements, suggesting that age-related FVIII increase did not influence baseline FVIII:C. The higher baseline FVIII:C levels may be caused by stress or by the physiological variation of FVIII.

In conclusion, the majority of patients with historically lowest FVIII:C levels <0.10 IU/ml had a FVIII:C response ≥0.30 IU/ml after desmopressin. Even though the response was short, desmopressin can be considered as a treatment option for small surgery or minor injury in these patients. This may avoid a more costly treatment with FVIII clotting factor concentrates, without loss of quality of care and furthermore will not increase the risk of inhibitor development. It is recommended to perform a desmopressin test dose in all non-severe hemophilia A patients to evaluate its therapeutic use.

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CHAPTER 3

Response to desmopressin is strongly dependent on *F8* gene mutation type in mild and moderate hemophilia A

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SUMMARY

Background: Desmopressin causes 2-6 fold increase of factor VIII (FVIII) in mild or moderate hemophilia A patients. However, responses are variable and little is known whether this is associated with *F8* gene mutation.

Objective: To assess the relationship between *F8* gene mutation and desmopressin response in hemophilia A patients.

Methods: Desmopressin response (*absolute* and *relative*) was determined in 97 hemophilia A patients. Four amino acid changes (Arg2169His, Pro149Arg, Asn637Ser, and Arg612Cys) and a number of other mutations leading to an aberrant FVIII protein or FVIII deficiency were analyzed.

Results: Patients with Arg2169His showed significantly lower FVIII levels before and after desmopressin compared to all other mutations ($p<0.001$). Pro149Arg amino acid change showed significantly lower FVIII levels 1 hour after desmopressin compared to all other mutations ($p<0.005$). An absolute response with FVIII $\geq$0.50 IU/ml after 1 hour was observed in 41% (9 of 22) of patients with Arg2169His; however this was not sustainable after 6 hours in any of these subjects. No patients with Pro149Arg mutation (n=6) showed an absolute response with FVIII $\geq$0.50 IU/ml. Patients with other mutations showed significantly more complete and partial responses. Relative responses did not differ between mutations.

Conclusion: Our study shows that hemophilia A patients with amino acid change Arg2169His or Pro149Arg have a decreased desmopressin response with regard to FVIII levels as compared to other mutations. Our results indicate that response to desmopressin is dependent on the *F8* gene mutation type, despite the fact that multiple factors influence the desmopressin response, even within families.

INTRODUCTION

Hemophilia A is a rare X-linked bleeding disorder caused by a variety of mutations in the *F8* gene resulting in reduced clotting factor VIII (FVIII) levels. The *F8* gene is situated at the distal end of the long arm of the X-chromosome (Xq28).[1,2] FVIII has a domain structure of A1-a1-A2-a2-B-a3-A3-C1-C2.[3] Gene mutations can lead to an aberrant FVIII protein or a deficiency of FVIII resulting in varying clinical severity.[4,5] Mild and moderate hemophilia A are most often caused by missense mutations.[6] Hemophilia A patients can be treated with FVIII clotting factor concentrates or desmopressin (1-deamino-8-D-arginine vasopressin, DDAVP). Desmopressin is a synthetic analogue of the hormone vasopressin that causes release of von Willebrand factor from the Weibel-Palade bodies in the vascular endothelium with a simultaneous rise in FVIII.[7,8] In addition it has a strong antidiuretic effect. In order to prevent bleeding during surgical procedures, FVIII levels should be above 0.50 IU/ml.[9-11] In case of minor surgery, FVIII levels above 0.30 IU/ml suffice.[11-13] The average rise in FVIII and von Willebrand factor after desmopressin infusion is 3 to 5-fold, but varies strongly between individuals. The half-life of released FVIII after desmopressin administration varies from 5 to 8 hours. Because of the great inter-individual variation, desmopressin should be tested in an elective setting in every patient before actual treatment, a so called desmopressin test dose. In general, desmopressin is a less expensive therapy compared to administration of FVIII concentrates and is also safer due to absence of the risk of inhibitor development. Furthermore, there is no risk of viral transmission.[14] Therefore, desmopressin is an important treatment option for patients who are at risk for inhibitor development and especially also for patients with limited resources. Several factors have been reported to be predictive for desmopressin response, such as age and baseline FVIII coagulation activity (FVIII:C).[15-17] Responses in family members are often considered predictive as consistency is seen in family members with regard to desmopressin infusion.[15] However, it is unknown if the mutation in the *F8* gene itself determines this response. Recent studies suggested such an association in mild and moderate hemophilia A patients.[15,18] In the present study we therefore investigated the relationship between type of *F8* gene mutation and desmopressin response in a large group of mild and moderate hemophilia A patients.

MATERIALS AND METHODS

Subjects

Patients with mild or moderate hemophilia A visiting the Hemophilia Treatment Center for adults and children of the Erasmus University Medical Center - Sophia Children's

Hospital in Rotterdam, the Netherlands, were included. Inclusion criteria were male sex, documented *F8* gene mutation, previous intravenous or intranasal administration of desmopressin and the availability of FVIII:C levels at baseline and at peak level after desmopressin infusion. Exclusion criterion was the presence of a FVIII inhibitor at time of desmopressin administration. FVIII:C levels were available in 74 of 97 patients 3 hours after desmopressin and in 69 of 97 patients 6 hours after desmopressin. Patients who received a desmopressin infusion prior to surgery only had FVIII:C measurement before and 1 hour after desmopressin. 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 Center Rotterdam.

Desmopressin administration

Patients received a desmopressin test dose or a desmopressin infusion before surgery with measurement of FVIII levels at baseline and subsequently after desmopressin administration. Desmopressin was administered intravenously (0.3 µg/kg bodyweight) or intranasally (2 sprays of 150 µg; total dose 300 µg). The desmopressin test dose was followed by a maximum of 4 blood samples; before infusion, 1, 3, and 6 hours after desmopressin infusion.

Laboratory methods

Venous whole blood was collected in 0.105 M sodium citrate tubes (Becton, Dickinson and Company (BD) Vacutainer) and centrifuged at 2000 x *g* for 10 min at 4°C. The plasma was centrifuged additionally at 14000 x *g* for 10 min at 4°C and stored at -80°C until analysis. FVIII:C was measured by a one-stage clotting assay, using FVIII-deficient plasma (Siemens Healthcare, Marburg/Germany) and reference plasma (Cryocheck, Precision Biologic, Kordia) on an automated coagulation analyzer (Sysmex CA1500, Siemens, Breda).

Mutation analysis

Sequencing of the complete coding sequence, including exon-intron boundaries, for the *F8* gene was performed at the Department of Clinical Genetics of the Leiden University Medical Center, Academic Medical Center Amsterdam and University Medical Center Utrecht in the Netherlands. When an index patient in the family was known, only the exon of the familial mutation was sequenced, including exon-intron boundaries. In this study, the four mutations that occurred most frequently were grouped, namely c.6505G>A p.Arg2169His, c.1834C>T p.Arg612Cys, c.1910A>G p.Asn637Ser and c.446C>G p.Pro149Arg. Patients with the remaining mutations (in exon 2, 6, 7, 8, 11, 14, 15, 18 and 25) were combined in one group and named "other mutations".

In the above mentioned mutation descriptions, 'c' stands for coding DNA reference sequence and 'p' is the protein reference sequence.[19]

Response criteria

Desmopressin response was expressed as an absolute response and as a relative response, based on definitions described in earlier studies.[15,20] An *absolute* response was defined as complete if FVIII:C increased to ≥0.50 IU/ml, partial if FVIII:C ≥0.30 but <0.50 IU/ml or no response if FVIII:C <0.30 IU/ml. The absolute response was measured 1 hour (initial response), 3 hours (sustained 3 hr response) and 6 hours (sustained 6 hr response) after desmopressin administration. The same response definitions as for the initial response were used for the sustained responses, namely complete, partial and no response. The FVIII:C level determined after 1 hour was considered the FVIII peak level. FVIII relative increase was calculated as FVIII:C peak level divided by FVIII:C baseline level. A *relative* response was defined as complete if a three or more fold increase of FVIII as compared to the baseline FVIII value was calculated, partial if two or more fold but less than three-fold FVIII increase over baseline was demonstrated or as no response if a less than two-fold FVIII increase over baseline was measured. Response was defined as inconsistent when at least one family member had a different response to desmopressin compared to the other family members.

Statistical analysis

Descriptive statistics for continuous variables are presented as mean ± standard deviation in case of normal distribution and as median and 25-75% interquartile range (IQR) in case of non-normal distribution. For comparison of FVIII:C levels between groups the non-parametric Kruskal-Wallis test was used. In case of significant differences the Mann-Whitney U test was performed. For comparison of proportions a Chi-square test was used and Fisher's exact test when necessary. A p-value <0.05 was considered statistically significant. Bonferroni correction for multi-comparison tests was used when testing different mutations by Mann-Whitney U test; a p-value <0.005 was considered statistically significant. Bonferroni correction was also used when testing different *F8* gene mutation domains by Mann-Whitney U test; a p-value <0.003 was considered statistically significant. SPSS version 17.0 (SPSS Inc. Chicago, Illinois, USA) was used.

RESULTS

In the Hemophilia Treatment Center of the Erasmus University Medical Center in Rotterdam, 176 patients are known with mild or moderate hemophilia A of whom

168 received desmopressin in the past. In 99 of these 168 patients, the *F8* gene mutation has been determined. After exclusion of patients with a FVIII inhibitor, 97 patients were eligible and were included in our observational study (Figure 1). Of the 97 patients, 83 patients had mild hemophilia A (FVIII:C baseline 0.19 IU/ml, IQR 0.11-0.29 IU/ml) and 14 had moderate hemophilia A (FVIII:C baseline 0.04 IU/ml, IQR 0.03-0.05 IU/ml). Table 1 shows the patient characteristics. The median age at time of desmopressin administration was 27 years (range 7-72). Ninety patients (93%) received desmopressin intravenously and 7 (7%) intranasally.

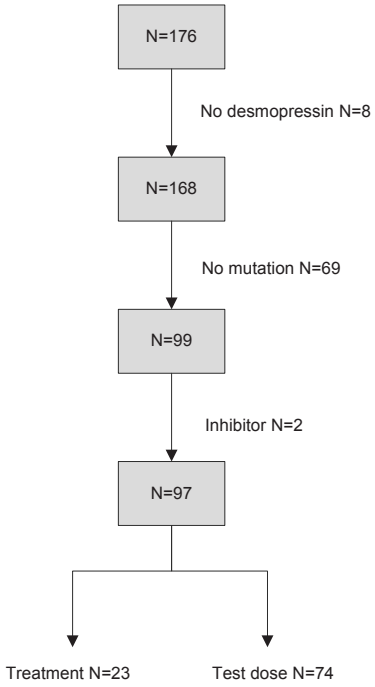

Figure 1. Flowchart of the included patients with mild or moderate hemophilia A.

Initial and sustained absolute response

Twenty-two patients had amino acid change Arg2169His, thirteen Arg612Cys, ten Asn637Ser and six patients Pro149Arg. In the remaining 46 patients, mutations occurred only in one or two patients. In this set of other mutations, there were 24 missense mutations in a variety of exons (exon 2, 6, 7, 8, 11, 14, 15, 18 and 25), one inversion in exon 6 and one type 1 inversion of intron 22. Figure 2A-B shows FVIII:C levels after desmopressin infusion in all patients with amino acid change Arg2169His and Pro149Arg. Of the 22 patients with Arg2169His, 9 (41%) showed a complete initial absolute response. None of these patients sustained in this response after 6

Table 1. Characteristics of the study population

	Patients N=97
Age* (years)	27 (7-72)
BMI†	25.4 ± 4.7
Hemophilia A	
Mild	83 (85.6%)
Moderate	14 (14.4%)
Administration	
Intravenous	90 (92.8%)
Intranasal	7 (7.2%)
Blood group‡	
0	36 (45.6%)
non 0	43 (54.4%)
FVIII:C baseline (IU/ml)	0.17 (0.08-0.27)
range	0.01-0.69
FVIII:C peak (IU/ml)	0.61 (0.41-0.95)
range	0.07-3.20
FVIII:C 3 hours (IU/ml)§	0.49 (0.31-0.77)
range	0.09-1.63
FVIII:C 6 hours (IU/ml)¶	0.38 (0.25-0.61)
range	0.06-1.24
FVIII:C relative increase over baseline**	4.15 (3.19-5.50)
range	1-19

BMI, body mass index; FVIII:C, Factor VIII coagulation activity.
Categorical data are presented as n (%). Summary statistics for continuous variables are presented as mean ± standard deviation in case of normal distribution; median and 25-75% interquartile range when non-normal distribution, except for age: median (range). Range is shown for FVIII:C.
*age at time of desmopressin test dose/administration.
†n=84 based on patients in whom both height and weight were available.
‡n=79 based on patients in whom data were available.
§n=74 based on available FVIII levels 3 hours after desmopressin administration.
¶n=69 based on available FVIII levels 6 hours after desmopressin administration.
** FVIII:C relative increase was calculated as FVIII:C peak level divided by FVIII:C baseline level.

hours. A partial initial absolute response (FVIII:C ≥0.30 but <0.50 IU/ml) was seen in 8 of 22 patients (36%) and in 2 of 14 patients the response sustained. Five of 22 patients (23%) had no response (Table 2). None of the patients with mutation Pro149Arg achieved a complete initial absolute response. Four of 6 patients (67%) had a partial initial absolute response, the response sustained in one patient after 6 hours (Table 2). The responses seen in patients with the other mutations (Asn637Ser, Arg612Cys and the less frequently occurring other mutations) are shown in Table 2 and Figure 2C-E. Of the 46 patients with other mutations, 32 patients (70%) had a complete initial absolute response. The response sustained in 15 of 32 patients

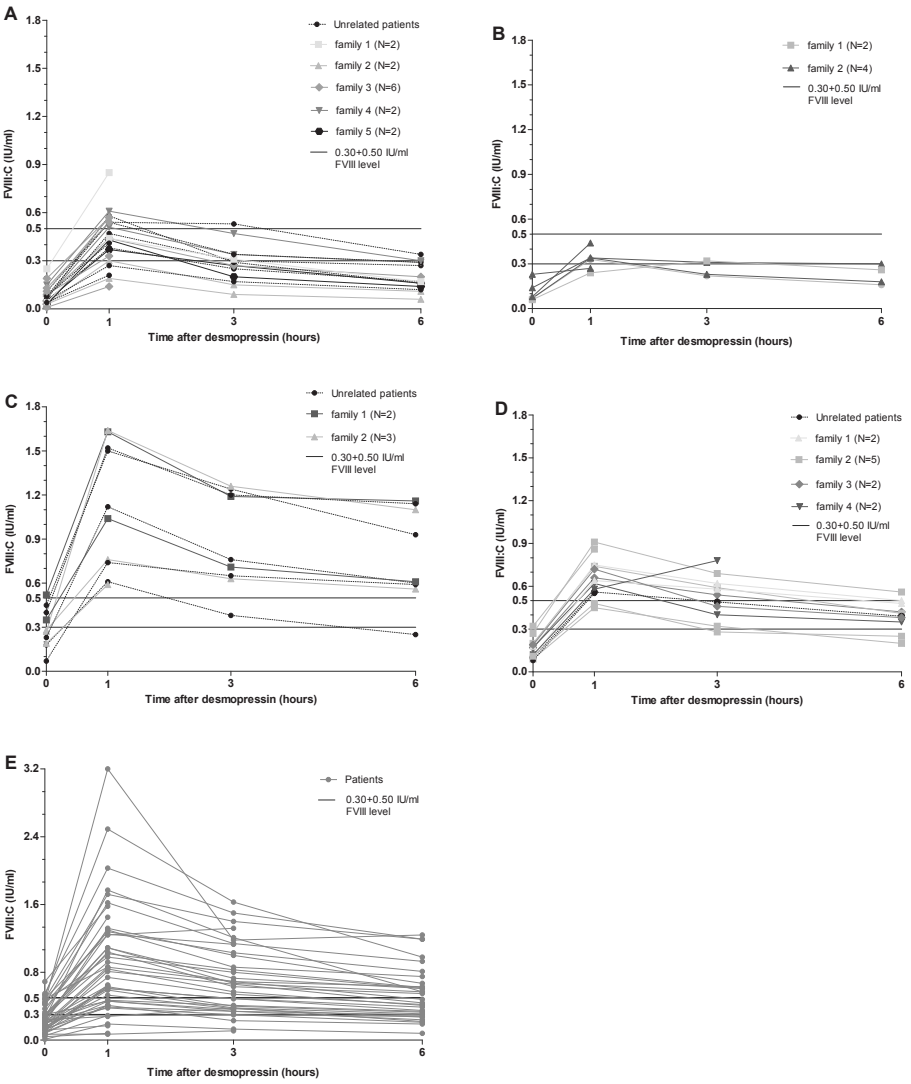

Figure 2. FVIII:C in hemophilia A patients before and after desmopressin.
A) Amino acid change Arg2169His (N=22).
B) Amino acid change Pro149Arg (N=6).
C) Amino acid change Asn637Ser (N=10).
D) Amino acid change Arg612Cys (N=13).
E) Other mutations (N=46).
Related individuals are highlighted.

Table 2. Absolute response to desmopressin in different amino acid changes

Initial response*	Arg2169His	Pro149Arg	Asn637Ser	Arg612Cys	Other	Total
1 h	N=22	N=6	N=10†	N=13†	N=46†	N=97
Complete	9 (40.9)	0 (0.0)	10 (100.0)	11 (84.6)	32 (69.6)	62 (63.9)
Partial	8 (36.4)	4 (66.7)	0 (0.0)	2 (15.4)	8 (17.4)	22 (22.7)
No response	5 (22.7)	2 (33.3)	0 (0.0)	0 (0.0)	6 (13.0)	13 (13.4)
Sustained response*						
3 h	N=14	N=4	N=9‡	N=11	N=36‡	N=74
Complete	1 (7.1)	0 (0.0)	8 (88.9)	7 (63.6)	21 (58.3)	37 (50.0)
Partial	5 (35.7)	2 (50.0)	1 (11.1)	3 (27.3)	12 (33.3)	23 (31.1)
No response	8 (57.1)	2 (50.0)	0 (0.0)	1 (9.1)	3 (8.3)	14 (18.9)
6 h	N=14	N=4	N=9‡	N=10	N=32‡	N=69
Complete	0 (0.0)	0 (0.0)	8 (88.9)	2 (20.0)	15 (46.9)	25 (36.2)
Partial	2 (14.3)	1 (25.0)	0 (0.0)	6 (60.0)	10 (31.3)	19 (27.5)
No response	12 (85.7)	3 (75.0)	1 (11.1)	2 (20.0)	7 (21.9)	25 (36.2)

h, hours.
Data are presented as n (%).
*complete *absolute* response, FVIII:C ≥0.50 IU/ml; partial *absolute* response, FVIII:C ≥0.30 but <0.50 IU/ml; no *absolute* response, FVIII:C <0.30 IU/ml.
† p<0.005 compared to amino acid change Pro149Arg.
‡ p<0.001 compared to amino acid change Arg2169His.

(47%) after 6 hours (Table 2). All patients with amino acid change Asn637Ser and 11 of 13 patients with Arg612Cys had a complete initial absolute response. This response sustained after 6 hours in 8 of 9 patients (86%) with Asn637Ser and in 2 of 10 patients (20%) in patients with Arg612Cys (Table 2). As expected, patients with mild hemophilia A showed more complete absolute responses compared to moderate hemophilia A patients after desmopressin (p<0.001). There was no difference in type of response between patients who had a desmopressin test dose (n=74) and patients who received desmopressin for therapy (n=23). In total, 77% of patients with amino acid change Arg2169His, 67% with Pro149Arg, 100% of patients with Asn637Ser and Arg612Cys and 87% of patients with other mutations had a response to desmopressin (complete and partial initial absolute response). After 6 hours 14% of the patients with Arg2169His, 25% of patients with Pro149Arg, 89% of Asn637Ser, 80% of Arg612Cys and 78% of patients with other mutations still had a response (complete and partial sustained absolute response) (Table 2, Figure 2C-E).

FVIII levels and desmopressin response with regard to mutations

Patients with amino acid change Arg2169His had significantly lower FVIII:C levels at baseline, at peak, 3 hours and 6 hours after infusion compared to patients with other mutations (p<0.001), except for patients with Pro149Arg. Patients with Pro149Arg had significantly lower FVIII:C levels 1 hour after infusion compared to patients with

other mutations (p<0.005), except for patients with Arg2169His (Figure 3). Patients with amino acid change Arg612Cys, Asn637Ser and the set of other mutations did not have significantly different FVIII:C levels at baseline, peak level and 3 hours after desmopressin compared to each other. Six hours after desmopressin infusion, patients with amino acid change Arg612Cys had significantly lower FVIII:C levels compared to Asn637Ser (p=0.003) (Figure 3). Because of the large number of patients with other mutations (n=46) we made a subdivision into 5 groups based on the location of the mutations in the *F8* gene domain. Patients with a mutation in the B-domain had significantly higher FVIII:C levels at peak level compared to patients with mutations in the A2-domain (p=0.001) (data not shown). No significant difference in FVIII:C peak level was found between patients who had desmopressin as test dose and patients who had desmopressin for therapy.

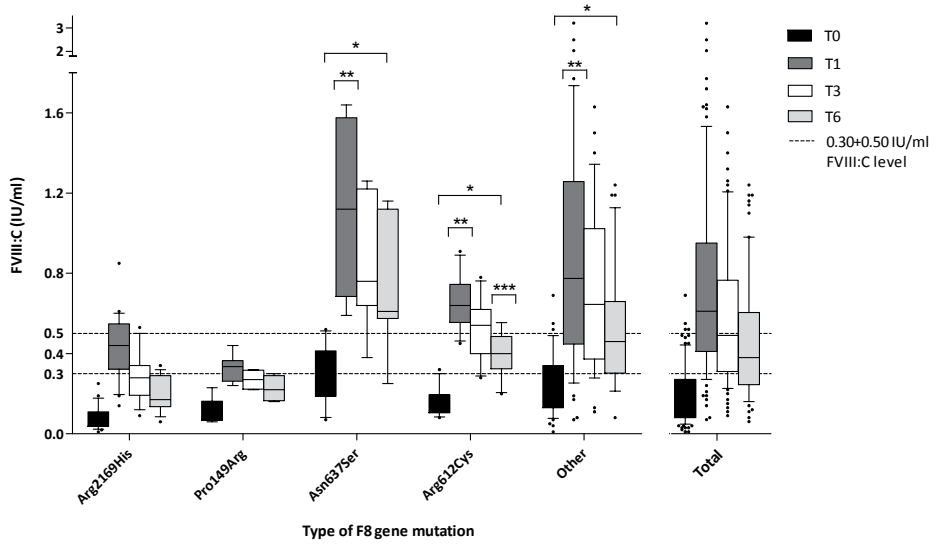

Figure 3. FVIII:C before, 1, 3 and 6 hours after desmopressin in mild and moderate hemophilia A patients with different *F8* gene mutations.

The boxplots indicate the median, 25-75% interquartile range and 10-90% range. Outliers are indicated as •.

*p<0.001 compared to Arg2169His; **p<0.005 compared to Pro149Arg; ***p=0.003 compared to Asn637Ser.

Familial consistency of absolute response

Among the included patients in our study there were several relatives. We investigated the consistency of their absolute responses. In total, there were 20 families consisting of two or more individuals distributed in 12 mutations. Eleven families showed a consis-

tent initial response to desmopressin (9 complete responses, 2 partial responses). These families had the following amino acid changes: Arg2169His, Asn637Ser, Arg612Cys, Thr154Ala, Phe698Ser, Arg717Gln, Arg2178Cys and c.787+6T>C (Supplementary table 1). Nine families had inconsistent initial responses to desmopressin and these families had amino acid changes: Arg2169His, Pro149Arg, Arg612Cys, Val181Met, Arg550Cys and Gly1960Val. Families with Arg2169His and Arg612Cys amino acid change had both consistent and inconsistent responses. In two of the five families with Arg2169His, the initial and sustained response between relatives was similar after desmopressin. Patients in the other three families showed inconsistent responses. Of the three families with amino acid change Arg612Cys, two families had consistent initial responses after desmopressin, whereas one family showed inconsistent initial and sustained responses after desmopressin (Supplementary table 1).

Relative response

Eighty of all 97 patients (83%) showed a complete relative response (three or more fold FVIII increase over baseline). Nine patients (9%) had a partial relative response (two or more fold but less than three-fold FVIII increase over baseline) and another 8 patients (8%) showed no relative response (less than two-fold FVIII increase over baseline). All patients with amino acid change Arg2169His, 67% of the patients with Pro149Arg, 90% of the patients with Asn637Ser, 92% of the patients with Arg612Cys and 72% of the patients with other mutations had a complete relative response. Of the 22 patients with a partial initial absolute response (FVIII:C levels ≥0.30-<0.50 IU/ml), 17 (77%) had a complete relative response. The relative response did not differ between

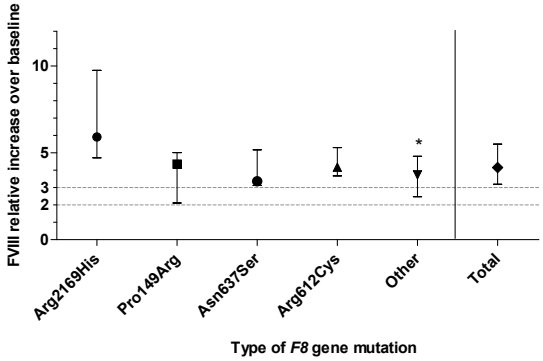

Figure 4. Relative increase over baseline of FVIII:C after desmopressin in different *F8* gene mutations.

Median and 25-75% interquartile range are depicted. The gray lines depict a 2 or 3-fold FVIII increase over baseline.

*p<0.001 compared to Arg2169His.

moderate and mild hemophilia A patients. No difference in relative response was seen between the different mutations (p=0.154) (Figure 4). However, a significantly higher relative increase over baseline was seen in patients with Arg2169His, who had the lowest FVIII:C baseline levels, compared to the set of other mutations (p<0.001). In amino acid change Pro149Arg, Asn637Ser and Arg612Cys, no statistically significant difference was seen in relative increase over baseline (Figure 4).

Potentially influencing factors

We studied factors that might influence the response to desmopressin. No difference was found between the intravenous and intranasal administration routes and the absolute and relative FVIII:C increase in the total study population and for the different mutations (Supplementary table 2). The patients who received desmopressin intranasally were distributed in all groups of mutations. We did not find a statistically significant difference in type of initial response and type of sustained response between patients with blood group 0 and non-0 (data not shown). Blood group 0 was not overrepresented in patients with no response to desmopressin. Patients with no initial response to desmopressin were significantly younger than patients with an initial response, defined as FVIII:C ≥0.30 IU/ml (p=0.035) in the total study population.

DISCUSSION

Desmopressin is often used as treatment for bleeding or prophylaxis prior to surgery in patients with mild hemophilia A. However, the response seen after administration shows a high inter-individual variability. In this study we evaluated both the absolute and relative response to desmopressin in relation to the *F8* gene mutation in 97 mild and moderate hemophilia A patients. We observed that patients with amino acid change Arg2169His had significantly lower FVIII:C levels at baseline, at peak level, 3 hours and 6 hours after desmopressin compared to all other mutations. Patients with Pro149Arg had significantly lower FVIII:C levels at peak level after desmopressin compared to the other mutations. Although desmopressin caused a three to five fold FVIII increase over baseline and although more than 67% of patients had an initial *absolute* response, in most patients with Arg2169His and Pro149Arg this response did not sustain, defined as FVIII:C levels of ≥0.30 IU/ml after 3 and 6 hours. As expected, mild hemophilia A patients showed more complete absolute responses after desmopressin compared to moderate hemophilia A patients.

A possible explanation for the short duration of response in patients with amino acid change Arg2169His may be a reduced binding capacity between von Willebrand factor and FVIII. Jacquemin et al. showed that in patients with a mutation in the C1

domain of the *F8* gene, FVIII has a reduced affinity for von Willebrand factor.[21] They also reported a reduced secretion rate of functional FVIII molecules in patients carrying amino acid change Arg2169His.

The baseline FVIII:C levels we observed in patients with amino acid change Arg2169His and Arg612Cys are similar to FVIII:C levels reported by Castaman et al.[15] However, in their case series amino acid change Arg2169His did not show poorer results compared to other mutations. Novel in our study is the significant lower *sustained* response after 6 hours in patients with this type of mutation compared to others. Moreover, we found that individuals carrying the Arg2169His amino acid change demonstrated the highest FVIII relative increase over baseline compared to the other mutations in our study; this phenomenon was also previously reported by d'Oiron et al.[22] Thus, although FVIII:C baseline levels are low, patients with this mutation have an unexpectedly high FVIII relative increase. Overall, the absolute FVIII:C level after desmopressin administration is more important than the FVIII relative increase, as conditions for surgical interventions are mainly based on this absolute level.[9-13]

Patients with amino acid change Arg2169His had baseline FVIII:C ranging from 0.01 to 0.25 IU/ml. In the HAMSTeRS database Arg2169His is associated with mild to severe hemophilia A, similar to our results.[6] An explanation for this might be an effect of age. We studied this in our patients and found that patients with high FVIII:C levels (0.08-0.25 IU/ml) were significantly older than patients with lower FVIII:C levels (0.01-0.07 IU/ml) (p=0.037), median age respectively 39 years, IQR 32-48 years and 17 years, IQR 16-43 years.

We have studied the difference in FVIII:C levels between patients with amino acid change Arg2169His and Pro149Arg. FVIII:C baseline levels did not differ, but FVIII:C peak levels showed borderline significance (p=0.08) (data not shown). It seems that the type of *F8* gene mutation has an added value to the prediction of response to desmopressin. However, numbers are small and future studies with a larger number of patients carrying the same type of *F8* gene mutation, should be performed.

Castaman et al.[15] showed a consistent response in families with the same mutation and a reproducible similar pattern of response in unrelated patients. In our study, 11 families out of 20 families had a consistent response. However, 9 of them had an inconsistent response (Supplementary table 1). For amino acid change Arg2169His this inconsistency is also reported by Seary et al.[18] As shown in Figure 3 the response to desmopressin in patients with the same mutation was variable in our cohort. This inconsistency in response in patients carrying the same mutation was also found by d'Oiron et al.[22] This variability supports the need for a desmopressin test dose in each individual patient and indicates that not only mutations in the *F8* gene, but multiple factors influence the response to desmopressin.

We have studied potentially influencing factors for the response to desmopressin; e.g. blood group and age. We did not find a difference in type of initial and sustained response (after 3 and 6 hours) between patients with blood group 0 and non-0 (data not shown). However, at 6 hours more patients with blood group non-0 still had a complete or partial response (n=20 versus n=13 blood group 0), although this was borderline significant (p=0.05). Also there was no overrepresentation of blood group 0 in the non-responder group. This might be expected based on the relationship between von Willebrand factor and blood group 0; individuals with blood group 0 have 25% lower levels than non-0 individuals.[23] Patients who did not have an initial response to desmopressin were significantly younger than patients with a response in the total study population (p=0.035). A larger study should be performed to elucidate the relationship between age and type of desmopressin response.

We found that patients with a mutation in the B-domain had significantly higher FVIII:C levels 1 hour after desmopressin compared to the patients with a mutation in the A2 domain. The mutations in our patients arising from the B-domain are all, except for one (unclassified variant), associated with a mild phenotype according to the HAMSTeRS database.[6] This might be an explanation why the FVIII:C levels in these patients were significantly higher.

The overall prevalence of inhibitors after treatment with FVIII concentrate is 5-7% in an unselected population of mild/moderate hemophilia patients.[24] Missense mutations that result in changes in the A2 domain and in the junction of the C1 and C2 domain of the *F8* gene (e.g. Arg612Cys, Asn637Ser and Arg2169His in our population) have a four-fold higher risk of inhibitor development after intensive treatment with FVIII clotting factor concentrates, compared to mutations in other regions.[25-27] Because of this higher inhibitor risk desmopressin should be considered as a treatment option, not only to prevent inhibitor development, but also when an inhibitor is present that is only directed towards exogenous FVIII.

Novel in our study is that we had a large study population and hence more patients with the same type of *F8* gene mutation than has previously been reported.[15,18] This enables us to draw better conclusions about the relation between *F8* gene mutation and the response to desmopressin. Besides, the mutations studied in our cohort were in part different from previous studies.[15,18] Most importantly, in the present study data on both pre-desmopressin and peak levels of all 97 patients as well as 6 hour FVIII:C levels in the majority (71%) of the patients were available. The 6 hour *sustained absolute* response is particularly important to predict whether the desmopressin effect is sufficient for surgical intervention. A drawback of our study is that the number of patients in our cohort was relatively small when subdivided into different groups for comparison between mutation types. Therefore, further research is needed with more patients carrying the same mutation. Another limitation is that we have included

7 patients with intranasal administration of desmopressin. Previously, it has been reported that these patients may have a reduced response compared to intravenous administration.[28] However, in our study we did not observe a difference between intravenous and intranasal administration (Supplementary table 2). Even after excluding patients who had intranasal administration of desmopressin, the results remained similar (data not shown).

In conclusion, we have shown that compared to other mutations, hemophilia A patients with amino acid change Arg2169His or Pro149Arg have a reduced initial and most importantly, a reduced sustained response to desmopressin. This indicates that the FVIII response to desmopressin in mild and moderate hemophilia A is dependent on the underlying mutation type in the *F8* gene.

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Supplemental table 1. Consistency of absolute responses in related patients

	Age	FVIII:C (IU/ml)	FVIII:C (IU/ml)	Type of response	FVIII:C (IU/ml)	Type of response	FVIII:C (IU/ml)	Type of response
	(years)	baseline	peak	peak*	3 hours	3 hours*	6 hours	6 hours*
ARG2169HIS								
Fam.1	44	0,09	0,33	PR	-	-	-	-
	67	0,19	0,57	CR	-	-	-	-
	57	0,08	0,44	PR	0,27	NR	0,20	NR
	29	0,01	0,14	NR	-	-	-	-
	35	0,11	0,57	CR	-	-	-	-
	34	0,13	0,54	CR	-	-	-	-
Fam.2	17	0,03	0,30	PR	0,15	NR	0,11	NR
	16	0,03	0,19	NR	0,09	NR†	0,16	NR†
Fam.3	41	0,25	0,85	CR	-	-	-	-
	17	0,04	0,44	PR	0,31	PR	0,17	NR
Fam.4	52	0,15	0,61	CR	0,47	PR	0,30	PR
	63	0,07	0,51	CR†	0,34	PR†	0,29	NR
Fam.5	49	0,03	0,43	PR	0,20	NR	0,14	NR
	47	0,05	0,37	PR†	0,27	NR†	0,16	NR†
PRO149ARG								
Fam.6	67	0,14	0,34	PR	0,31	PR	0,30	PR
	14	0,07	0,34	PR	0,23	NR	0,18	NR
	15	0,23	0,27	NR	-	-	-	-
	19	0,08	0,44	PR	-	-	-	-
Fam.7	21	0,07	0,33	PR	0,22	NR	0,16	NR
	23	0,06	0,24	NR	0,32	PR	0,26	NR†
ASN637SER								
Fam.8	72	0,52	1,63	CR	1,19	CR	1,16	CR
	9	0,35	1,04	CR†	0,71	CR†	0,61	CR†

Supplemental table 1. Consistency of absolute responses in related patients (continued)

	Age	FVIII:C (IU/ml)	FVIII:C (IU/ml)	Type of response	FVIII:C (IU/ml)	Type of response	FVIII:C (IU/ml)	Type of response
	(years)	baseline	peak	peak*	3 hours	3 hours*	6 hours	6 hours*
Fam.9	28	0,27	1,64	CR	1,26	CR	1,10	CR
	41	0,28	0,76	CR	0,63	CR†	0,56	CR†
	16	0,19	0,59	CR†	-	-	-	-
ARG612CYS								
Fam.10	56	0,17	0,66	CR	0,54	CR	0,42	PR
	7	0,19	0,72	CR†	0,46	PR	0,38	PR†
Fam.11	20	0,09	0,45	PR	0,32	PR	0,20	NR
	17	0,20	0,74	CR	0,59	CR	0,41	PR
	39	0,27	0,91	CR	0,69	CR	0,56	CR
	55	0,32	0,86	CR	-	-	-	-
	14	0,11	0,48	PR	0,28	NR	0,25	NR
Fam.12	23	0,17	0,62	CR	0,40	PR	0,35	PR
	43	0,12	0,58	CR†	0,78	CR	-	-
THR154ALA								
Fam.13	31	0,13	0,81	CR	0,66	CR	0,44	PR
	14	0,25	1,04	CR†	0,63	CR†	0,55	CR
VAL181MET								
Fam.14	28	0,16	0,53	CR	0,37	PR	0,27	NR
	25	0,12	0,46	PR	0,34	PR†	0,24	NR†
ARG550CYS								
Fam.15	53	0,49	0,86	CR	-	-	-	-
	17	0,13	0,39	PR	0,34	PR	0,30	PR
	53	0,12	0,63	CR	-	-	-	-
PHE698SER								
Fam.16	27	0,29	0,92	CR	0,69	CR	0,63	CR
	36	0,19	0,61	CR†	0,49	PR	0,39	PR

Supplemental table 1. Consistency of absolute responses in related patients (continued)

	Age	FVIII:C (IU/ml)	FVIII:C (IU/ml)	Type of response	FVIII:C (IU/ml)	Type of response	FVIII:C (IU/ml)	Type of response
	(years)	baseline	peak	peak*	3 hours	3 hours*	6 hours	6 hours*
ARG717GLN								
Fam.17	20	0,52	2,49	CR	1,63	CR	0,98	CR
	17	0,55	2,03	CR†	1,50	CR†	1,19	CR†
GLY1960VAL								
Fam.18	18	0,08	0,41	PR	-	-	-	-
	19	0,05	0,29	NR	0,30	PR	0,21	NR
	35	0,24	0,28	NR	0,38	PR†	0,22	NR†
	24	0,21	0,41	PR	-	-	-	-
ARG2178CYS								
Fam.19	47	0,18	0,84	CR	0,57	CR	0,41	PR
	33	0,20	0,86	CR	0,69	CR	0,48	PR
	14	0,13	0,59	CR†	0,38	PR	0,31	PR†
c.787+6T>C								
Fam.20	28	0,21	0,47	PR	0,41	PR	0,34	PR
	14	0,20	0,38	PR†	0,30	PR†	0,28	NR

Fam, family; -, unknown.

*NR, no response (FVIII <0.30 IU/ml); PR, partial response (FVIII ≥0.30 IU/ml but <0.50 IU/ml); CR, complete response (FVIII ≥0.50 IU/ml).

†consistent response in family.

Supplemental table 2. Route of administration and FVIII:C levels in the study population

	Intravenous route	Intranasal route	
	N=90	N=7	P-value
FVIII:C baseline (IU/ml)	0.18 (0.08-0.27)	0.13 (0.08-0.19)	0.399
FVIII:C peak (IU/ml)	0.61 (0.41-1.03)	0.57 (0.44-0.65)	0.427
FVIII:C 3 hours (IU/ml)*	0.49 (0.31-0.77)	0.66	0.590
FVIII:C 6 hours (IU/ml)†	0.37 (0.27-0.61)	0.44	0.725
FVIII:C relative increase over baseline	4.14 (3.29-5.45)	4.45 (3.00-6.23)	0.900

FVIII:C, Factor VIII coagulation activity.
Data are presented as median (25-75% interquartile range).
*n=74 (n=73 intravenous, n=1 intranasal).
†n=69 (n=68 intravenous, n=1 intranasal).

CHAPTER 4

Side effects of desmopressin in patients with bleeding disorders

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SUMMARY

Introduction: Desmopressin is frequently used in patients with bleeding disorders because of its pro-hemostatic effects. In recent years desmopressin use increased due to reported high incidence of inhibitors in mild hemophilia after clotting factor infusion and the rising costs of clotting factor concentrates. The safety and frequency of side effects has hardly been assessed in well-designed studies.

Aim: We therefore prospectively evaluated side effects of desmopressin in a large unselected cohort of bleeding disorder patients, who received a desmopressin test-dose.

Methods: Blood was drawn prior to, one, three, six and 24 hours after desmopressin. Primary outcome was change in serum sodium, hematocrit, serum- and urine osmolality, body weight and vital signs. Self-reported side effects were evaluated as secondary outcome.

Results: In total 108 patients were included, median age 30 years, the majority of whom had von Willebrand disease type 1 (76%). A significant change in water balance parameters was observed. Four patients (4%) had hyponatremia (≤135mmol/L) after 24 hours but no severe hyponatremia occurred (≤125mmol/L). After infusion, 41 (38%) patients were hypotensive (≤90mmHg SBP and/or ≤60mmHg DBP) and 10 (9%) presented with tachycardia (>100/min). However, none of these effects sustained at 24 hours. Infusion was discontinued in one patient because of tachycardia, nausea and malaise. Self-reported side effects included: headache, fatigue, flush and dizziness.

Conclusion: Observed side effects correspond with the known antidiuretic and vasomotor effects of desmopressin. Changes in parameters were temporary and not clinically relevant. In conclusion, our study supports desmopressin use as a safe treatment option in patients with various bleeding disorders.

INTRODUCTION

Desmopressin is widely used as a pro-hemostatic agent in patients with bleeding disorders as it releases von Willebrand factor (VWF) from the Weibel-Palade bodies in the endothelium and causes a subsequent rise in Factor VIII (FVIII).[1] In addition it improves platelet function.[2] Since the use of desmopressin many reports have been published focusing on its effect and sporadically on its side effects.[3-7] However, it is important to be well informed of its safety. Especially as its use is rising, e.g. in the surgical setting to specifically lower transfusional needs[8] and is expected to increase even further now the World Federation of Hemophilia recently applied to have desmopressin added to the World Health Organization's Essential Medicines list. Besides, a recent study showed a high incidence of neutralizing antibodies in mild hemophilia after supplementation with FVIII concentrates[9], and, in general, clotting factor concentrates are restrictedly available and result in high costs of for society.

Desmopressin selectively stimulates the vasopressin-2 receptors, hence causing a transient decrease in blood pressure and an antidiuretic effect.[10] As a result, reported side effects are headache, flushing, nausea.[4,5,11] Multiple case-reports have been published describing severe side effects in patients with bleeding disorders receiving desmopressin, giving the impression that desmopressin use often results in potential dangerous situations.[12-22] Despite the fact that this may occasionally occur, underlying factors other than desmopressin may be responsible. Until now, large studies primarily aimed to assess side effects have not been performed, and side effects have only been reported in small selected population.[3,4,11,23,24] Desmopressin can be administered intravenously, intranasally or subcutaneously. It is likely that vasoactive side effects occur more often after intravenous administration.[25] In the Netherlands, desmopressin is only administered via the intravenous or intranasal route, suggesting that our patients may experience more side effects. Therefore, we aimed to prospectively evaluate side effects of desmopressin in a large cohort of patients with bleeding disorders. The primary aim of the study was to measure objective parameters associated with the side effects of desmopressin. Secondary aim was to evaluate self-reported side effects.

MATERIALS AND METHODS

Patients

Patients with a bleeding disorder who were planned to undergo a desmopressin test-dose at the outpatient department of the Erasmus University Medical Centre-Sophia Children's Hospital, Rotterdam, the Netherlands, were eligible for the study. Between March 2011 and August 2013 all consecutive patients were included after written

informed consent. The study was not subject to the Medical Research Involving Human Subjects Act (WMO) and was approved by the Medical Ethics Committee of the Erasmus University Medical Centre Rotterdam.

Outcome

The primary outcome was change in laboratory values and vital parameters after desmopressin administration; serum sodium (mmol/L), hematocrit (L/L), serum- and urine osmolality (mOsm/kg), body weight (kg) and vital signs (i.e. blood pressure and heart rate). Self-reported side effects were our secondary outcome.

Desmopressin test-dose protocol

Desmopressin was administered intravenously as a single bolus of 0.3 µg/kg or intranasally as 300 µg (150 µg in each nostril). In adults blood was sampled prior to (T0), and one (T1), three (T3), six (T6) and 24 hours (T24) after desmopressin infusion. In children no blood was sampled 24 hours after desmopressin and the final follow-up blood sample was 6 hours. Each patient was instructed to drink a maximum of 1.5 liters for 24 hours after desmopressin, starting after the first urination after infusion.

Laboratory methods

Sodium was measured with the Ion-Selective Electrode method on the COBAS8000 (Roche Diagnostics, Almere, the Netherlands). Hematocrit was measured on an XN-analyzer (Sysmex) by means of the Direct Current Sheat-flow method in the RBC-channel. Osmolality in serum and urine was determined via freezing point depression osmometry on the Advanced osmometer 3320 (Hettich Instrumenten, Geldermalsen, the Netherlands).

Data collection

Sodium, hematocrit, serum- and urine osmolality were determined at T0, T6 and T24 except in children treated in the Sophia Children's hospital (n=11) as no extra blood was sampled for this purpose. Body weight was assessed at T0, T6 and T24 with the same scale. At all blood sampling time points blood pressure (BP, systolic SBP, diastolic DBP) and heart rate (HR) were measured. Additionally, patients were asked for possible side effects at all blood sampling moments; headache, flush, itching eyes, nausea, stomach-ache, tremor, fatigue, perspiration, dizziness. In case of headache or stomach-ache, patients not only confirmed whether they had an ache, but also assigned a number to the severity based on the 0-10 numeric pain rating scale (NRS).[26] The use of co-medication was assessed by the investigator.

Statistical analysis

Continuous variables are shown as mean ± standard deviation when normally distributed or as median [25-75% interquartile range, IQR] when skewed. Categorical variables are displayed as frequencies. Repeated-measures ANOVA with polynomial contrast was used to calculate statistical significance with Bonferroni correction for 95% confidence interval adjustment. When assumption of sphericity was violated the Greenhouse-Geisser correction was used. Comparisons between groups were performed using an Independent Samples T-test or Chi-square test. Vital signs were stratified for age (<18 and ≥18 years). SPSS version 21.0 (IBM Corp. Armonk, NY, USA) was used.

RESULTS

Study population

In total 108 patients were included with a median age of 30 years (range 5-68). The majority had type 1 von Willebrand disease (VWD) (82, 76%) or hemophilia A (16, 15%) (Table 1). The mean body weight was 72.8 ± 19.9kg and the total dose

Table 1. Baseline characteristics study population

	Patients (N=108)
Age (years)	29.50 (5-68)
Sex	
Male	41 (38%)
Female	67 (62%)
Body weight (kg)	72.80 (19.92)
Blood group O*	65 (66%)
Total dose desmopressin (µg)	21.37 (5.89)
Tilt during infusion	22 (20%)
Vital signs baseline	
SBP (mmHg)	122 (16)
DBP (mmHg)	74 (11)
Heart rate (/min)	76 (14)
Diagnosis	
VWD 1	82 (76%)
VWD 2†	2 (2%)
Hemophilia A	16 (15%)
Carrier hemophilia A	8 (7%)

SBP, systolic blood pressure; DBP, diastolic blood pressure; VWD, von Willebrand disease.
Data are presented as mean (standard deviation) or n (%), except for age: median (range).
In all but one patient desmopressin was intravenously administered.
*n=9 missing.
†VWD2A and VWD2N.

infused desmopressin was 21.4 ± 5.9 µg, indicating that patients were correctly dosed upon body weight with 0.3 µg/kg. In 70 patients (66%) fluid intake was 1.5L as was instructed (mean 1.0 ± 0.4L), but 36 (34%) drank >1.5L (mean 2.0 ± 0.4L). First urination after infusion was after a median of 90 minutes (IQR 70-165 min). None of the patients was known with cardiovascular disease. Forty-nine patients (45%) used co-medication of which the most frequently occurring were: antihypertensive drugs (11, 10%), antidepressant medication (10, 9%), oral contraceptives (9, 8%), respiratory inhalation medication (7, 6%) and antihistamines (6, 6%). Nineteen patients used a combination of drugs. All patients with antihypertensive medication except for one, continued their medication the morning of the test-dose.

Objective parameters

In adults blood pressure (BP) significantly decreased by 10 ± 11 mmHg (SBP) and 11 ± 9 mmHg (DBP) during infusion and remained lower at T24, 6 ± 11 mmHg and 2 ± 9 mmHg compared to T0. Heart rate (HR) significantly increased 7 ± 8/min after infusion but recovered at T1 (Figure 1). In children DBP but not SBP significantly decreased after infusion but recovered to baseline at T6. HR significantly increased 7 ± 7/min during infusion in children and recovered at T1 (Figure 1). Immediately after infusion 41 patients (30 adults, 11 children) (38%) were hypotensive (≤90 mmHg SBP and/or ≤60 mmHg DBP) and 10 (7 adults, 3 children) (9%) had tachycardia (HR>100/min). Children had significantly more hypotension after infusion than adults (11/16, 69% vs. 30/92, 33%, p=0.01). However, their BP was also significantly lower at T0 (111 ± 16/63 ± 9 mmHg vs. 124 ± 16/76 ± 11 mmHg, p<0.01). As expected sodium, hematocrit and serum osmolality significantly decreased after desmopressin, whereas urine osmolality increased (Table 2). None of these parameters exceeded reference values except for sodium: ≤135 mmol/L in 4 patients at T24. There were no patients with severe hyponatremia (≤125 mmol/L). We stratified vital signs and body weight for age: children <18 years (N=16) and adults ≥18 years (N=92). Body weight did not significantly change during 24 hours in adults: 76.7 ± 16.9 kg, 77.1 ± 17.0 kg and 77.3 ± 16.8 kg at T0, T6 and T24 respectively (p=0.06). In children body weight was significantly increased at T6: 50.3 ± 22.4 kg versus 51.1 ± 22.5 kg (p=0.01), but not clinically relevant.

Potential influencing factors

In the 36 patients (33%) who exceeded the advised fluid restriction of 1.5L (2.0 ± 0.4L), sodium at T24 was similar compared to patients in whom fluid intake was <1.5L (140.1 ± 3.1 mmol/l vs. 140.4 ± 1.9 mmol/L, p=0.74).

Water balance parameters, e.g. sodium, hematocrit and osmolality, were all significantly higher at baseline in males (sex appropriate for hematocrit). However, the

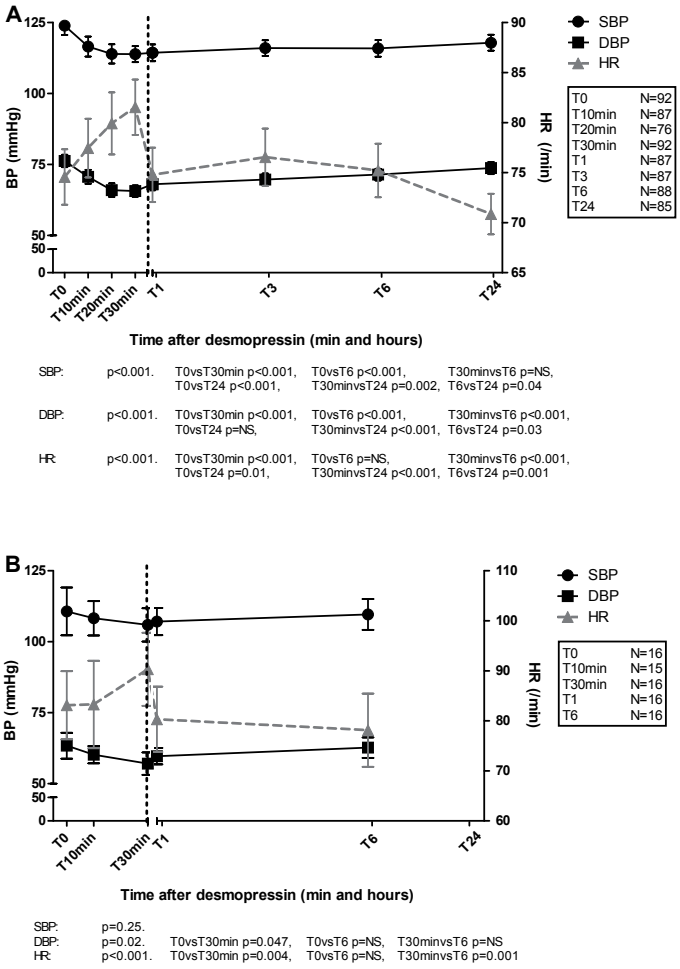

Figure 1. Change in vital signs after desmopressin infusion in N=92 adults (A) and N=16 children (B).

Mean and 95% confidence intervals are shown. BP, blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate.

In children (B), T20min and T3 are not depicted as the number of patients in whom these measurements were performed was <n=10. However, the mean and 95% CI of these measurements were within the course of the shown parameters.

change (decrease in sodium, hematocrit, serum osmolality, increase in urine osmolality) did not statistically differ with sex, except for serum osmolality: between T0-T24 serum osmolality increased 0.27 ± 5.11 mOsm/kg in males and decreased 3.44 ± 5.12 mOsm/kg in females, p<0.01. All 4 cases of hyponatremia (≤135mmol/L) were in female patients (4%). The change in vital signs was similar in both sexes.

Table 2. Desmopressin effect on water balance

	Sodium† (mmol/L)	Hematocrit‡ (L/L)	Serum osmolality§ (mOsm/kg)	Urine osmolality¶ (mOsm/kg)
Reference values	136-145	0.40-0.50 (M) 0.36-0.46 (F)	275-300	50-1200*
T0	141.26 (1.70)	0.40 (0.03)	284.79 (4.62)	657.44 (276.42)
T6	141.61 (1.75)	0.37 (0.03)	286.90 (6.51)	934.40 (139.85)
Sodium ≤125 (T6)	0 (0)			
Sodium ≤135 (T6)	0 (0)			
T24	140.28 (2.42)	0.38 (0.04)	282.78 (6.03)	857.93 (195.95)
Sodium ≤125 (T24)	0 (0)			
Sodium ≤135 (T24)	4 (4)			

T0, before desmopressin; T6, 6 hours after desmopressin; T24, 24 hours after desmopressin; M, male; F, female.

Data are presented as mean (standard deviation) or n (%); reference values are from the lab in the Erasmus University Medical Center.

*normal reference range for urine osmolality depends on fluid intake.

†valid N=90, p<0.001 → T0vsT6 p=0.148, T0vsT24 p<0.001, T6vsT24 p<0.001.

‡valid N=89, p<0.001 → T0vsT6 p<0.001, T0vsT24 p<0.001, T6vsT24 p=0.001.

§valid N=91, p<0.001 → T0vsT6 p=0.001, T0vsT24 p=0.002, T6vsT24 p<0.001.

¶ valid N=86, p<0.001 → T0vsT6 p<0.001, T0vsT24 p<0.001, T6vsT24 p=0.001.

During infusion, 22 patients (20%) went from an upright to supine position because of self-reported dizziness or low BP (on average after 14 minutes). Infusion was interrupted in one patient because of this and prematurely discontinued in another patient due to persistent tachycardia (HR 137/min), nausea and malaise. In 99% of patients desmopressin infusion was eventually completed. Vital signs after desmopressin infusion were similar between non-tilted and tilted patients, and hypotension did not occur more frequently in the latter group.

Eleven patients (10%) used antihypertensive drugs. As expected they had higher BP at T0 (140 ± 17/86 ± 13 mmHg vs. 120 ± 15/73 ± 10 mmHg, p<0.01), were older (50 years [range 20-68] vs. 28 years [range 5-65], p<0.01) and had higher body weight (88.2 ± 20.6 kg vs. 71.1 ± 19.2 kg, p<0.01). There was no difference in water balance parameters compared to patients without antihypertensive treatment. Vital signs were significantly higher at all times, but the change (decrease in BP, increase in HR) did not differ between antihypertensive medication and no antihypertensive medication. Ten patients (9%) used antidepressant medication. They had higher BP prior to and after infusion compared to patients without (T0 133 ± 18/84 ± 12 mmHg vs. 121 ± 16/73 ± 11 mmHg and T24 128 ± 15/82 ± 12 mmHg vs. 116 ± 12/72 ± 9 mmHg, p<0.01). Consequently, they showed less hypotension after infusion (1/10 (10%) vs. 40/98 (41%)), although not statistically significant (p=0.09). Water balance parameters were comparable.

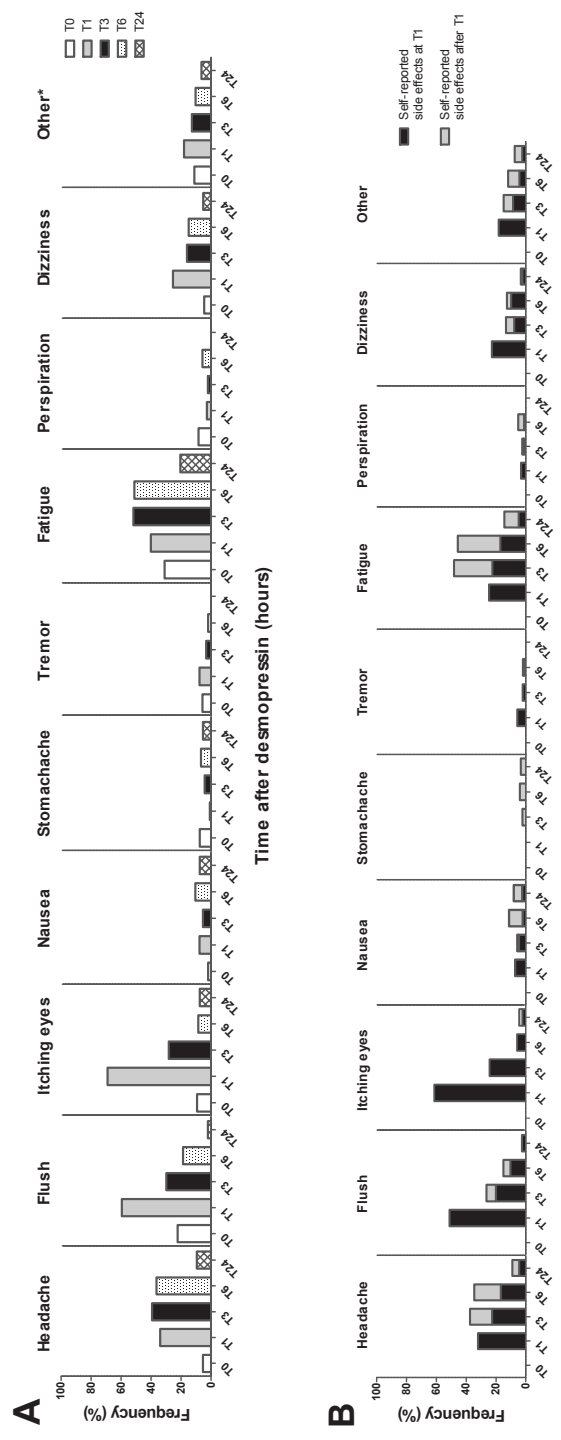

Figure 2. Self-reported side effects after desmopressin administration in the total cohort (A) and after exclusion of self-reported side effects at T0 (B), highlighting patients in black who reported side effects after one hour (T1) and the course of their self-reported side effects.
*Other consisted of: being cold, palpitations, dry mouth, diarrhea, burning sensation while urinating, thirst, cramps in calves, being warm, blurry vision, chest pain.

Self-reported side effects

Most self-reported side effects were headache, fatigue, flush, itching eyes and dizziness that generally subsided after 24 hours (Figure 2A). At T0, 71 patients (66%) reported some kind of discomfort. Symptoms were not related to the bleeding disorder, such as urinary infection, laryngitis, (menstruation-related) back pain, kidney stone, muscle ache or having a cold. The total number of patients with self-reported side effects after infusion was 103 (97%), 76 (81%), 79 (74%) and 45 (48%) at T1, T3, T6 and T24, respectively. Figure 2B shows all patients who did not report complaints at T0 and highlights those with self-reported side effects at T1 and the course of those side effects (in black). Thirty-two patients (32%) without complaints at T0 reported headache at T1 with a median score of 3 (IQR 2-4) on the 0-10 numeric pain rating scale, and a score of 2 (IQR 0-5), 2 (IQR 0-5) and 0 (0), at T3, T6 and T24, respectively (Friedman ANOVA, $p<0.001$). Patients tilted during infusion reported more dizziness at T1 and more headache and flush at T3 ($p<0.03$) compared to non-tilted patients.

Association self-reported side effects and objective parameters

Sodium levels at T6 and T24 were not associated with duration of antidiuresis (i.e. time to first urination). Patients with hyponatremia (≤135mmol/L) (n=4) at T24 reported more nausea than patients with normal sodium levels (50% vs. 6%, p=0.03). Conversely, sodium levels were lower in patients who reported nausea after desmopressin ($p<0.03$). Patients with hypotension after infusion (n=41) reported less flush at T1 and T3 (38% and 10% vs. 64% and 38%, $p<0.02$) compared to patients without hypotension. Patients with tachycardia after infusion (n=10) reported more dizziness at T3 (50% vs. 11%, p=0.03).

DISCUSSION

Desmopressin is frequently used as pro-hemostatic agent in patients with bleeding disorders to treat or prevent bleeding. It is an inexpensive drug without risk of development of inhibitory antibodies or transmission of viral infections and is considered to be a safe treatment option with mostly mild side effects. Our current knowledge on the safety of desmopressin is predominantly based on case reports, small studies or on selected patient populations. Therefore we conducted a large prospective study aiming primarily at changes of objective laboratory and vital signs and substantiated this with self-reported side effects.

Desmopressin was well tolerated in over 100 patients and in all but one patient desmopressin infusion was completed. We observed a significant change in water balance parameters, a decrease in blood pressure and an increase in heart rate after desmopressin. These changes were only temporary and correspond with the

antidiuretic and vasomotor effects of the drug and are in line with what has previously been reported.[25,27] Our results suggest that hyponatremia is rare and that after 24 hours, desmopressin may be administered safely again. However, when administering after only 12 hours one should be aware of the late onset of hyponatremia: time to reach the lowest sodium concentration after desmopressin intravenously is 30 hours (range 6-48).[3] Indeed in our population none had hyponatremia after 6 hours but four did experience mild hyponatremia (≤135 mmol/L) after 24 hours, none of whom were severely affected (<125 mmol/L). Notably, previous studies demonstrated that most symptoms of low sodium levels, such as nausea and seizures, occur when levels fall below 120 mmol/L.[28] Therefore, we advise measurement of serum osmolality or sodium concentration when repeated doses are administered.[29] In our study, patients who reported nausea had lower sodium levels compared to non-nauseous patients.

Other studies and case-reports reporting hyponatremia after desmopressin were performed mostly in young children or in cases where fluid intake was not controlled.[21,22] The youngest patient in our study was 5 years old and all patients were carefully instructed with respect to fluid intake. Despite this instruction we observed that 34% of patients drank more than 1.5L. These patients did not differ with regard to laboratory values and vital signs or self-reported side effects when compared to patients who drank <1.5L. Lethagen and colleagues have used fluid restriction up to 2L without severe side effects being reported.[4] Patients tilted during infusion because of low RR or self-reported dizziness, showed similar vital signs and water balance parameters after desmopressin to non-tilted patients, suggesting that tilting is not indicative of hypotension or hyponatremia after desmopressin.

A relatively high number of patients reported other side effects, often associated with pre-existing complaints prior to infusion. This is a phenomenon also shown by Miesbach et al.[4] Therefore, we focused on patients without complaints at T0 to study the incidence of desmopressin-related side effects. In general, self-reported side effects at T1 subsided during the subsequent 24 hours. The incidence remained high for several complaints (i.e. headache, flush and fatigue) and for some the highest frequency of occurrence was at T3, but these were considered mild side effects and were not related to hyponatremia. It is suggested that side effects are most pronounced with intravenous administration.[25] Indeed frequencies in our population were higher than what is reported with subcutaneous[30] (30% flush) or intranasal[31] (3% flush, 4% headache, 2% dizziness) administration and are in line with findings by Miesbach et al. who also specifically studied side effects of intravenously administered desmopressin.[4] Based upon the severity classification of side effects by Leissinger et al.[25] there were no life-threatening events (i.e. thrombotic complications) in our population. Thrombotic complications after desmopressin treatment have been described.[13,17-20] It can be assumed that patients with cardiovascular risk factors are more prone to develop such

an event. Patients in our study had no previous cardiovascular disease and were not likely to experience thrombotic events. We consider cardiovascular disease as contra indicator for its use.

Simultaneous use of desmopressin and drugs that disturb secretion of the antidiuretic hormone may increase the risk of water intoxication.[32] In our cohort ten patients (9%) used tricyclic antidepressants and selective serotonin reuptake inhibitors at time of desmopressin infusion but did not have lower sodium levels compared to patients without antidepressant therapy (data not shown). Sex showed to be of influence on serum sodium levels as women had significantly lower sodium levels at all blood sampling times and consequently more hyponatremia. A sex-specific effect on sodium concentrations has been reported[33] and also a gender difference in antidiuretic response to desmopressin.[34] Nonetheless, the magnitude of the decrease in sodium levels was similar between sexes.

A recent study investigated the effect of a subcutaneous capped dose of desmopressin (15μg) in patients with bleeding disorders.[35] It showed to be as effective as dosing based upon 0.3μg/kg. Some suggest a maximum dose of 20μg intravenously.[7,36] In our study we did not use a capped dose not even in patients with BMI≥30 (mean dose 29 ± 4μg). These patients (n=15) did not experience more change of laboratory values, vital signs, clinically relevant side effects (i.e. hyponatremia and hypotension) and self-reported side effects. This suggests that from the side effects perspective a capped dose is not necessary.

A drawback of our study is that only a limited number of children was included and that no extra blood was sampled to evaluate water balance parameters in this group. It would be useful to study desmopressin side effects in a larger cohort of children. Especially as we observed a higher rate of hypotension after infusion in our cohort compared to adults. Also, our data only evaluated intravenous and not intranasal desmopressin administration. However, previous studies have showed a similar efficacy between the two administrations with possibly less side effects with intranasal use.[37,38] The clinical experience with desmopressin is largely based on small case series or selected populations. The strength of our study is that it consisted of a large number of patients with bleeding disorders with an indication for desmopressin, including children and was primarily focused on laboratory values and vital sign changes next to self-reported side effects.

Based on our results we conclude that desmopressin is a safe treatment option in patients with bleeding disorders, based on objective parameters that were not clinically relevant, such as severe hyponatremia. Nonetheless patients did have a decrease in blood pressure and corresponding increase in heart rate. Although many patients self-reported side effects after infusion, almost all were minor and subsided to base-

line state after 24 hours or even earlier. This study supports the use of desmopressin as a safe treatment option in patients with inherited or acquired bleeding disorders.

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CHAPTER 5

Desmopressin in hemophilia: a proposal for a clinical response definition and individualized test-dose regimen

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Submitted

SUMMARY

Introduction: Due to inter-individual variation in desmopressin responses, non-severe hemophilia A patients undergo a desmopressin test-dose. International guidelines do not define an adequate desmopressin response nor wat blood sampling protocol should be used, resulting in various definitions and protocols. Notably, definitions do not contain information on the response duration.

Aim: To assess the desmopressin response in non-severe hemophilia A patients according to currently used definitions and to propose a uniform response definition and blood sampling protocol.

Methods: We included 105 non-severe hemophilia A patients who received a desmopressin test-dose, which included blood sampling before, 1, 3 and 6 hours after infusion. Response definitions were based on clinical hemostatic cut-offs or additionally contained FVIII:C fold increase over baseline.

Results: Desmopressin response rates in our cohort varied depending on the used definition. FVIII:C 1 hour after infusion was subdivided into four categories (<0.30, ≥0.30-0.49, ≥0.50-0.79 and ≥0.80 IU/ml) and showed to be indicative of the response after 6 hours (complete, partial or none) in 40% of patients, whom subsequently required no additional blood sampling. FVIII:C fold increase showed no association with desmopressin response based on FVIII:C after 3 and 6 hours.

Conclusion: We propose to use a response definition based on FVIII:C levels used in clinical practice (0.30 and 0.50 IU/ml). Patients with FVIII:C after 1 hour <0.30 (non-responder) or ≥0.80 IU/ml (durable responder), do not need additional blood sampling. Patients with 0.30-0.79 IU/ml FVIII:C after 1 hour, require an additional blood sample after 6 hours to determine the desmopressin response duration.

INTRODUCTION

Hemophilia A is an X-linked inherited bleeding disorder characterized by a deficiency of coagulation factor VIII (FVIII). Desmopressin (1-deamino-8D-arginine vasopressin, DDAVP) is commonly used in patients with non-severe hemophilia A to temporarily improve hemostasis and to prevent or treat bleeding.[1] The effect of desmopressin has been extensively reported in the literature as are the potential side effects.[2-5] Because of inter-individual variation in its response, it is recommended to perform a so called test-dose prior to treatment.[6] However, in the international treatment guidelines no statement is made what is considered an adequate response and what kind of blood sampling protocol after the test-dose should be followed.[6] This is important as the future use of desmopressin in a patient is generally based on the results of this single test-dose.

Multiple studies have evaluated desmopressin response in hemophilia A and in many of them different definitions of response were used. Some were based solely on clinical cut-offs, i.e. FVIII activity level (FVIII:C) ≥0.30 for minor and ≥0.50 IU/ml for major bleeds or surgery respectively[7-9], whereas others also incorporated a FVIII:C fold increase over baseline to not only evaluate clinical but also biological efficacy.[1,10-14] As a consequence, comparison of responses between studies is difficult and patients are classified differently depending on the type of definition. A uniform response definition would offer a solution to these issues.

The drawback of the current response definitions is that they do not contain information on the duration of the response. Almost all above mentioned definitions were used to evaluate the response one hour after administration. Based on this, the duration and course of FVIII:C increase after desmopressin administration was estimated but not confirmed. Perhaps by including FVIII:C fold increase over baseline into the response, more information can be obtained on the duration. However, this has not yet been proven. Alternatively, an extensive blood sampling protocol can be used to specifically study the duration of the response but this is inconvenient for the patient. It is generally accepted that blood samples should be taken at least prior to and one hour after desmopressin administration and due to increased clearance in some patients, also after four hours.[14]

Because of the variation in response it is likely that some patients require extensive blood sampling to evaluate the response, whereas others can suffice with limited blood sampling. The aim of our study was to investigate the response to desmopressin in a large cohort of non-severe hemophilia A patients using various previously published response criteria and to provide an overview of the desmopressin response definitions currently used in the literature in order to make a recommendation for a uniform response definition. Additionally, we aimed to evaluate the blood sampling

protocol in our study population to see whether we can individualize the optimal sampling procedure and minimize blood sampling for the patients.

MATERIALS AND METHODS

Patients

Mild or moderate hemophilia A patients who previously had undergone a desmopressin test-dose in the Hemophilia Treatment Centre of the Erasmus University Medical Centre or Sophia Children's Hospital, Rotterdam, were included. An exclusion criterion was the presence of a FVIII inhibitor at time of desmopressin administration. 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.

Desmopressin test-dose and response definitions

In our cohort, desmopressin was administered either intravenously (0.3 µg/kg bodyweight) or intranasally (2 sprays of 150 µg each; total dose 300 µg). The desmopressin test-dose was accompanied by a maximum of four blood samples: before infusion (T0), 1 (T1), 3 (T3), and 6 hours (T6) after desmopressin infusion. FVIII:C level at T3 was missing in 4 patients.

An overview of the most commonly reported desmopressin response definitions in hemophilia A patients is shown in Table 2. All definitions were used to assess a desmopressin response approximately one hour after desmopressin infusion, at the FVIII:C peak level. Seary et al[8], Nolan et al[7], and ourselves[9] have used response definitions based on clinically relevant hemostatic cut-offs without incorporating a FVIII:C fold increase over baseline, although other definitions have been used in other studies.[1,10-14]

For the evaluation of the blood sampling protocol, we used clinical response definitions: no response (NR) FVIII:C <0.30 IU/ml, partial response (PR) FVIII:C ≥0.30-0.49 IU/ml and complete response (CR) FVIII:C ≥0.50 IU/ml. A durable response was defined as FVIII:C ≥0.30 IU/ml at T6 and a short-term response as FVIII:C <0.30 IU/ml at T6.

Outcome

Primary outcome was the difference in distribution of an adequate desmopressin response based on the currently used definitions of desmopressin response reported in literature. Secondary outcome was the number of patients in whom blood sampling after T0 and T1 provided no additional information with regard to the duration of the desmopressin response.

Statistical analyses

Descriptive statistics for continuous variables are summarized as median (25-75% interquartile range). Categorical variables are shown as proportions. FVIII:C level at T1 was divided into four groups (<0.30 IU/ml, ≥0.30-0.49 IU/ml, ≥0.50-0.79 IU/ml and ≥0.80 IU/ml) to evaluate the desmopressin response at T3 and T6. For comparison of FVIII:C levels between groups the non-parametric Kruskal-Wallis test was used. In case of significant differences the Mann-Whitney U test was used. A p-value <0.05 was considered statistically significant. SPSS version 21.0 (IBM Corp. Armonk, NY, USA) was used.

RESULTS

Study population

In total, 105 non-severe hemophilia A patients were included with a median age of 30 years (range 6-75). The majority of patients had mild hemophilia A (n=92, 88%). Desmopressin was administered intravenously in all but one patient (Table 1).

Table 1. Study population characteristics

	Patients N=105
Age at desmopressin administration	30 (6-75)
Hemophilia severity	
Mild	92 (88%)
Moderate	13 (12%)
Blood group 0	38 (41%)
Body weight (kg)	74 (67-84)
Desmopressin administration	
Intravenous	104 (99%)
Intranasal	1 (1%)
Lowest historical FVIII:C measured	0.13 (0.07-0.20)
Baseline FVIII:C (just before desmopressin; T0)	0.18 (0.10-0.27)

FVIII:C, Factor VIII coagulation activity.
Data are presented as median (25-75% interquartile range) or n (%) except for age: median (range).
Blood group missing in 12 patients, total N=93. Body weight missing in one patient, total N=104.

Response definitions

Figure 2 shows the distribution of desmopressin responses in the study population according to the response criteria reported in the literature. We evaluated patients who were 'mismatched' based on the two definitions: i.e. only clinical FVIII:C cut-off or additional FVIII:C fold increase over baseline. Based solely on the clinical FVIII:C ≥0.30

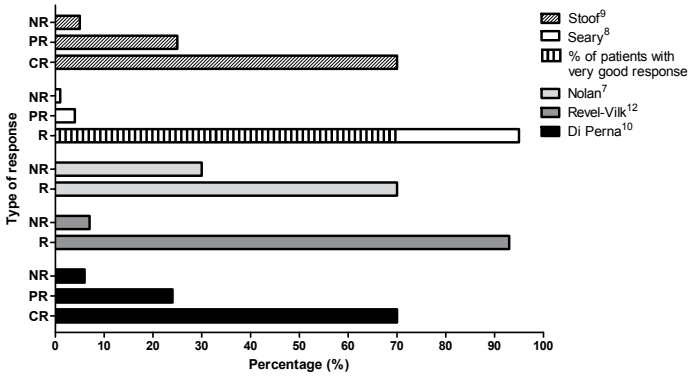

Figure 1. Distribution of desmopressin responses in the present study population of hemophilia patients (N=105) according to the different response criteria reported in the literature.
CR, complete response; PR, partial response; R, response; NR, non-response (response definitions explained in Table 2).

IU/ml cut-off at T1, 100 patients (95%) had a response to desmopressin. However, when the ≥2-fold increase is included in the definition, two of these patients did not meet the criteria one of whom had a FVIII:C level at T1 of 1.03 IU/ml but only increased 1.4-fold. Conversely, five patients who had ≥2-fold increase over baseline did not reach FVIII:C ≥0.30 IU/ml at T1.

Table 2. Desmopressin peak response definitions in hemophilia reported in literature

References	Response definitions*
Di Perna et al.[10]	CR = FVIII:C ≥0.50 IU/ml
Siew et al.[13]	PR = FVIII:C ≥0.30 but <0.50 IU/ml but have ≥2-fold increase over baseline
Castaman et al.[14]	NR = none of the above
Revel-Vilk et al.[12]	Response = FVIII:C ≥0.30 IU/ml and ≥2-fold increase over baseline
Leissinger et al.[1]	
Dunn et al.[11]	
Nolan et al.[7]	Response = FVIII:C ≥0.50 IU/ml
Seary et al.[8]	Very good response = FVIII:C ≥0.50 IU/ml
	Response = FVIII:C ≥0.30 IU/ml
	PR = FVIII:C ≥0.20 but <0.30 IU/ml
	NR = FVIII:C <0.20 IU/ml
Stoof et al.[9]	CR = FVIII:C ≥0.50 IU/ml
	PR = FVIII:C ≥0.30 but <0.50 IU/ml
	NR = FVIII <0.30 IU/ml

CR, complete response; PR, partial response; NR, non-response; FVIII:C, Factor VIII coagulation activity.
*definitions generally used to evaluate a desmopressin response one hour after desmopressin administration (T1).

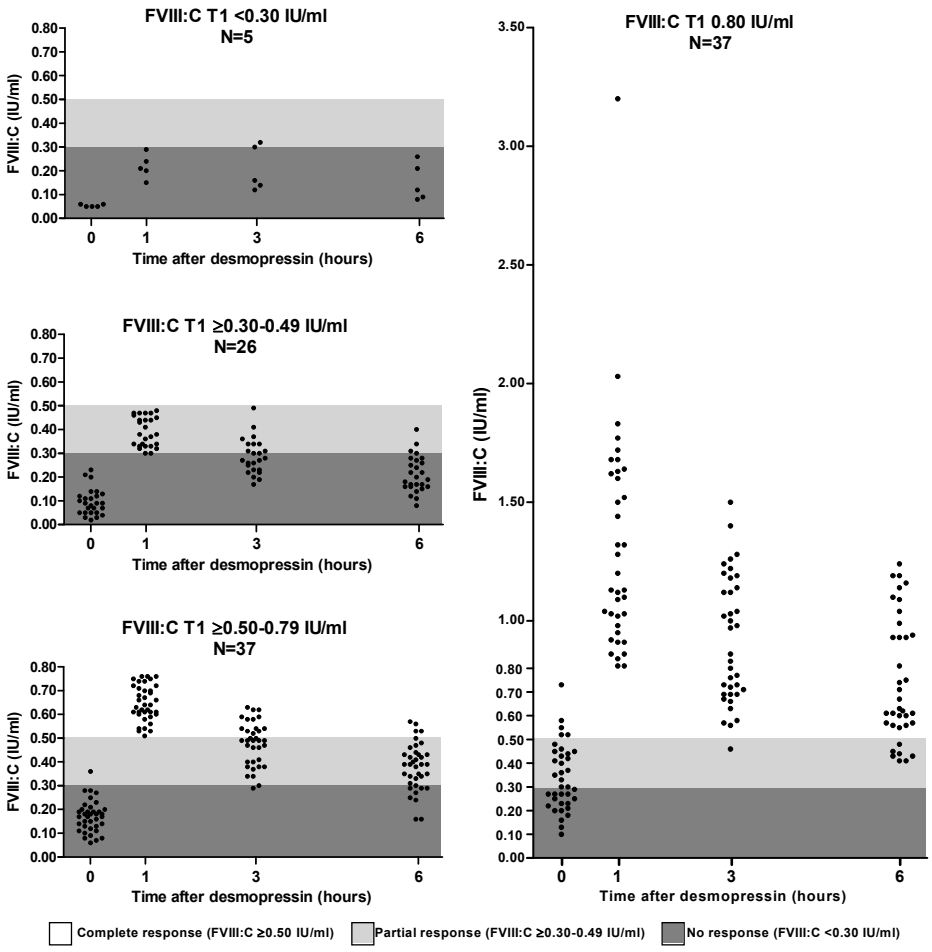

Figure 2. FVIII:C before and after desmopressin divided by FVIII:C levels one hour (T1) after desmopressin.

FVIII:C at T3 missing in 4 patients (N=2 with FVIII:C at T1 ≥0.30-0.49 IU/ml and N=2 with FVIII:C ≥0.50-0.79 IU/ml).

Determinants of duration of response

As all currently used response definitions primarily focused on the response at T1, we studied potential determinants indicative of the duration of response. For this we used the clinical response definition (no response (NR) FVIII:C <0.30 IU/ml, partial response (PR) ≥0.30-0.49 IU/ml and complete response (CR) ≥0.50 IU/ml). We studied FVIII:C fold increase over baseline at T1 in relation to achieved desmopressin responses at T3 and T6, for which we divided fold increase into tertiles; 1st tertile 1.4-3.4 fold increase, 2nd tertile 3.5-4.6 and 3rd tertile 4.7-23.5. FVIII:C level at T3 did not significantly differ

between the fold increase tertiles (1st tertile 0.54 IU/ml (0.41-0.81), 2nd tertile 0.54 IU/ml (IQR 0.36-0.72), 3rd tertile 0.38 IU/ml (IQR 0.28-0.71), p=0.12. FVIII:C level at T6 significantly differed between the 1st and 3rd fold increase tertile (0.44 IU/ml (IQR 0.34-0.62) vs. 0.29 IU/ml (IQR 0.17-0.47), p=0.01) but not between the other tertiles. There was no association between fold increase and type of responses at T3 and T6 and no trend was seen towards better responses with higher fold increase over baseline.

Subsequently, we studied FVIII:C at T1 in relation to the response at T3 and T6. We divided FVIII:C at T1 in four groups based on clinically relevant cut-offs: <0.30 IU/ml (N=5), ≥0.30-0.49 IU/ml (N=26), ≥0.50-0.79 IU/ml (N=37) and ≥0.80 IU/ml (N=37). The response to desmopressin was strongly dependent upon the baseline FVIII:C level as FVIII:C at T0 was higher in individuals with an increased response to desmopressin: 0,05 IU/ml (IQR 0.05-0.06), 0.09 IU/ml (IQR 0.05-0.12), 0.17 IU/ml (IQR 0.13-0.20) and 0.30 IU/ml (IQR 0.23-0.45) respectively (p<0.001). Figure 2 shows FVIII:C before and after desmopressin based on the four groups determined by T1 FVIII:C levels. All patients with FVIII:C ≥0.80 IU/ml at T1 had a durable response at T3 and T6 (Figure 2), of whom 81% still had FVIII:C ≥0.50 IU/ml at T6. Thirty-four patients (97%) who had FVIII:C ≥0.50-0.79 IU/ml at T1, had FVIII:C ≥0.30 IU/ml at T3. However, at T6 only 29 patients (78%) showed a durable response and 8 (22%) had FVIII:C levels below 0.30 IU/ml (Figure 2). Eleven patients (46%) with FVIII:C ≥0.30-0.49 at T1 had FVIII:C ≥0.30 IU/ml at T3 which decreased to only 4 patients (15%) with a durable response at T6. As can be expected, none of the patients with FVIII:C <0.30 IU/ml at T1 had a durable response at T6 (FVIII:C ≥0.30 IU/ml).

DISCUSSION

International guidelines recommend to perform a desmopressin test-dose in each non-severe hemophilia A patient to assess the extent and duration of the FVIII:C response. This should be performed in every individual patient because of an inter-individual variation in the response. However these guidelines differ in the definition of an adequate response or the blood sampling protocol that should be used. We studied the response to desmopressin in a large cohort of non-severe hemophilia A patients and assessed the response rate according to the various reported definitions. Additionally, based on our findings we propose an individualized blood sampling protocol following the desmopressin test-dose.

The currently used response definitions are based on clinical FVIII:C cut-offs (i.e. FVIII:C ≥0.30 IU/ml for minor bleeds or surgery and ≥0.50 IU/ml for major bleeds or surgery) or additionally include FVIII:C fold increase over baseline. A response definition based solely on clinical cut-offs seems most relevant to establish the desmopressin

effect as we found that FVIII:C fold increase over baseline is not associated with the FVIII:C peak level or duration of the response. Two of 100 patients with FVIII:C ≥0.30 IU/ml 1 hour after desmopressin, were considered non responders to desmopressin when including ≥2-fold increase over baseline in the response definition, one of whom had a FVIII:C level at 1 hour above ≥0.50 IU/ml (1.03 IU/ml). Previous studies that evaluated the FVIII:C fold increase over baseline in relation to FVIII:C peak level, also showed a 'mismatch' between these two variables with several patients having ≥2-fold increases of FVIII:C , but a peak level of FVIII:C <0.30 IU/ml and vice versa.[12,14-16] Also, in these studies a wide range of FVIII:C fold increases over baseline were reported, however with similar FVIII:C peak levels.[12,14,15] Additionally, we found that fold increase over baseline was not indicative of the response duration 6 hours after infusion. This suggests that FVIII:C fold increase over baseline has limited value in classifying a patient's desmopressin response. Besides, the FVIII:C fold increase over baseline evaluates the biologic effect of desmopressin, whereas in the clinical setting the achieved FVIII:C level is used for treatment decisions.

By using clinical cut-offs based on absolute FVIII:C levels achieved 1 hour after infusion to define a desmopressin response, a decision can be made about the type of bleeding or surgery that can be treated with desmopressin. The FVIII:C cut-offs 0.30 and 0.50 IU/ml are generally accepted as the hemostatic thresholds to prevent bleeding in case of minor and major surgery and/or treatment of minor and major bleeds respectively.[6]

To estimate the duration of a desmopressin response in hemophilia patients, we divided FVIII:C levels 1 hour after infusion into groups related to the above described

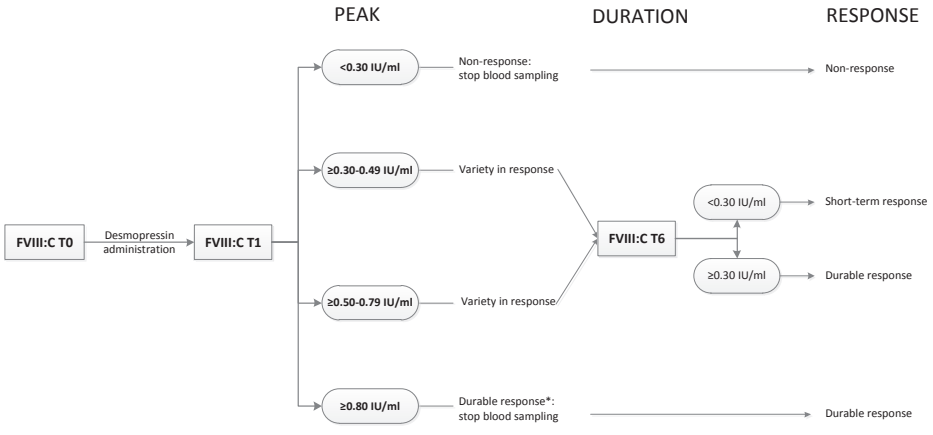

Figure 3. Decision tree of test-dose regimen based on achieved FVIII:C levels one hour after desmopressin administration.
*durable response, FVIII:C ≥0.30 IU/ml at T6.

clinical cut-offs. Patients with FVIII:C <0.30 IU/ml are considered non-responders and additional blood sampling was of no use. Patients with FVIII:C after one hour ≥0.30-0.79 IU/ml showed a variety of responses and required additional blood sampling at 6 hours as no clear estimation of the duration could be made based on FVIII:C at 1 hour. This includes patients with FVIII:C ≥0.50 IU/ml who are generally thought to have an adequate desmopressin response. An additional cut-off at 1 hour of FVIII:C ≥0.80 IU/ml was therefore introduced, since 100% of patients with FVIII:C ≥0.80 IU/ml at 1 hour had durable desmopressin responses at 6 hours. In patients with FVIII:C ≥0.30-0.79 IU/ml at 1 hour, FVIII:C at 3 hours had no added value for prediction of the duration of the desmopressin response as the variety of responses seen at this time point were not indicative for the response at 6 hours (Figure 3).

We found that by using these FVIII:C cut-offs at 1 hour (<0.30, ≥0.30-0.49, ≥0.50-0.79, ≥0.80 IU/ml), no further blood sampling is needed in 40% of patients, whereas the physician is informed about the duration of the desmopressin response. As a better estimation about the duration of the response can be made, the clinical applicability of desmopressin may be improved. Additional support for the use of desmopressin is important as it is inexpensive and carries no risk of viral transmission or the development of inhibitory antibodies. Classification of desmopressin responses based on FVIII:C at 1 hour implies that FVIII:C should be measured immediately following sampling. If this is not possible, we suggest that a follow-up sample at 6 hours should always be obtained.

The hemostatic level for FVIII:C is considered to be around 0.30-0.40 IU/ml.[17,18] There is limited evidence that proves the importance of a durable response (FVIII:C ≥0.30 IU/ml at 6 hours) and an optimal hemostatic response in minor interventions or surgery. However, as suggested by McMillan et al, maintenance levels after surgical procedures should be at least 0.30 IU/ml for several days.[19] Therefore, it is of importance to know the duration of the FVIII:C increase at 6 hours, and not only before and 1 hour after desmopressin. Based on FVIII:C at 6 hours, responses can be classified as short-term or durable. Although a short-term response consists of FVIII:C <0.30 IU/ml after 6 hours, desmopressin in these patients may suffice in case of minor surgery or trauma. Subsequently, a durable response may suffice in case of major surgery or trauma.

In the international literature, blood sampling 4 hours after administration is advised which was not included in our test-dose regimen. However, it is plausible that FVIII:C at T4 (just as measurement after 3 hours, as we performed) is not conclusive in the determination of the duration of the response. This, as varying responses could still be seen at 6 hours, as we especially observed in the patients with FVIII:C ≥0.50-0.79 IU/ml at 1 hour. We identified the FVIII:C clinical cut-offs at 1 hour in relation to the desmopressin response at 6 hours in our hemophilia population. Further studies in other non-severe hemophilia A patient populations are needed to prospectively validate the

FVIII:C clinical cut-offs after 1 hour that we propose. In addition, prospective studies evaluating the association between achieved FVIII:C response after desmopressin and bleeding outcome should be performed. An important strength of our study is that we sampled blood at multiple occasions after desmopressin use. Therefore, we were capable of evaluating the response duration and thereby can suggest an individualized desmopressin test-dose regimen.

Based on our results we conclude that a clinical response definition based on FVIII:C levels after 1 hour is more useful than the use of a FVIII:C fold increase over baseline. By evaluating the achieved FVIII:C level at T1 according to the FVIII:C cut-off levels of 0.80, 0.50 and 0.30 IU/ml, a better estimation of the duration of the desmopressin response can be made without the need for more blood sampling in approximately 40% of hemophilia A patients. Therefore, one size does not fit all when it comes to blood sampling for desmopressin test-doses and a more individualized approach can be followed.

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CHAPTER 6

Infusion of desmopressin does not improve primary hemostasis in patients with cirrhosis

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SUMMARY

Background and aims: Cirrhosis frequently affects multiple components of hemostasis. Reversal of the coagulopathy of these patients is frequently required in case of bleeding episodes, or as prophylaxis before invasive procedures. Although 1-deamino-8-D-arginine vasopressin (desmopressin) is widely used as a pro-hemostatic agent in patients with cirrhosis, it is unclear whether desmopressin truly enhances hemostasis in these patients. Here we investigated the hemostatic effects of a single bolus of desmopressin in patients with cirrhosis.

Methods: Ten patients with cirrhosis (child B or C) and ten patients with mild hemophilia A received an intravenous single bolus of 0.3 microgram/kg desmopressin. Plasma was collected prior to and at 1, 3, 6, and 24 hours after desmopressin administration. Levels of Von Willebrand factor (VWF), VWF propeptide, factor VIII (FVIII), and ADAMTS13 were measured in all plasma samples, whereas VWF multimers and functional VWF-dependent platelet adhesion were determined in the samples pre- and 1 hour after desmopressin administration.

Results: Following desmopressin administration, VWF, FVIII, and VWF propeptide levels increased in patients with hemophilia, while patients with cirrhosis only showed an increase in VWF propeptide and FVIII levels. High molecular weight VWF multimers and VWF-dependent platelet adhesion increased in patients with hemophilia one hour after desmopressin administration, but did not change in the patients with cirrhosis. Levels of ADAMTS13 were unaffected in both patient groups after desmopressin.

Conclusion: The lack of relevant effects of desmopressin on laboratory indices of primary hemostasis in patients with cirrhosis is in line with previous clinical study results in these patients.

INTRODUCTION

Cirrhosis is associated with multiple changes in the hemostatic system including thrombocytopenia and platelet function defects, decreased circulating levels of pro- and anticoagulant factors, increased levels of von Willebrand factor (VWF) and factor VIII, (FVIII) and decreased levels of fibrinolytic proteins.[1] Cirrhosis has long been considered as a bleeding disorder, but it has become generally accepted that the hemostatic changes in cirrhosis may result in both bleeding and thrombotic complications.[2] Nevertheless, reversal of the coagulopathy of these patients is frequently required in case of bleeding episodes, or as prophylaxis before invasive procedures.

Administration of 1-deamino-8-D-arginine vasopressin (desmopressin) has been shown to correct the skin bleeding time in patients with cirrhosis.[3-5] Not much is known on the mechanism by which desmopressin would shorten the bleeding time in cirrhosis. The efficacy of desmopressin in patients with mild hemophilia A or type 1 von Willebrand disease has been ascribed to an elevation of circulating levels of VWF and factor FVIII.[6] However, since VWF and FVIII levels in cirrhosis are already substantially elevated[7,8], it is unclear whether a further elevation in levels would exert any relevant pro-hemostatic effect. It has been demonstrated previously that VWF and FVIII levels increase in patients with cirrhosis after an intravenous, but not after a subcutaneous injection of desmopressin.[3-5] Clinical data available from controlled studies indicate a lack of efficacy in patients with bleeding varices and in patients undergoing liver transplantation.[9,10]

The multimeric composition of VWF is controlled by a VWF-cleaving protease, ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13). A complete deficiency of this protein results in diffuse microthrombosis as a result of spontaneous platelet clumping by ultra-large VWF multimers, a disease referred to as thrombotic thrombocytopenic purpura.[11] Infusion of desmopressin has been shown to result in a transient decrease of ADAMTS13 plasma levels, which may reflect consumption of ADAMTS13 in the process of active proteolysis of the desmopressin -induced release of ultra-large VWF molecules.[12]

We investigated the potential pro-hemostatic effects of desmopressin in patient with cirrhosis to provide a rationale for the use of desmopressin as a pro-hemostatic agent in such patients.

MATERIALS AND METHODS

Ten adult patients with stable cirrhosis (Child-Pugh score B or C) and ten adult patients with hemophilia A were included in this study between October 2011 and August

2013. Exclusion criteria for both groups were known malignancies, active infection, renal failure, congenital hemostatic disorders (other than hemophilia A), recent transfusion of blood products, and the use of vitamin-K antagonist therapy. A small questionnaire was used to collect demographic information.

Written informed consent was obtained from every subject participating in this study. The study was approved by the local Medical Ethics Committee from the University Medical Center Groningen. Study procedures were in accordance with the Helsinki Declaration of 1975. Hemophilia patients were recruited from the Erasmus University Medical Center in Rotterdam, and the study was approved by the local ethical review board.

Intervention

All participating patients received a single bolus dose of desmopressin (0,3 µg/kg) through an intravenous catheter.

Plasma samples

Prior to, and 1, 3, 6, and 24 hours after desmopressin administration blood samples were drawn by vena-puncture and collected into vacuum tubes containing 3.8% trisodium citrate as an anticoagulant, at a blood to anticoagulant ratio of 9:1. Platelet poor plasma was prepared by double centrifugation at 2000g and 10.000g respectively for 10 min. Plasma was snap-frozen in liquid nitrogen and stored at -80 °C until use.

VWF and ADAMTS13 assays

Plasma levels of VWF were determined with an in-house enzyme-linked immunosorbent assay (ELISA) using commercially available polyclonal antibodies (DAKO, Glostrup, Denmark). VWF propeptide levels were determined using a commercially available ELISA from GTI Diagnostics (Aachen, Germany) according to the manufacturer's protocol.

ADAMTS13 activity was measured in plasma which was pretreated with bilirubin oxidase (10U/mL; Sigma-Aldrich, Zwijndrecht, The Netherlands) to avoid interference of bilirubin with the assay.[13] ADAMTS13 activity was assessed using the FRETS-VWF73 assay (Peptanova, Sandhausen, Germany) based on method described by Kokame et al.[14] The antigen levels of VWF and the activity of ADAMTS13 in pooled normal plasma were set at 100%, and values obtained in test plasmas were expressed as a percentage of pooled normal plasma.

VWF multimers

VWF multimer analysis was performed by sodium dodecyl sulfate agarose gel electrophoresis followed by western blotting. The blots were incubated with rabbit anti-VWF antibody (DAKO) and goat anti-rabbit IRDye 800 CW (LI-COR Biosciences, Lincoln,

NE). The first five bands were considered as low-molecular weight multimers, whereas other bands were considered as high molecular weight (HMW) multimers. The blots were scanned by the Odyssey Imager (Westburg, Leusden, The Netherlands) and were quantified by morphometric analysis using the ImageScope software package (Aperio, Vista, CA). After shading correction and interactive thresholding, the selected positive pixels were measured. The positive area was the sum of the area of positive pixels of low-molecular weight and HMW bands. Data was expressed as the percentage of HMW multimers per total VWF multimers, which equals the percentage of positive pixels in the HMW band area per total positive pixel area.

Factor VIII

Levels of FVIII were measured on an automated coagulation analyzer (ACL 300 TOP) with reagents and protocols from the manufacturer (Hemosil (R) SynthASil and FVIII depleted plasma; Instrumentation Laboratory, Breda, the Netherlands).

Platelet Adhesion Assay

The ability of VWF to support platelet adhesion was studied under flow conditions in a reconstituted blood model. Red blood cells and platelets were isolated from whole blood of healthy volunteers who had blood group O as described previously.[15] Cells were mixed with patient plasma or plasma from healthy volunteers to obtain reconstituted blood with a hematocrit of 40% and a platelet count of 250.000/μL. VWF-dependent platelet adhesion in reconstituted blood samples was assessed using a cone and plate viscometer (Diamed Impact R, Turnhout, Belgium). Uncoated Diamed wells were perfused at shear rate of 1,800/second for 2 minutes according to the instructions of the manufacturer. Platelet adhesion was quantified using May-Grünwald staining followed by software-assisted morphometric analysis using the Diamed apparatus and software delivered by the manufacturer.

Statistical analyses

Data are presented as medians with interquartile range (IQR) or as numbers with percentages. The one-way ANOVA with Dunnett's post hoc test was used to compare levels of VWF, VWF propeptide, FVIII, and ADAMTS13 at the various time points after desmopressin administration to the baseline values. Correlations between VWF levels or differences in VWF level after desmopressin and the Child-Pugh score were determined by Pearson's correlation coefficient. The paired t-test was used to analyze differences in VWF multimer release and VWF-dependent platelet adhesion between baseline and 1 hour after desmopressin administration. A p-value <0.05 was considered statistically significant. Analyses were performed using GraphPad Prism (San Diego, USA) and the statistical software package SPSS 15.0 (SPSS Inc., Chicago, IL).

RESULTS

Patient characteristics

Patient characteristics are shown in Table 1. Patients with cirrhosis had higher levels of VWF, FVIII and VWF propeptide at baseline compared to patients with mild hemophilia A. Baseline levels of VWF in patients with cirrhosis increased with the severity of disease as assessed by the Child-Pugh scores (r=0.85, p=0.002). Baseline levels of ADAMTS13 were comparable in both groups. VWF propeptide/antigen ratio was strongly increased at baseline in patients with cirrhosis (Table 1).

Table 1. Patient characteristics

	Hemophilia A N=10	Cirrhosis N=10
Age (years)	46 (40-55)	55 (47-58)
Male/female ratio	10/0	8/2
BMI	26 (23-32)	26 (24-35)
Child-Pugh classification		
Child B	N/A	6 (60%)
Child C	N/A	4 (40%)
Etiology of liver disease (number of patients)		
Biliary cirrhosis	N/A	1 (10%)
Alcoholic cirrhosis	N/A	5 (50%)
Hemachromatosis	N/A	1 (10%)
Alcohol + NASH	N/A	2 (20%)
NASH	N/A	1 (10%)
Laboratory values (at baseline)		
Hemoglobin (mmol/L)	Not determined	7.2 (6.4-8.3)
Platelets (10^9/L)	242.0 (168.8-206.3)	95.5 (58.5-148.5)
INR	Not determined	1.4 (1.3-1.8)
Creatinine (µmol/L)	Not determined	66.5 (53.8-88.3)
Bilirubin (µmol/L)	Not determined	89.5 (53.0-126.0)
Albumin (g/L)	Not determined	31 (26.5-33.5)
VWF (%)	114 (94.5-164.3)	521 (238-643.8)
ADAMTS13 (%)	76.6 (57.3-87.9)	93.9 (59.2-130.4)
VWF propeptide (U/dL)	108.5 (97.3-121.3)	284 (236.5-392.8)
FVIII (%)	20 (12.3-38)	152.5 (117.3-193.8)

BMI, body mass index; NASH, non-alcoholic steatotic hepatitis; INR, international normalized ratio; VWF, Von Willebrand factor; ADAMTS13, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; FVIII, Factor VIII; N/A, not applicable.
Data are presented as medians (25-75% interquartile range), ratio or n (%).

VWF, VWF propeptide, FVIII, and ADAMTS13 after desmopressin administration

Following administration of desmopressin, patients with hemophilia showed a significant increase in levels of VWF after 1 hour, followed by a steady decrease over time (Figure 1). In patients with cirrhosis, there was no significant change in levels of VWF following desmopressin administration. However, in some patients with cirrhosis, VWF levels slightly increased. The difference in VWF levels between baseline and 1 hour after desmopressin administration was inversely correlated with Child-Pugh scores (r=0.72, p=0.019). In other words, only in those patients with a low Child-Pugh score, a slight increase in VWF levels was detected.

Despite the absence of an increase in VWF plasma levels in patients with cirrhosis, levels of VWF propeptide did increase significantly 1 hour after desmopressin administration, although the relative increase in VWF propeptide following desmopressin in patients with hemophilia was much more pronounced (Figure 1).

Both patients with hemophilia and patients with cirrhosis showed an increase in levels of FVIII 1 hour after desmopressin administration, but the increase in the patients with cirrhosis did not reach statistical significance (Figure 2).

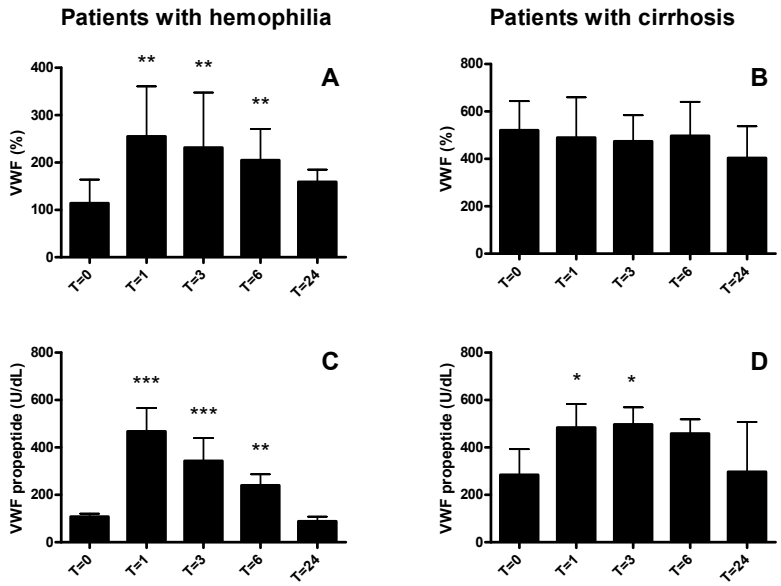

Figure 1. VWF antigen (A,B) and VWF propeptide (C,D) levels in patients with hemophilia (panels A,C) or cirrhosis (panels B,D) at baseline (T0), and 1, 3, 6, and 24 hours (T1, T3, T6, T24) after desmopressin administration. Bars indicate medians, error bars indicate 25-75% interquartile range.

*p<0.05; **p<0.01; ***p<0.001, all versus baseline.

ADAMTS13 activity did not change over time following desmopressin administration in both patients with cirrhosis and patients with hemophilia (Figure 2).

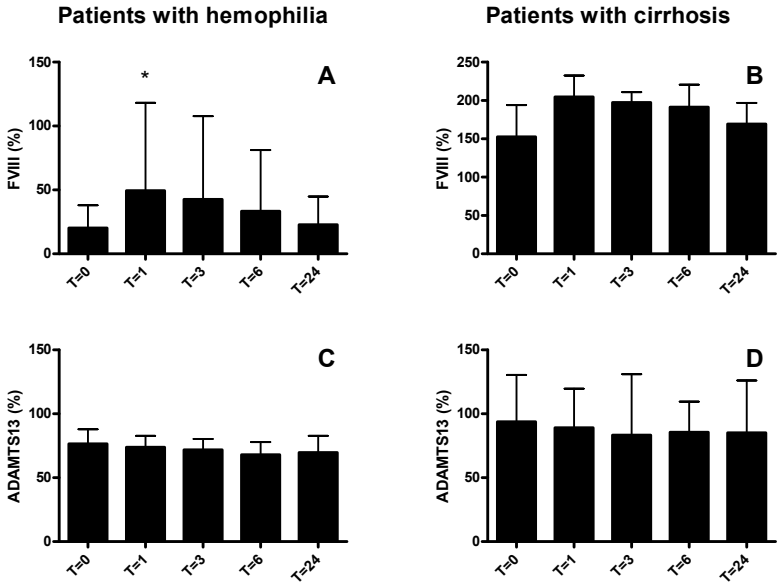

Figure 2. FVIII (A,B) and ADAMTS13 (C,D) levels in patients with hemophilia (panels A,C) or cirrhosis (panels B,D) at baseline (T0), and 1, 3, 6, and 24 hours (T1, T3, T6, T24) after desmopressin administration. Bars indicate medians, error bars indicate 25-75% interquartile range.
*p<0.05 versus baseline.

VWF multimer pattern and VWF-dependent platelet adhesion following administration of desmopressin

One hour following desmopressin administration, the proportion of high molecular weight VWF multimers increased significantly in patients with hemophilia but not in the patients with cirrhosis (Figure 3). In line with the increase in the proportion of high molecular weight VWF multimers, VWF-dependent platelet adhesion and aggregation under conditions of flow substantially increased 1 hour after desmopressin administration in patients with hemophilia. Although there was a slight increase in platelet adhesion and aggregation in the patients with cirrhosis, this difference did not reach statistical significance.

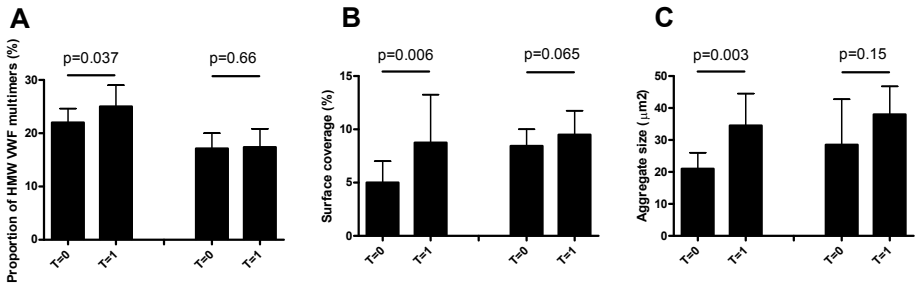

Figure 3. (A) Proportion of high molecular weight (HMW)-VWF multimers in plasma from patients with hemophilia (left) or patients with cirrhosis (right) taken at baseline (T0) and 1 hour after desmopressin administration (T1). (B,C) Capacity of plasma from patients with hemophilia (left) or patients with cirrhosis (right) at baseline (T0) and 1 hour after desmopressin administration (T1) to support platelet adhesion (B) and aggregation (C) under conditions of flow. Bars indicate medians, error bars indicate IQR.

DISCUSSION

The combined results of our investigation show that administration of a single standardized dose of desmopressin to patients with cirrhosis resulted in minor changes in indices of primary hemostasis compared to changes observed following desmopressin administration to patients with mild hemophilia A. The hemostatic effect of desmopressin is assumed to be dependent on elevation of circulating levels of VWF and FVIII. Whereas VWF and FVIII substantially increased in patients with mild hemophilia A, the effects in patients with cirrhosis were marginal. Although these results and published clinical studies[9,10] suggest a lack of hemostatic effect of desmopressin in patients with cirrhosis, we did observe a slight, although not statistically significant, improvement of the capacity of patient plasma to support platelet adhesion in a flow-based model. The latter results are consistent with the improvement in skin bleeding time following administration of desmopressin.[3-5]

Although there are a number of studies showing a lack of clinical effect of desmopressin in patients with cirrhosis, a recent randomized controlled study suggested that desmopressin is as effective as transfusion of blood products in preventing bleeding in patients.[16] However, a drawback of this study was the absence of a non-treated control group, which makes it impossible to determine whether desmopressin and blood products are equally effective in preventing blood loss, or that neither intervention is effective. The latter possibility is plausible in view of the recently changed insights in the hemostatic management of patients with cirrhosis.[1,17]

Two previous studies in which desmopressin was administered intravenously to patients with cirrhosis showed significant elevations of VWF plasma levels[3,5], whereas

one study employing subcutaneous desmopressin reported no increase in VWF plasma levels.[4] Although we cannot fully explain the discrepancy between studies, we speculate that only in those patients with relatively mild disease and consequently moderately elevated VWF levels, administration of desmopressin can result in a slight elevation of VWF plasma levels. Indeed, in the two studies in which desmopressin administration did result in significant elevations of VWF plasma levels, baseline VWF levels were much lower than in the present study as well as the study in which subcutaneous desmopressin was used.

The lack of VWF increase in patients with more advanced cirrhosis might be related to continuous endothelial cell stimulation or dysfunction as described in cirrhotic patients.[18,19] Continuous activation of endothelial cells in cirrhosis might lead to exhaustion of VWF in the endothelial cells rendering desmopressin stimulation ineffective. It has to be noted that the level of endothelial cell activation (and thus the baseline VWF level) may differ according to the etiology of disease, but to our knowledge this has not been studied in detail. As our small patient cohort consisted primarily of patients with alcoholic liver disease, and as our cohort did not include patients with hepatitis-associated cirrhosis, it is unclear whether our results are valid for cirrhosis of all etiologies.

Another explanation for the lack of increase in plasma VWF levels might be that desmopressin-mediated released VWF in cirrhotic patients is instantly consumed. Such a consumptive process may either occur systemically, or within the diseased liver. Thrombi within the cirrhotic liver have been demonstrated previously, providing support for intrahepatic consumption.[20] Alternatively, desmopressin-induced VWF may remain attached to the activated endothelial cells which are known to have the capacity to bind VWF.[21] Consumption or endothelial attachment of desmopressin-released VWF would explain the increase in VWF propeptide levels in patients with cirrhosis, which is released simultaneously with VWF from endothelial cells.[22]

In contrast to a previous study in healthy volunteers and patients with type 1 von Willebrand disease[12], we did not find a decrease in ADAMTS13 plasma levels following desmopressin administration. We have no explanation for this discrepancy, but do note that there is no reason to assume that ADAMTS13 is consumed, inhibited, or cleared following VWF proteolysis. The normal ADAMTS13 levels in patients with cirrhosis are at variance with some papers describing decreased levels of ADAMTS13 in cirrhosis.[23,24] Nevertheless, also normal to elevated levels of ADAMTS13 in cirrhosis have been described in humans[15] and experimental animal models[25], which may be related to the fact that the stellate cell is the primary site of ADAMTS13 biosynthesis.[26]

We studied the effects of desmopressin administration in plasma-based assays of primary hemostasis, as the desmopressin-induced increases in VWF and FVIII are thought to be important determinants of its hemostatic effect. However, desmopres-

sin also appears to have direct stimulatory effects on platelets[27,28], and future studies will be required to assess effects of desmopressin on platelets from patients with cirrhosis which is of particular interest as cirrhosis may be associated with platelet function defects.[29]

Our combined results suggest desmopressin to have minimal hemostatic effects in patients with cirrhosis. Desmopressin may be effective in patients with mild disease and relatively low baseline VWF plasma levels, but clinical studies with relevant clinical endpoints will be required to ascertain this.

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CHAPTER 7

Primary postpartum hemorrhage in women with von Willebrand disease or carriership of hemophilia despite specialized care: a retrospective survey

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SUMMARY

Introduction: Pregnant women with bleeding disorders require specialized peripartum care to prevent postpartum hemorrhage (PPH). If third trimester coagulation factor levels are <0.50 IU/ml, prophylactic treatment is indicated and administered according to international guidelines. However, optimal dose and duration are unknown and bleeding may still occur.

Aim: To investigate the outcome in women with von Willebrand disease (VWD) or hemophilia carriership treated according to current practice guidelines.

Methods: From the period 2002-2011, 185 deliveries in 154 VWD women or hemophilia carriers were retrospectively included. Data on blood loss, bleeding disorder characteristics and obstetric risk factors were obtained. The outcome was primary PPH, defined as blood loss ≥500ml within 24 hours postpartum and severe PPH as blood loss ≥1000ml.

Results: Primary PPH was observed in 62 deliveries (34%), 14 (8%) of which resulted in severe PPH. In 26 deliveries prophylactic treatment was administered due to factor levels below the 0.50 IU/ml cut-off in the third trimester, 14 of which (54%) were complicated by PPH. We found an increased PPH risk in deliveries given prophylactic treatment compared with deliveries without (OR 2.7, 95%CI 1.2-6.3).

Conclusion: PPH incidence was highest in deliveries with the lowest factor levels in the third trimester. Currently, delivery outcome in women with bleeding disorders is unsatisfactory, given the high PPH incidence despite specialized care. Future studies are needed to optimize management of deliveries in this patient population.

INTRODUCTION

Von Willebrand disease (VWD) is the most common inherited bleeding disorder caused by quantitative (type 1 and 3) or qualitative (type 2) abnormalities of von Willebrand factor (VWF).[1] Hemophilia A and B are X-linked bleeding disorders characterized by a deficiency of coagulation factor VIII (FVIII) or factor IX (FIX), respectively. About 80% of hemophilia carriers have coagulation factor levels that are sufficient for normal hemostasis. However, the range in factor levels is wide and an increased bleeding tendency has been observed in carriers with low factor levels.[2]

Pregnancy is considered as a hypercoagulable state due to changes in all aspects of hemostasis including remarkable increases of VWF and FVIII and minimal increase of FIX.[3,4] During pregnancy, levels of these coagulation factors usually do not increase to the same extent in women with bleeding disorders as in those without.[5,6] It is stated in international guidelines that coagulation factor levels, such as FVIII, FIX and VWF, below 0.50 IU/ml in the third trimester are insufficient for normal hemostasis and that prophylactic replacement therapy with factor concentrate during delivery is indicated to prevent postpartum hemorrhage (PPH).[7,8] However, optimal dose and duration are unknown and bleeding may still occur.

PPH is the leading cause of almost a quarter of all maternal deaths worldwide.[9] The incidence of severe primary PPH (≥1000ml blood loss within 24 hours postpartum) in the general Dutch population is 4.5%.[10] In a previous study of our group, 51% of women with moderate and severe VWD self-reported PPH[11]; other studies documented an incidence of 20-40% in VWD and carriers of hemophilia.[6,12-16] The aim of our study was to investigate outcome in pregnant women with bleeding disorders in three Dutch Hemophilia Treatment Centers treated according to current practice guidelines.

MATERIALS AND METHODS

Study design and patients

A retrospective cohort-study was conducted in three Hemophilia Treatment Centers (HTCs) in the Netherlands: the Erasmus University Medical Centre in Rotterdam, the Leiden University Medical Centre in Leiden and the Academic Medical Centre in Amsterdam. Eligible patients were pregnant women with VWD and hemophilia A or B carriers who gave birth between January 1st 2002-December 31st 2011. We included pregnancies that resulted in a delivery after at least 16 weeks of gestation and until the first occurring PPH. Exclusion criteria were: diagnosis of a bleeding disorder as a consequence of previous PPH, diagnosis of a bleeding disorder after pregnancy,

and unknown gestational age. Women were identified through monthly reports of hemophilia meetings where pregnancy management is discussed, and by searching patient files of all women with VWD or hemophilia carriership registered at the HTCs for their obstetric history. Diagnosis of the bleeding disorder was based on assessment of coagulation factor levels, DNA analysis and/or pedigree analysis. A uniform pregnancy management protocol has been used over the 10-year observation time based on the Dutch guideline.[17,18]

Outcome

Primary outcome was primary PPH defined as blood loss ≥500ml within 24 hours postpartum. Severe primary PPH was defined as blood loss ≥1000ml within 24 hours postpartum based on the WHO definition.[9] In one delivery blood loss of 500ml was reported while 4 units of red blood cells were transfused. This delivery was classified as severe PPH as we found it unlikely someone bleeds <1000ml but does require transfusion of 4 units red blood cells. Secondary outcome was use of prophylactic replacement therapy with factor concentrate. Besides primary PPH, secondary PPH is also a serious complication of birth in women with bleeding disorders, defined as blood loss occurring between 24 hours-6 weeks postpartum.[7] It has been suggested that most cases of secondary PPH are dealt within the community or in an emergency setting.[19] With our approach of data collection we therefore did not obtain objective information on secondary PPH and hence could not use it as an outcome.

Data collection

The amount of blood loss was obtained from obstetric records received by the HTCs and was based on visual estimates. Apart from blood loss, data were also collected on obstetric risk factors documented in literature.[20,21] (supplemental table 2), factor level in the third trimester of pregnancy (25-40 weeks of gestation) as well as, if available, non-pregnant and in early pregnancy, and administration of prophylactic treatment with factor concentrates and use of antifibrinolytic agents. In general, prophylactic treatment is administered at the end of the first stage of labor (around 7 centimeters dilation) and is dosed based upon body weight (units/kg), third trimester factor levels and desired target levels of 1.00 IU/ml. Information on hemoglobin level or platelet count was only rarely available and too limited to assess. Coagulation factor levels were obtained: VWF activity (VWF:Act) in VWD (FVIII coagulant activity (FVIII:C) in VWD type 2N), FVIII:C in hemophilia A carriers and FIX coagulant activity (FIX:C) in hemophilia B carriers. VWF:Act included either VWF ristocetin cofactor activity (VWF:RCo) or VWF activity assay (Instrumentation Laboratory BV, Breda, the Netherlands). VWF activity assay uses latex particles coated with a monoclonal murine antibody directed against the GpIbα binding domain of VWF. These latex particles are incubated with patient

plasma and agglutination of the particles, proportionally to the GpIbα binding activity of VWF, is measured. These two measurements have been reported to be strongly correlated.[11] The measurements were performed in the local HTCs.

Statistical analysis

Descriptive statistics for continuous variables are presented as mean ± standard deviation (SD) or median with 95% confidence interval (95%CI). Categorical variables are expressed as proportions. Logistic regression was performed to model the association between bleeding disorder characteristics and PPH; odds ratios (ORs) and 95% confidence intervals (CI) were calculated. Additionally a propensity score was calculated based on risk estimates reported in previous literature[20,21] to act as a weighted score representing obstetric risk factors. This was used in the model as adjustment for known obstetric risk factors. SPSS version 21.0 (IBM Corp. Armonk, NY, USA) was used.

RESULTS

Study population

In total, 470 pregnancies were identified between 2002-2011 (Figure 1). One-hundred-and-twelve were excluded because they ended before 16 weeks of gestation, i.e. 91 spontaneous abortions, 19 elective abortions and 2 ectopic pregnancies. Another 102 pregnancies were excluded based on the described criteria. Of the remaining 256 pregnancies, 227 were managed by HTCs, ranging from total obstetrical and hematological care during pregnancy to an advisory consultation with the attending physician in a non-HTC hospital. In 42 pregnancies there was no information on PPH. Therefore the final study population consisted of 185 pregnancies in 154 women, mostly hemophilia A carriers (78, 51%) and type 1 VWD patients (49, 32%). There were no pregnancies in women with type 3 VWD. The median age at delivery was 31 years (10-90th 24-37 years) and median parity was zero (10-90th 0-2) (Table 1).

Postpartum hemorrhage and bleeding disorders

The overall incidence of primary PPH (≥500ml) was 62/185 (34%), including 14 (8%) with severe PPH (≥1000ml) (Figure 1). In 8 deliveries (4%) a red blood cell transfusion was given; in four <4 units, in three ≥4 units and in one number of units was unknown. The median estimated blood loss was 300ml (95%CI 300-400ml) and did not differ between bleeding disorders; 300ml (95%CI 300-400ml) hemophilia A carrier, 400ml (95%CI 300-500ml) hemophilia B carrier, 400ml (95%CI 300-500ml) VWD type 1 and 300ml (95%CI 200-1000ml) VWD type 2 (Table 2).

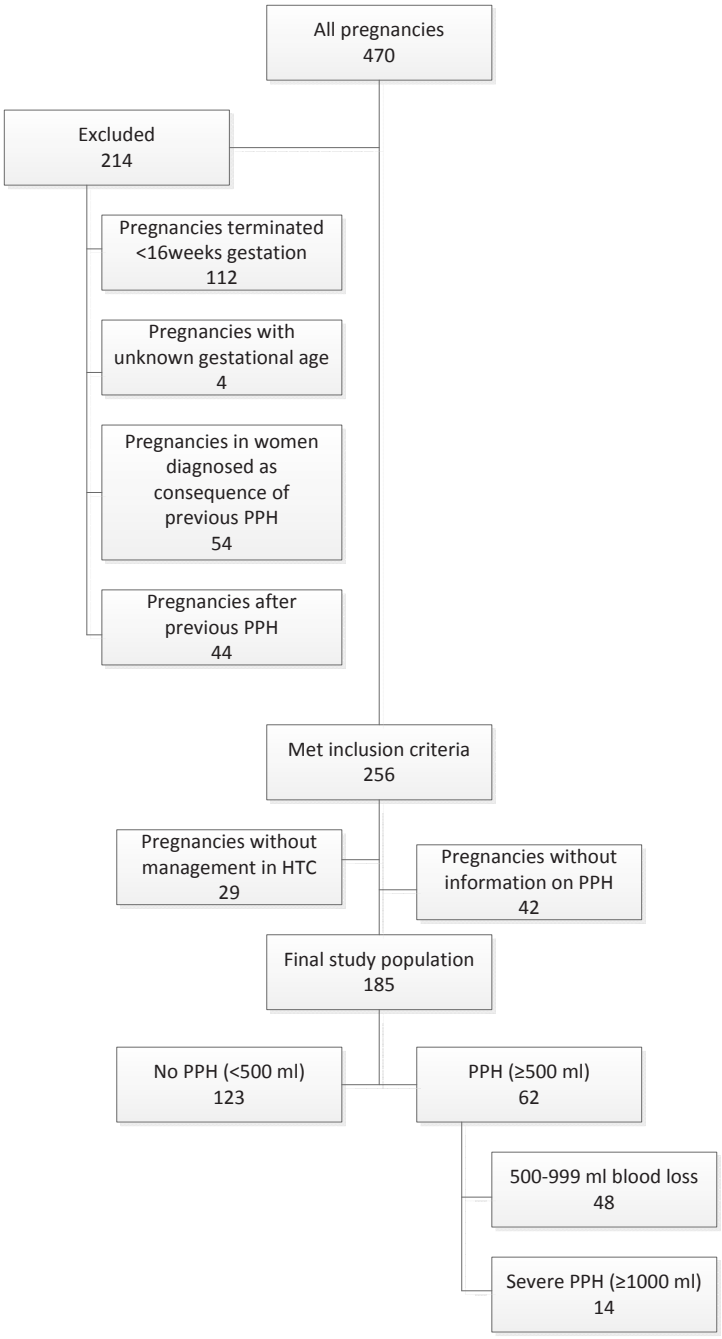

Figure 1. Pregnancy identification and selection procedure.
Pregnancies with management in a Hemophilia Treatment Center (HTC) and information on the occurrence of postpartum hemorrhage (PPH), included until first occurring PPH, formed the study group and all analyses were performed in this group.

Table 1. Characteristics of study population

	Patients (N=154)	Deliveries (N=185)	Factor level non-pregnant period*
Median age at delivery, y		31 (24-37)	
Median parity		0 (0-2)	
Bleeding disorder			
VWD type 1	49 (31.8)	56 (30.3)	0.48 (0.22-0.78)
VWD type 2	12 (7.8)	15 (8.1)	0.10 (0.05-0.30)
Carriership hemophilia A	78 (50.6)	95 (51.4)	0.65 (0.36-1.15)
Carriership hemophilia B	15 (9.7)	19 (10.3)	0.67 (0.22-0.93)
Number of deliveries per woman in ten-year follow-up			
1		126	
2		25	
3		3	

Y, years; VWD, von Willebrand disease.
Data are presented as n (%) or median (10^{th}-90^{th} percentile).
*VWF (VWF:Act) in VWD (FVIII coagulant activity (FVIII:C) in VWD type 2N), FVIII:C in hemophilia A carriers and FIX coagulant activity (FIX:C) in hemophilia B carriers.

Factor level and prophylactic replacement therapy

Factor levels increased during pregnancy in all bleeding disorders (Figure 2). The mean factor level at 25-40 weeks of gestation was 1.21 ± 0.60 IU/ml in pregnancies without PPH, 1.21 ± 0.66 IU/ml in pregnancies with blood loss 500-999ml and 0.92 ± 0.70 IU/ml in pregnancies with severe PPH. In 163 pregnancies a factor level in the non-pregnant period was known and in 69 (42%) this was <0.50 IU/ml. Of these 69, only 23 pregnancies persisted in a factor level <0.50 IU/ml in the third trimester (mean factor level 0.22 ± 0.13 IU/ml), the cut-off for prophylactic treatment, and were consequently treated according to guidelines (Table 2); 6 VWD type 1, 12 VWD type 2, one hemophilia A carrier and 4 hemophilia B carriers. Another 3 pregnancies received prophylactic treatment; one in which factor level was unknown; one in a VWD type 2N patient with FVIII:C 0.53 IU/ml and one in a VWD type 1 patient with VWF:Act 1.03 IU/ml but VWF:Ag 0.40 IU/ml. Prophylactic treatment consisted of VWF-FVIII concentrate in case of VWD and FVIII- or FIX-concentrate in case of hemophilia A or B carriership, respectively. Prophylactic treatment dosage was based upon third trimester factor levels, body weight (units/kg) and desired target levels of ≥1.00 IU/ml. Desmopressin was not administered. In total, prophylactic treatment with coagulation factor concentrate was given in 26 of 185 pregnancies (14%) in 23 different women (Table 2) and in all patients the predefined dose was administered (Supplemental table 1).

Table 2: Bleeding disorder characteristics and postpartum hemorrhage

	Total N	Median estimated blood loss in ml (95%CI)	PPH (≥500ml) N (%)	500-999ml N (%)	Severe PPH (≥1000ml) N (%)	No PPH N (%)
Disease	185		62	48	14	123
Carriership hemophilia A	95	300 (300-400)	28 (29.5)	23 (24.2)	5 (5.3)	67 (70.5)
Carriership hemophilia B	19	400 (300-500)	7 (36.8)	5 (26.3)	2 (10.5)	12 (63.2)
Type 1 VWD	56	400 (300-500)	21 (37.5)	18 (32.1)	3 (5.4)	35 (62.5)
Type 2 VWD	15	300 (200-1000)	6 (40)	2 (13.3)	4 (26.7)	9 (60)
Prophylactic coagulation factor replacement therapy						
No	159	300 (300-400)	48 (30.2)	39 (24.5)	9 (5.7)	111 (69.8)
Yes	26	500 (300-600)	14 (53.8)	9 (34.6)	5 (19.2)	12 (46.2)
Factor level in 3rd trimester*						
≥1.00 IU/ml	93	300 (300-400)	30 (32.3)	24 (25.8)	6 (6.5)	63 (67.7)
0.50-0.99 IU/ml	33	400 (300-500)	11 (33.3)	9 (27.3)	2 (6.1)	22 (66.7)
<0.50 IU/ml	23	400 (300-600)	11 (47.8)	7 (30.4)	4 (17.4)	12 (52.2)

VWD, von Willebrand disease; PPH, postpartum hemorrhage.

Data are presented as median (95% CI) or n (%).

*no data on factor level in 3rd trimester in n=36; measured before prophylactic replacement therapy.

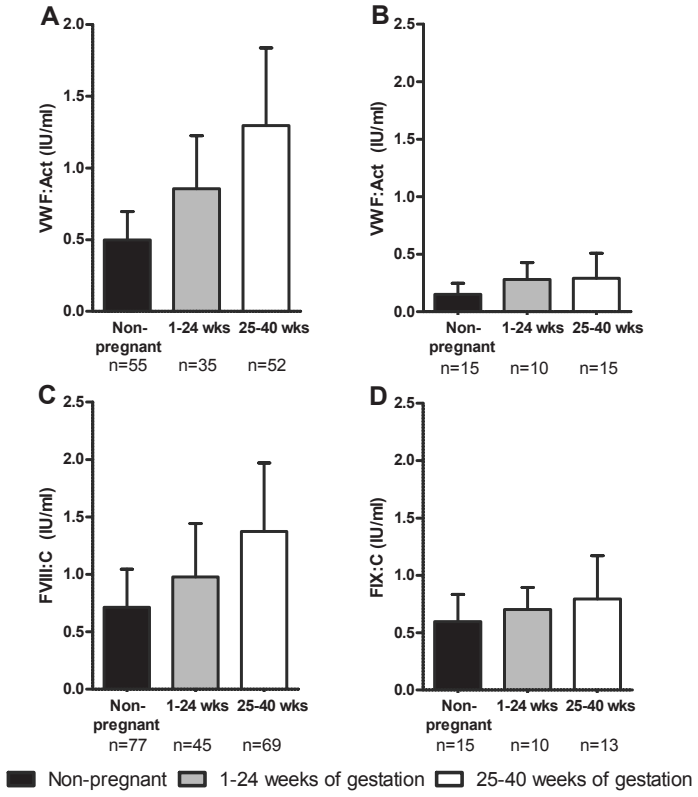

Figure 2. Coagulation factor level increase during pregnancy in patients with VWD type 1 (A) and VWD type 2 (B) or carriership of hemophilia A (C) and carriership of hemophilia B (D).
The vertical axes depict coagulation factor activity levels, including VWF:Act in VWD (FVIII:C in VWD 2N), FVIII:C in carriership of hemophilia A and FIX:C in carriership of hemophilia B. The horizontal axes depict weeks of gestation.

Postpartum hemorrhage, factor level in third trimester and prophylactic replacement therapy

With decreasing third trimester factor level the median estimated blood loss and hence the incidence of PPH, increased: factor level ≥1.00 IU/ml, 300ml (95%CI 300-400ml); 0.50-0.99 IU/ml, 400ml (95%CI 300-500ml) and <0.50 IU/ml, 400ml (95%CI 300-600ml) (Table 2). As all pregnancies with factor level <0.50 IU/ml received prophylactic replacement therapy it was not possible to adjust for this mediator and study the direct effect of factor level on bleeding risk. Fourteen of 26 deliveries (54%) that received prophylactic treatment resulted in PPH, 5 (19%) of which were severe (Table 2). These were distributed over the different bleeding disorders; 4 VWD type 1, 6 VWD type 2, 3 hemophilia B carriers and one hemophilia A carriers. Eleven of 23 pregnancies (48%)

Table 3: Risk estimates of bleeding disorder characteristics for postpartum hemorrhage

	PPH (≥500ml) vs. no PPH			500-999ml vs. no PPH			Severe PPH (≥1000ml) vs. no PPH		
	OR_{crude} 95%CI	OR_{adj}† 95%CI	OR_{adj}‡ 95%CI	OR_{crude} 95%CI	OR_{adj}† 95%CI	OR_{adj}‡ 95%CI	OR_{crude} 95%CI	OR_{adj}† 95%CI	OR_{adj}‡ 95%CI
Disease									
Carrier hem. A	1*		1*	1*		1*	1*		1*
Carrier hem B	1.4 (0.5;3.9)		0.9 (0.3;3.0)	1.2 (0.4;3.8)		0.9 (0.3;3.3)	2.2 (0.4;12.9)		1.2 (0.1;13.1)
Type 1 VWD	1.4 (0.7;2.9)		0.9 (0.4;2.2)	1.5 (0.7;3.1)		1.0 (0.4;2.5)	1.1 (0.3;5.1)		0.5 (0.1;4.1)
Type 2 VWD	1.6 (0.5;4.9)		1.4 (0.4;5.7)	0.6 (0.1;3.2)		0.9 (0.3;3.3)	6.0 (1.3;26.4)		3.8 (0.4;34.0)
Prophylactic coagulation factor replacement therapy									
No	1*	1*	1*	1*	1*	1*	1*	1*	1*
Yes	2.7 (1.2;6.3)	3.9 (1.2;12.4)	3.2 (0.9;11.0)	2.1 (0.8;5.5)	3.9 (1.1;13.2)	2.9 (0.8;10.6)	5.1 (1.5;17.8)	3.0 (0.4;20.0)	5.2 (0.4;64.7)
Factor level in 3rd trimester									
≥1.00 IU/ml	1*	1*	1*	1*	1*	1*	1*	1*	1*
0.50-0.99 IU/ml	1.1 (0.5;2.4)	1.1 (0.4;2.6)	0.8 (0.3;2.3)	1.1 (0.4;2.7)	1.2 (0.5;3.1)	1.0 (0.3;2.9)	1.0 (0.2;5.1)	0.5 (0.1;3.3)	0.02 (0;5.7)
<0.50 IU/ml	1.9 (0.8;4.9)	2.0 (0.6;7.0)	1.8 (0.4;7.8)	1.5 (0.5;4.4)	2.6 (0.7;10.0)	1.6 (0.3;7.7)	3.5 (0.9;14.3)	0.8 (0.1;8.6)	1.6 (0.05;53.4)

VWD, von Willebrand disease; hem, hemophilia; PPH, postpartum hemorrhage; OR, odds ratio.

*reference category.

†prophylactic replacement therapy adjusted for type of disease; factor level in 3rd trimester adjusted for type of disease.

‡ adjusted for above mentioned factors and propensity score.

with third trimester factor level <0.50 IU/ml resulted in PPH (≥500ml) despite prophylactic replacement therapy. In 7 of these 11 pregnancies (64%) factor level had not or only minimally increased during pregnancy and was <0.20 IU/ml in the third trimester, mostly in women with VWD type 2. We found an increased risk of PPH (≥500ml) in case of prophylactic treatment compared to no treatment after adjustment for type of disease (OR 3.9, 95%CI, 1.2-12.4) (Table 3).

Postpartum hemorrhage in relation to obstetric characteristics

Risk estimates of known obstetric risk factors for PPH are shown in supplemental table 2.[20,21] In our population significant risk factors for PPH were: cesarean section (elective and emergency), prolonged third stage of labor (≥30 min), retained placenta, episiotomy, multiple gestation, labor induction and nulliparity. PPH frequency per obstetric risk factor is shown in supplemental table 3. In almost all pregnancies (166/173, 96%, 12 unavailable data) ≥1 obstetric risk factor was present. A propensity score was calculated based on previously reported risk estimates[20,21] and the median score in our population was 6.1 (10-90th 1.9-12.0). After adding the propensity score into our logistic regression model the risk of PPH (≥500ml) in case of prophylactic treatment compared to no treatment was OR 3.2 (95%CI, 0.9-11.0). The risk for PPH between the types of diseases and with decreasing third trimester factor levels did not significantly change (Table 3). Table 4 shows characteristics of all deliveries resulting in severe PPH. There is a variety of obstetric risk factors present next to normal or low third trimester factor levels.

DISCUSSION AND CONCLUSION

Postpartum hemorrhage (PPH) is the leading cause of almost a quarter of all maternal deaths worldwide.[9] To prevent PPH women with bleeding disorders need specialized care during delivery, ideally in a Hemophilia Treatment Centre. It is recommended to check coagulation factor levels during pregnancy at least once in the third trimester when these reach the highest levels in the normal population, in case of VWF and FVIII around 2.00-3.00 IU/ml.[4,12] Also procedures that could potentially increase risk of hemorrhage in affected fetuses, like prolonged labor and instrumental deliveries, should be avoided.[7,8,22] In our study we found a primary PPH incidence of 34% in deliveries in VWD patients or hemophilia carriers, 8% of which were severe. Increased incidence of primary PPH (≥500ml) in hemophilia carriers and VWD patients has also been reported in other studies (6-59%).[12-16] It should be noted that most of these studies were based on patient recall and therefore incidences could have been overestimated.

Table 4: Characteristics of 14 pregnancies with severe postpartum hemorrhage

No	Type of disease	Uterus atony	Type of delivery*	Prolonged 3rd stage of labor (≥30min)	Retained placenta	Pre-eclampsia	Episiotomy or perineal lacerations	Multiple gestation	Instrumental delivery
1	VWD 1	No	Emer. CS	No	N/A	No	No	No	No
2	VWD 1	-	Vag	-	Yes	-	-	No	-
3	VWD 1	No	Vag	Yes	Yes	No	Laceration	No	No
4	VWD 2A	No	Vag	No	Yes	Yes	Epi	No	No
5	VWD 2B	No	Vag	No	No	No	Laceration	No	No
6	VWD 2N	No	Vag	Yes	Yes	No	Laceration	Yes	No
7	VWD 2N	No	Elective CS	No	N/A	No	N/A	No	N/A
8	C hem A	No	Emer. CS	No	N/A	No	No	No	No
9	C hem A	No	Vag	No	No	No	Epi	No	No
10	C hem A	No	Elective CS	No	N/A	No	N/A	No	N/A
11	C hem A	Yes	Elective CS	No	N/A	No	N/A	Yes	N/A
12	C hem A	No	Vag	No	No	No	Epi	Yes	No
13	C hem B	No	Vag	No	No	No	Both	No	No
14	C hem B	-	Elective CS	No	N/A	-	N/A	No	N/A

VWD, von Willebrand disease; C hem A, carrier hemophilia A; C hem B, carrier hemophilia B; Emer CS, emergency cesarean section; Vag, vaginal delivery; Epi, episiotomy; N/A, not applicable; -, unknown.
*indications for elective CS were: placenta praevia, breech position 2x, carriership hemophilia B.
†indication for induction of labor were: decreased fetal movements 2x, serotinity, hypertension/pre-eclampsia 2x.
‡factor activity level; VWF:Act for VWD type 1, 2A and 2B, FIX:C for carrier hemophilia B and FVIII:C for carrier hemophilia A and VWD type 2N.

Our results suggest an association between low third trimester factor level and PPH. In 23 of 185 pregnancies (12%) coagulation factor concentrate was given prophylactically during labor because third trimester factor levels were <0.50 IU/ml, aiming at target levels of 1.00 IU/ml according to the national guideline. Despite this, 11 deliveries (48%) were complicated by PPH; eight (71%) in women with VWD type 1 and 2, 6 of which had factor level <0.20 IU/ml in the third trimester. For VWD type 2 this can be explained by the pathophysiology; although VWF:Ag may increase, VWF remains dysfunctional and VWF:Act marginally increases.[23] One VWD type 1 patient with very low VWF levels was included who had minor VWF:Act increase during pregnancy. This has been described before and may be due to mutations in the VWF gene that are associated with increased VWF clearance or are compound heterozygous for different mutations.[24] The other way around, in pregnancies complicated by PPH mean third trimester factor levels were lower compared to pregnancies without complications (1.14 ± 0.67 IU/ml versus 1.21 ± 0.60 IU/ml). Non-pregnant factor level in the study population did not seem to be indicative of third trimester factor level and PPH.

We hypothesized there would not be an increased PPH risk in women with bleeding disorders compared to the general population when treated with prophylactic

Macrosomy (>4kg)	Induced labor†	Augmented labor	Gravidity	Parity	Age (years)	Blood loss (mL)	Factor level non-pregnant (IU/ml)‡	Factor level early pregnancy (IU/ml)‡	Factor level 3rd trim (IU/ml)‡
No	Yes	No	2	1	29	1100	0.28	1.11	1.77
No	-	-	1	0	32	3000	0.79	-	1.64
No	Yes	No	1	0	37	2000	0.78	1.01	-
No	Yes	No	2	0	31	1400	0.05	0.06	0.15
No	No	No	2	0	29	2500	0.05	0.12	0.12
No	No	No	1	0	30	2000	0.13	0.37	0.53
No	N/A	N/A	5	2	39	1000	0.05	-	0.06
No	Yes	No	1	0	34	1300	0.85	-	1.48
No	Yes	No	4	1	36	1500	0.89	0.90	1.65
No	N/A	N/A	3	2	33	1000	1.36	-	-
No	N/A	N/A	2	1	32	2000	0.74	-	1.65
No	No	No	1	0	34	1400	0.50	-	1.12
Yes	No	No	1	0	23	1600	0.17	-	0.17
No	N/A	N/A	2	0	38	1000	0.72	0.76	0.75

replacement therapy according to the guidelines. Remarkably, we found an increased risk for PPH in deliveries receiving prophylactic treatment compared to deliveries without (OR 3.9, 95%CI, 1.2-12.4). This is in line with a recent study by James et al. who reported significantly greater blood loss in women receiving prophylaxis compared to untreated women (615ml (473,758) vs. 448ml (379,517)).[25] Simultaneously, the majority of our total study population had ≥1 obstetric risk factor. After adjusting for this by means of a propensity score the OR for PPH in deliveries receiving prophylactic treatment compared to deliveries without was 3.2 (95%CI, 0.9-11.0). Notably, PPH incidence was highest in pregnancies with third trimester factor level <0.20 IU/ml suggesting that current interventions with prophylactic replacement therapy are not sufficient for optimal delivery and to prevent PPH. As achieved factor levels after coagulation factor infusions were not measured routinely, it is possible that these did not rise to peak levels of 1.00 IU/ml resulting in blood loss. Subsequent dose adjustments to maintain adequate levels were not performed routinely and could have led to under-dosing. Nonetheless, in all patients the advised dose of prophylactic treatment was administered based on target levels of at least 1.00 IU/ml. Desmopressin was not administered according to the Dutch guideline that states to only administer desmopressin after cord clamping.[17] Hence, it cannot be used as prophylactic treatment during labor.

In this study we collected data from 3 HTCs over a 10-year period and as a result obtained information on a large cohort of pregnancies in women with bleeding disorders. Deliveries were managed with routine care as advised by current guidelines.

Selection of pregnancies because of PPH was prevented by inclusion of all consecutive pregnant women with bleeding disorders and by excluding women who were diagnosed with a bleeding disorder as a consequence of previous PPH. Pregnancies occurring after a previous PPH were also excluded as it is likely that treatment in these cases was intensified in the subsequent delivery. This selection could explain the higher risk for PPH in deliveries in nulliparous women than previously reported (OR 3.1 vs 1.5, supplemental table 2) as we selected deliveries in multiparous women with no previous bleeding predisposition in earlier pregnancies. Due to a retrospective design not all data could be collected. Because of missing data on PPH in 42 pregnancies, PPH incidence may have been overestimated. However, when we assume that these 42 pregnancies had no PPH, the calculated incidence of primary severe PPH would still have been 14 in 227 pregnancies (6.2%). The incidence of primary severe PPH in the Dutch population is 4.5%. Therefore, the actual incidence will likely be somewhere between 6.2-8%. The exact timing of PPH within 24 hours was unknown and speculation on the influence of declining factor levels was therefore not possible. The use of visual estimated blood loss introduces potentially significant inter-observer bias as also different centers are involved, and overestimation of blood loss volume, especially when considering women receiving prophylactic treatment that are probably more carefully monitored. However, in general visual estimated blood loss tends to underestimate blood loss volume[26] and as a result, cases of PPH could have been missed. It should be noted however that visual estimated blood loss has not been evaluated in women with bleeding disorders before. Yet, in the Netherlands these data are routinely obtained for nearly all deliveries and have been used previously for management evaluation.[27,28]

Our results suggest that prophylactic treatment with factor level measurements is necessary. Also, because third trimester and immediate postpartum factor levels are increased with 50-100% compared to non-pregnant levels in women without bleeding disorders, target levels of VWF and FVIII for prophylactic replacement therapy in pregnant women with bleeding disorders should perhaps be closer to 1.50-2.00 IU/ml at time of delivery instead of the current target of 1.00 IU/ml. Additionally, concurrent anti-fibrinolytic therapy and use of double uterotonics in women with third trimester levels <0.50 IU/ml should be considered. Our missing data indicate that feedback between obstetrician and hematologist should be improved.

To conclude, this study shows that the outcome of the current management in women with bleeding disorders is unsatisfactory, because there is a high incidence of primary PPH despite specialized care in HTCs, particularly in women with low factor levels in the third trimester. Future prospective studies are needed to confirm our findings and optimize management of deliveries in women with bleeding disorders.

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Supplemental table 1. Prophylactic replacement therapy protocol

Disease	Product	Tranexamic acid*	Protocol	Dose†	Factor level‡ before admin (IU/ml)	Factor level‡ after admin (IU/ml)	Body weight (kg)	PPH
VWD type 1	Hemate-P	no	during dilation + pp 1 day	50E/kg + 25E/kg	-	-	-	yes
	Hemate-P	no	during dilation + pp 1 day	20E/kg + 20E/kg	-	-	95	no
	Hemate-P	yes	during dilation + pp 1 day	40E/kg	0,44	1,69	75	no
	Hemate-P	yes	during dilation + pp 1 day	40E/kg + 20E/kg	-	-	73	no
	Hemate-P	no	during dilation + pp 2 days	50E/kg + 25E/kg	-	-	64	yes
	-	-	-	-	-	-	77	yes
	Hemate-P	no	during dilation + pp 1 day	50E/kg + 50E/kg	0,29	-	47	yes
VWD type 2	Hemate-P	yes	during dilation + pp 1 day	30E/kg + 15E/kg	-	-	90	no
	Hemate-P	yes	during dilation + pp 1 day	50E/kg + 25E/kg	-	-	59	yes
	Hemate-P	no	during dilation + pp 1 day	50E/kg + 25E/kg	-	-	-	no
	Hemate-P	no	during dilation + pp 1 day	50E/kg + 25E/kg	-	-	-	yes
	Hemate-P	no	during dilation + pp 1 day	12E/kg + 12E/kg	0,73	1,53	83	no
	Hemate-P	yes	during dilation + pp 1 day	40E/kg + 20E/kg	0,72	1,53	70	no
	Hemate-P	yes	during dilation	25E/kg	-	-	71	no
	Hemate-P	no	during dilation + pp 1 day	50E/kg + 25E/kg	-	-	-	no
	Hemate-P	no	during dilation + pp 1 day	25E/kg	-	1,50	89	yes
	Hemate-P	yes	during dilation + pp 1 day	50E/kg + 25E/kg	-	-	-	yes
	Hemate-P	yes	during dilation + pp 1 day	25E/kg + 15E/kg 2dd	-	-	125	no
	-	-	-	-	-	-	-	yes
	-	-	-	-	-	-	-	yes
carrier hem A	FVIII	no	during dilation + pp 3 days	20E/kg + 20E/kg 3d	0,48	1,11	74,8	no
	Refacto	no	prior to cesarean section, pump every 8 hours	50E/kg	-	-	80	yes
carrier hem B	Benefix	no	during dilation	4000E	0,44	0,78	-	yes
	Benefix	no	during dilation	100E/kg	-	-	75	yes
	Benefix	no	during dilation	100E/kg	-	-	95	yes
	Benefix	no	during dilation	50E/kg	-	-	75	no

PPH, postpartum hemorrhage; VWD, von Willebrand disease; hem, hemophilia; pp, postpartum; -, unknown.
*4dd 1 gram for at least 7 days.
†dose: initial dose and subsequent dose(s) given every 12 hours following the previous dose.
‡factor activity level: VWF:Act for VWD type 1, 2A and 2B, FIX:C for carrier hemophilia B and FVIII:C for carrier hemophilia A.

Supplemental table 2. Risk factors for postpartum hemorrhage

	Odds ratio for PPH [20,21]	Odds ratio for PPH [study population]
1. Uterus atony	47.0	-
2. Placenta previa	13.1	-
3. Emergency cesarean section	9.0	3.2
4. Prolonged 3rd stage of labor (≥30min)	7.6	4.7
5. Retained placenta	5.2	5.8
6. Pre-eclampsia	5.0	1.6 (NS)
7. Episiotomy*	4.7	3.2
8. Multiple gestation	4.5	10.7
9. Elective cesarean section	4.0	3.1
10. Instrumental delivery*	2.4	0.9 (NS)
11. Perineal lacerations*	2.1	0.9 (NS)
12. Pyrexia in labor	2.0	2.2 (NS)
13. Macrosomy (>4kg)	1.9	0.8 (NS)
14. Induced labor*	1.7	2.6
15. Augmented labor	1.7	1.0 (NS)
16. Nulliparous	1.5	3.1
17. Age ≥35 years	1.4	1.0 (NS)

PPH, postpartum hemorrhage; -, statistical analysis not possible due to low numbers (n=1 or 2); NS, not significant.

NB. Uterus rupture or inversion did not occur, neither did placental abruption.

Supplemental table 3: Obstetric risk factors and postpartum hemorrhage

	Valid N	Total N (%)	Median estimated blood loss in ml (95% CI)	PPH (≥500 ml) N (%)	500-999 ml N (%)	Severe PPH (≥1000 ml) N (%)	No PPH N (%)
Obstetric risk factors		**185**		**62**	**48**	**14**	**123**
Uterus atony	173	2 (1.2)	1350 -	2 (100)	1 (50)	1 (50)	0 (0)
Placenta previa	175	1 (0.6)	1000 -	1 (100)	0 (0)	1 (100)	0 (0)
Emergency cesarean section	181	16 (8.8)	500 (300-800)	9 (56.3)	7 (43.8)	2 (12.5)	7 (43.8)
Prolonged 3rd stage of labor (≥30min)	176	9 (5.1)	500 (300-2000)	6 (66.7)	4 (44.4)	2 (22.2)	3 (33.3)
Retained placenta	176	7 (4)	1400 (300-3000)	5 (71.4)	1 (14.3)	4 (57.1)	2 (28.6)
Pre-eclampsia	174	7 (4)	300 (100-1400)	3 (42.9)	2 (28.6)	1 (14.3)	4 (57.1)
Episiotomy*	141	51 (36.2)	400 (300-500)	21 (41.2)	17 (33.3)	4 (7.8)	30 (58.8)
Multiple gestation	185	6 (3.2)	950 (100-2000)	5 (83.3)	2 (33.3)	3 (50)	1 (16.7)
Elective cesarean section	181	18 (9.9)	500 (300-600)	10 (55.6)	6 (33.3)	4 (22.2)	8 (44.4)
Instrumental delivery*	145	14 (9.7)	300 (300-500)	3 (21.4)	3 (21.4)	0 (0)	11 (78.6)
Perineal lacerations*	143	68 (47.6)	300 (300-400)	17 (25)	13 (19.1)	4 (5.9)	51 (75)
Pyrexia in labor	173	4 (2.3)	450 -	2 (50)	2 (50)	0 (0)	2 (50)
Macrosomy (>4kg)	179	21 (11.7)	300 (250-500)	6 (28.6)	5 (23.8)	1 (4.8)	15 (71.4)
Induced labor†	157	49 (31.2)	400 (300-500)	20 (40.8)	15 (30.6)	5 (10.2)	29 (59.2)
Augmented labor†	157	31 (19.7)	350 (300-450)	9 (29)	9 (29)	0 (0)	22 (71)
Nulliparous	185	109 (58.9)	400 (300-500)	47 (43.1)	38 (34.9)	9 (8.3)	62 (56.9)
Age ≥35 years	185	39 (21.1)	300 (300-500)	13 (33.3)	9 (23.1)	4 (10.3)	26 (66.7)

PPH, postpartum hemorrhage.

Obstetric risk factors ranked from highest to lowest RR for PPH; data are presented as n (%) or median (95% CI); valid N = number of pregnancies in which information was available.

*frequencies based on vaginal deliveries only (minus n=34 cesarean sections).

†frequencies based on vaginal deliveries and emergency cesarean sections (minus n=18 elective cesarean sections); induced labor n=18 oxytocin, n=25 prostaglandins, n=6 both; augmented labor n=31 oxytocin.

CHAPTER 8

GaitSmart as a new simple tool to measure gait variability in patients with and without hemophilic arthropathy: MOVE study

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Submitted

SUMMARY

Introduction: The main long-term complication of hemophilia is arthropathy development due to recurrent joint bleeds. Methods to detect arthropathy at an early stage are hardly available. Gait analysis may be useful as it evaluates patients during a weight-bearing situation and integrates the impact of multiple-joint arthropathy and muscle-bleed sequelae in contrast to methods containing separate joint assessment. Unfortunately, it is laborious and expensive. However, an easily applicable, portable device (GaitSmart™) has recently been developed that is able to evaluate joint function.

Aim: To evaluate the use of GaitSmart™ in hemophilia patients.

Methods: We included hemophilia patients ≥12 years without recent bleeds. Patients were compared to 46 healthy controls. The Hemophilia Joint Health Score (HJHS) was performed by a trained physical therapist in all patients. GaitSmart™ consists of six sensors applied on calves, thighs and pelvis which are activated during a walk up-and-down a corridor. Gait parameters include sagittal and coronal range of motion, stance flexion, joint symmetry and stride duration.

Results: We included 106 patients, 21 of whom had documented lower limb arthropathy (20%). Patients with knee arthropathy showed reduced stance flexion and sagittal knee ROM. Patients with hip arthropathy had reduced sagittal femur and hip ROM. Gait parameters in patients with solitary ankle arthropathy were within normal limits. Patients with a best possible HJHS had significantly different sagittal hip, femur and pelvis ROM compared to controls.

Conclusion: This pilot-study suggests that GaitSmart™ provides complementary information to the HJHS and is able to identify knee and hip arthropathy in hemophilia.

INTRODUCTION

Hemophilia A and B are X-linked inherited bleeding disorders caused by insufficient or absent clotting factor VIII (FVIII) or clotting factor IX (FIX), respectively. Hemophilia is classified as severe when <0.01 IU/ml clotting factor is present, moderate 0.01-0.05 IU/ml and mild when 0.05-0.40 IU/ml.[1] Depending on the severity, bleeding symptoms can range from hematomas and bleeding after minor trauma to spontaneous joint and muscle bleeds. Joint bleeds (hemarthrosis) are often located in the synovial joints of which the ankles are the most severely and most frequently affected. Nonetheless, also knees, hips and elbows are frequently affected.[2] Long-term consequences of hemarthroses and subsequent iron depositions are chronic synovitis, cartilage damage and eventually irreversible arthropathy, leading to gait alterations.[3,4] Prophylactic replacement therapy with clotting factor concentrates aims to prevent arthropathy. To assess whether prophylactic treatment is effective in preventing arthropathy, musculoskeletal assessment through the Hemophilia Joint Health Score (HJHS) can routinely be performed by a physical therapist. This is currently considered the international gold standard for clinical joint health evaluation.[5] Imaging with X-ray, ultrasound or MRI is also regularly performed.[6,7] Disadvantages of this approach are however that it is costly and does not integrate the effect of multiple-joint arthropathy and muscle bleeds. Moreover, imaging does not evaluate musculoskeletal function during weight-bearing conditions when pain and functional limitations often become more evident.

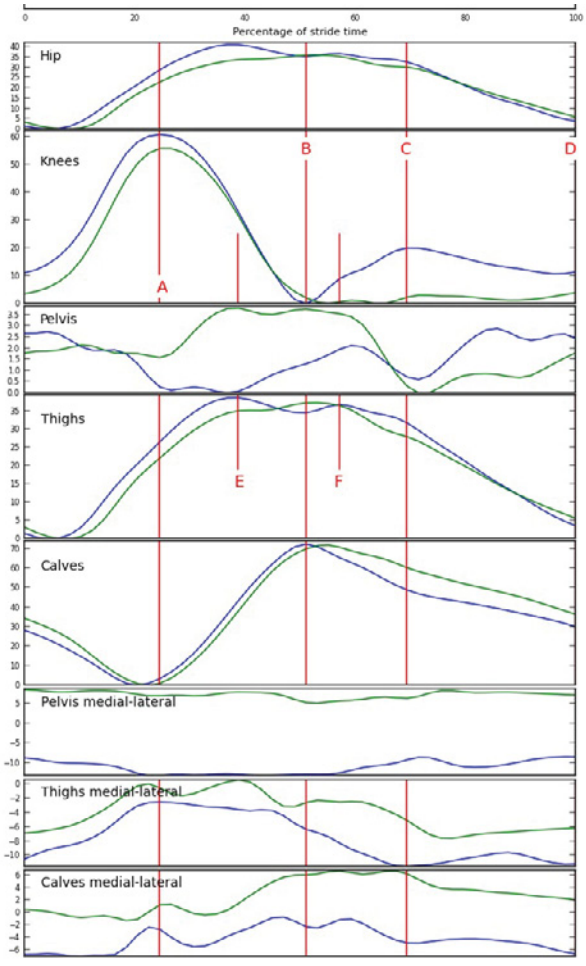
Bladen et al reported that a pressure sensitive walkway (GAITRite®) was able to identify subtle gait changes due to arthropathy.[8] Three-dimensional gait analysis also seems capable of monitoring hemophilic arthropathy.[9] These developments lead to the question if such techniques should be implemented in hemophilia care.

In general, gait analysis can be laborious, time-consuming and therefore expensive. Recently however, a novel, portable, sensor-based device was developed (GaitSmart™).[10,11] Previous studies have proven its applicability, accuracy and reproducibility in measuring joint and limb segment range of motion (ROM) in an ageing population[10] and in knee osteoarthritis.[12]

The aim of this pilot-study was to evaluate the use of GaitSmart™ in patients with and without hemophilic arthropathy. The hypothesis was that hemophilia patients with documented arthropathy produce abnormal gait patterns, and that hemophilia patients with clinically asymptomatic joints produce a normal gait pattern.

A

B



Typical stride from 410.11 to 411.37 seconds

Range of Motion Score %		85.7
	Left	Right
Range hip (°)	41.09	35.41
Range knee (°)	61.00	57.84
Range pelvis (°)	3.53	4.76
Range thigh (°)	38.61	37.28
Range calf (°)	72.42	73.65
Average Duration (s)	1.27	
Quality of Gait Score %		**67.9**
Pelvis medial-lateral (°)	4.67	4.92
Thigh medial-lateral (%)	23.26	22.96
Calf medial-lateral (%)	8.68	10.61
Swing Time (%)	50.00	54.00
Stance Flexion (°)	20.09	3.08
Symmetry Score %		**75.0**
Hip (%)	14.85	
Knee (%)	5.31	
Pelvis (%)	-29.58	
Thigh (%)	3.51	
Calf (%)	-1.68	
Stance (%)	84.65	
Swing Difference (%)	-4.00	

For symmetry scores negative values mean less movement on the left than the right; positive values mean less movement on the right than the left.

A score of 100% and no highlighted values means no concern.
Amber means caution and red means you should take action. Coloured borders indicate values approaching the next classification.
The blue line represents the left leg; the green line represents the right leg.

Figure 1. Positioning of sensors on the body (A) and example of generated output (B).
The blue trace represents the left leg, the green trace the right leg.

MATERIALS AND METHODS

Study design, patients and controls

This cross-sectional study (trial registration: NTR4561) was performed in 2014. All severities of hemophilia A or B patients from our Hemophilia Treatment Centre, ≥12 years without joint or muscle bleeds in the preceding 30 days visiting the outpatient department, were included after written informed consent. Patients unable to walk 20 meters without use of assistive devices were excluded. In addition, 46 controls of similar ages were measured to compare with the study population. Controls had no history of severe injuries of the lower extremities. The study was not subject to the Medical Research Involving Human Subjects Act (WMO) and was approved by the local Medical Ethics Committee.

Gait analysis and joint status assessment

GaitSmart™ analysis consists of six sensor modules with accompanying Velcro straps. The sensors are inertial measurement units and contain three orthogonal gyroscopes and three orthogonal accelerometers that measure both angular velocity and acceleration. The six sensors are first synchronized using dedicated software (Poseidon), then disconnected from the computer, switched on and mounted on the lateral sides of the hip, just above the iliac crest, the thigh, just below the greater trochanter and the belly of the gastrocnemius muscle of the calf (Figure 1). Patients were asked to stand still for ten seconds in order to calibrate the sensors. Subsequently, patients walked up-and-down a 20 meter corridor on standardized footwear (neutral running shoes, Saucony, Shadow 9) at their own self-selected speed. Walking velocity is not measured directly by GaitSmart™ but depends on cadence and stride length, parameters that are measured. Stride length is related to thigh segment angles and leg length and previous data indicated a relative constant thigh range of motion until the age of 80 years.[10] Poseidon software was used to choose a minimum of five strides where stride duration was continuous to within 5% of the mean for that set of strides. From this subset, the most representative stride of the gait pattern was calculated automatically, i.e. the stride with the lowest error compared to other strides. Gait parameters were then obtained for this stride and shown graphically and in tabular form (Figure 1).

Gait parameters consisted of a range of motion (ROM) score, including pelvis, hip, femur, knee and tibia ROM in the sagittal plane and stride duration, a quality of gait score consisting of femur and tibia ROM in the coronal plane and knee stance flexion, and a symmetry score reflecting joint and segment symmetry (Supplemental table 1). Limb segment ROM represents the bone movement and gives precise information as to how the joint angles were achieved. The mean value of left and right limb parameters were used in the controls, patients without lower limb arthropathy and patients with bilateral

arthropathy. In patients with unilateral arthropathy, limb parameters of the affected side were used. Supplemental table 2 shows left and right limb parameters separately for patients with arthropathy. GaitSmart has been validated against an optical system.[10]

Since GaitSmart™ has not been used previously in a hemophilia patient population, the Hemophilia Joint Health Score (HJHS) version 2.1 was concomitantly assessed in each patient by a trained physical therapist. HJHS scores consist of 20 points per joint and a global gait score (GGS) of maximum 4 points. GGS assesses walking, stairs, running and hopping on 1 leg with GGS 0 having all skills within normal limits and GGS 4 with no skills within normal limits. We only used the lower limb scores (knee and ankle) with a possible maximum score of 84. Higher scores represent a worse joint status.[13]

Outcome

The primary outcome was a difference in GaitSmart™ parameters, defined as range of motion and quality and symmetry of gait, between hemophilia patients with confirmed lower limb arthropathy and the controls. Secondary outcome was the number of clinically asymptomatic hemophilia patients (HJHS score of 0) with an abnormal gait pattern.

Data collection

Demographic information was collected including annualized bleeding rate (based on previous 5 years), treatment regimen and presence of arthropathy confirmed with X-ray. HJHS assessment included (duration of) swelling, muscle atrophy, crepitus on motion, loss of static range of motion, joint pain and strength for knees and ankles, and a global gait. The effect of musculoskeletal health on daily functioning was also evaluated. Therefore, patients additionally completed the Hemophilia Activities List (HAL) of which the basic (LOWBAS) and complex activities involving lower extremities (LOWCOM) component scores, were used.[14] Adult patients filled out the Rand-36 questionnaire[15,16] where only the physical functioning domain was analyzed and children the Haemo-QoL short version.[17] A WOMAC (Western Ontario and McMaster Universities Arthritis Index) score[18] was used to evaluate pain and stiffness of the ankle, knee and hip. All scores were transformed to a 0-100 scale, where 0 represented the worst possible score and 100 the best possible score.

Statistical analyses

Descriptive statistics for continuous variables are summarized as mean (standard deviation) in case of normally distributed data and as median [25-75% interquartile range] in case of skewed data. Categorical variables are shown as proportions. Differences between controls and the patients were analyzed with Independent Samples t-test. Questionnaire standardized scores were calculated according to manual instructions. Correlation between the different questionnaires and gait parameters were assessed

with Spearman's correlation and compared between groups using Mann-Whitney U test. Correlations of 0.20-0.39 were considered weak, 0.40-0.59 moderate, 0.60-0.79 strong and >0.79 very strong. A $p<0.05$ was considered statistically significant. SPSS version 21.0 (IBM Corp. Armonk, NY, USA) was used.

RESULTS

Study population

In total, 106 patients were included with a median age of 31 years (range 12-84). There were 46 controls of similar ages (median age of 28 (range 13-62), p=0.57) (Table 1). The majority of patients had hemophilia A (n=93, 88%), the others had

Table 1. Study population characteristics

	Patients N=106	Controls N=46
Age (years)	31 (12-84)	28 (13-62)*
Hemophilia A	93 (88%)	N/A
Severity		
Severe	31 (29%)	
Moderate	25 (24%)	
Mild	50 (47%)	
Treatment†		N/A
Prophylaxis	25 (25%)	
Annualized bleeding rate (5 years)‡	4.8 (2.5-6.6)	
On-demand	76 (75%)	
Annualized bleeding rate (5 years)‡	0.0 (0.0-0.6)	
Bleed in preceding year		N/A
Joint	26 (25%)	
Muscle	15 (14%)	
Arthropathy		N/A
Ankle	10 (9%)	
Knee	5 (5%)	
Multiple (knee, ankle, hip)	6 (6%)	
Elbow	8 (8%)	
Ankle arthrodesis	3 (3%)	N/A
Total knee replacement	4 (4%)	N/A
Orthotics	16 (15%)	4 (9%)
Sport exercise	70 (66%)	40 (87%)
Hours/week	2 (0-3)	4 (2-6)

N/A, not applicable.
Data are presented as median (25-75% interquartile range) or n (%) except for age: median (range).
*p=0.57 compared to patients.
†in 5 patients treatment regimen was unknown, total n=101.
‡annualized bleeding rate was calculated in 91 patients, in 15 patients information was missing.

hemophilia B. Thirty-one had severe hemophilia (factor level <0.01 IU/ml) and 25 moderate (factor level 0.01-0.05 IU/ml), 25 (25%) of whom used prophylactic replacement therapy. Fourteen patients (13%) used primary prophylaxis (i.e. regular continuous treatment, started before the second large joint bleed and age of 3 years [19]), 11 (10%) non-primary prophylaxis (n=8 secondary, n=3 tertiary) and in five patients start of prophylaxis was unknown. The median age at first joint bleed was one year in severe (IQR 1-2), five years in moderate (IQR 3-12) and 14 years (IQR 11-20) in mild hemophilia patients. Twenty-three (46%) of mild and five of the moderate hemophilia patients (20%) had never experienced a joint bleed. The annualized bleeding rate in patients on prophylaxis was 4.8 (IQR 2.5-6.6) compared to 0 (IQR 0.0-0.6) in patients who received on-demand therapy with clotting factor concentrate in case of a bleed.

Arthropathy and GaitSmart™

Twenty-nine patients (27%) showed clinical signs of arthropathy confirmed by X-ray, 21 cases (20%) of which concerned the lower limbs. The ankles were affected in ten patients, the knees in five, and multiple joints in six patients (Table 1). Sagittal ROM of the knee, tibia, femur and hip and stance flexion were all significantly lower in patients with lower limb arthropathy compared to the controls: 59.4° ± 9.5 vs. 65.9° ± 4.2, 73.4° ± 7.8 vs. 78.5° ± 4.1, 39.7° ± 7.4 vs. 43.7° ± 4.7, 37.5° ± 7.2 vs. 42.8° ± 5.1, and 16.6° ± 6.9 vs. 20.1° ± 4.6 respectively (all $p<0.05$) (Figure 2). In patients with solitary knee arthropathy, gait parameters reflecting knee function were diminished: ROM knee (50.4° ± 5.4 vs. 65.9° ± 4.2 in controls) and stance flexion (10.5° ± 4.2 vs. 20.1° ± 4.6 in controls). Also, for four of six patients with multiple joint arthropathy (including the knee joint) these knee parameters were abnormal (Supplemental table 2). In two patients with multiple joint arthropathy including the hips, both sagittal ROM of femur and hips were abnormal. In patients with solitary ankle arthropathy all gait parameters were normal (Supplemental table 2).

Hemophilia patients without confirmed lower limb arthropathy showed reduced sagittal femur and hip ROM compared to the controls (41.7° ± 4.0 vs. 43.7° ± 4.7 and 39.9° ± 4.3 vs. 42.8° ± 5.1), $p<0.05$ (Figure 2).

Hemophilia Joint Health Score and GaitSmart™

The median overall lower limb HJHS was 2 out of 84 (range 0-3), with 26 (27%) patients having a best possible score of 0 indicating clinically asymptomatic joints. Remarkably, in these 26, sagittal ROM of the hip and femur were significantly decreased and sagittal pelvis ROM significantly increased compared to the healthy controls (39.8° ± 4.2 vs. 42.8° ± 5.1, 41.5° ± 3.9 vs. 43.7° ± 4.7 and 4.6° ± 1.3 vs. 4.0° ± 1.1), $p<0.05$ (Figure 2).

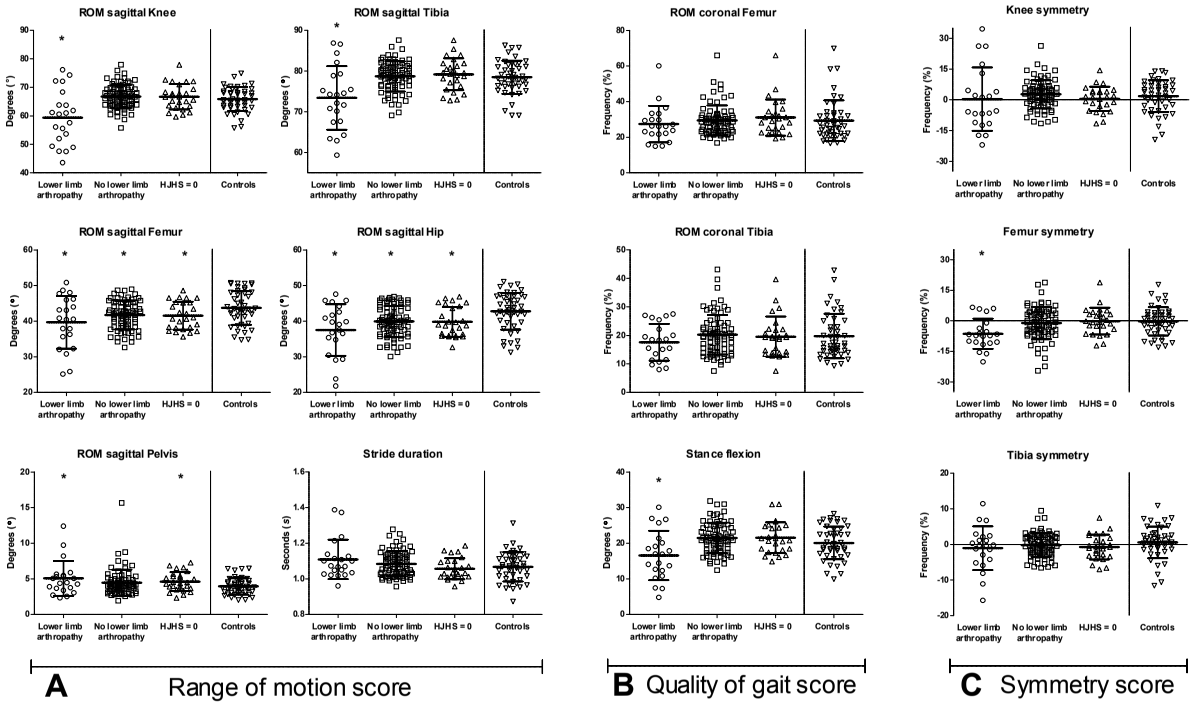


Figure 2. Gait parameters in patients with (N=21) and without lower limb arthropathy (N=77), HJHS=0 (N=26) and healthy controls (N=46). Scatter dot plot: black line represents mean and standard deviation. ROM, range of motion.

A) Parameters representing range of motion.

B) Parameters representing quality of gait.

C) Symmetry parameters; negative values mean less movement on left than right; positive values mean less movement on right than left.

* $p<0.05$ versus controls.

We studied the association between gait parameters and HJHS items representative of joint function, i.e. ROM score and extension/flexion loss (Figure 3). The ROM score showed moderate correlation to the HJHS (r=-0.41, p<0.01) directed towards higher HJHS scores with lowering ROM scores (Table 2). Sixty-four (67%) patients had best possible ROM scores and HJHS extension/flexion loss scores of 0. Ankle arthropathy patients had best possible ROM scores but HJHS extension/flexion loss >0. Patients with abnormal ROM scores but HJHS extension/flexion loss=0 mostly had reduced sagittal hip and femur ROM or a slow stride duration, items not assessed within the HJHS. Patients with knee arthropathy were detected by both GaitSmart™ and HJHS.

Further, we compared gait parameters between the HJHS global gait score (GGS). GGS was distributed as: GGS 0 n=70, GGS 1 n=7, GGS 2 n=5, GGS 3 n=4, GGS 4 n=10. No trend of worsening gait parameters with increasing GGS scores was observed. However, sagittal ROM of the tibia, femur, hip and pelvis were significantly lower in GGS 4 compared to GGS 0, p<0.02.

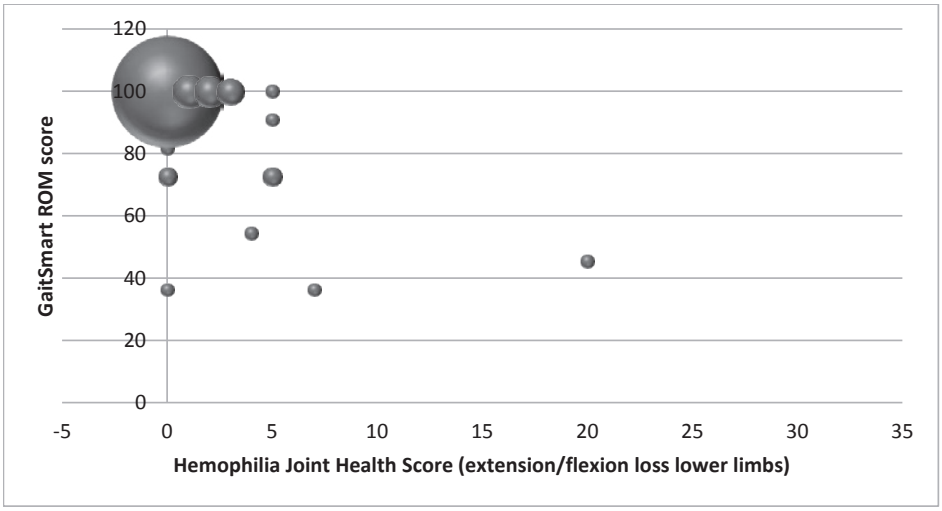


Figure 3. GaitSmart™ ROM score versus extension/flexion loss detected by the Hemophilia Joint Health Score (HJHS), i.e. parameters representative of joint function.
Bubble chart: the larger the bubble, the more patients with similar scores.
N=96 due to 10 missing HJHS. GaitSmart™ ROM score is a summary score of all sagittal range of motion parameters. The extension and flexion loss of lower limbs were combined for the HJHS score.

Disease characteristics and GaitSmart™

Gait patterns in severe and mild hemophilia patients were similar. Gait parameters were comparable between treatment regimes in hemophilia; gait patterns in patients receiving prophylactic treatment were as good as patients who were treated on-demand in case of a bleed. Within the group of patients on prophylaxis there was

Table 2. Daily and physical functioning questionnaires and correlation with GaitSmart™

	Score range	Total N=106	Patients with best possible score	Correlation GaitSmart™ ROM score	Lower limb arthropathy patients N=21	Patients without arthropathy N=77
	(worst – best possible)	Median [IQR]	N (%)	Spearman's rho (p-value)	Median [IQR]	Median [IQR]
GaitSmart™ ROM score	0-100	100 [100-100]	82 (77)	-	96 [77-100]†	100 [100-100]
HJHS lower limbs 2.1*	84-0	2 [0-3]	26 (27)	-0.41 (<0.01)	6 [4-8]†	1 [0-2]
HAL (LOWBAS)	0-100	100 [81-100]	55 (52)	0.46 (<0.01)	52 [36-83]†	100 [93-100]
HAL (LOWCOM)	0-100	98 [78-100]	45 (43)	0.42 (<0.01)	60 [30-83]†	100 [92-100]
Rand-36 physical functioning (n=90)	0-100	90 [75-100]	31 (34)	0.59 (<0.01)	70 [45-85]†	100 [90-100]
Haemo-QoL (n=16)	0-100	90 [84-98]	1 (6)	-0.38 (0.18)	-	12 [6-18]

HJHS, Hemophilia Joint Health Score; HAL, Hemophilia Activities List; LOWBAS, basic lower extremity activities; LOWCOM, complex lower extremity activities.
*n=96 in whom HJHS was performed.
†indicates statistically significant differences between patients with lower limb arthropathy and patients without (p<0.001).

lower knee sagittal ROM when comparing primary, secondary and tertiary prophylaxis: 66.0° ± 3.6, 63.8° ± 4.9 and 57.3° ± 7.3, respectively (p<0.02). Fifteen patients (14%) had ≥1 muscle bleed in the previous year and showed tibia sagittal ROM compared to patients without muscle bleeds: 75.0° ± 3.1 vs. 78.1° ± 5.0 (p=0.02). Nine of these 15 patients also had ≥1 joint bleed in the last year and 4 patients had documented arthropathy of the knee and ankle.

Quality of Life questionnaires and GaitSmart™

Overall outcomes in the hemophilia population were good as the medians were close or equal to the best possible score (Table 2). All quality of life scores showed a moderate correlation with GaitSmart™, except for the Haemo-QoL (Table 2). In patients with arthropathy outcome measures of the HAL and Rand-36 were significantly lower compared to patients without (p<0.001), as were the WOMAC scores for ankle pain and stiffness: 70 (IQR 55-90) vs. 100 (IQR 100-100) and 50 (IQR 38-100) vs. 100 (IQR 88-100), respectively, p<0.001.

DISCUSSION

In general, gait analysis provides objective information on a patient's functional musculoskeletal status and hence may be useful in hemophilia patients in which arthropathy is still the main long-term complication. This pilot-study was designed to evaluate the use of GaitSmart™, an easily applicable, sensor-based device, in hemophilia patients

with overt arthropathy and in clinically asymptomatic hemophilia patients and to compare findings with a healthy control population.

In comparison with healthy controls, all sagittal plane range of motion scores were significantly different in hemophilia patients with confirmed lower limb arthropathy. In patients with solitary knee arthropathy, variables previously reported as discriminative of knee osteoarthritis were affected. Thus indicating that GaitSmart™ is able to detect joint-specific arthropathy.[12,20] We compared GaitSmart™ to the current gold standard for clinical joint health evaluation in hemophilia, the HJHS. Although this score has only been validated in children up to 18 years, it is also suggested for use in adults.[21] We interpreted lower limb HJHS>0 as clinically symptomatic and HJHS=0 as clinically asymptomatic as the minimum clinically important difference is unknown.[13] Seventy-three percent of patients had HJHS>0 and only 20% had confirmed lower limb arthropathy. This 'discrepancy' is explained by the fact that certain HJHS items are unlikely to interfere with gait when they are mildly present but are incorporated in the HJHS score, such as crepitus on motion.

On average clinically asymptomatic hemophilia patients showed different sagittal hip, femur and pelvis ROM when compared to controls. Sagittal hip and femur ROM were similar to patients without lower limb arthropathy. This in line with what has previously been reported in hemophilia patients by Cayir et al[22], although they also found reduced sagittal knee ROM.

Stephensen et al[23] reported increased knee flexion in swing and during stance and a lack of leg straightening at the end of stance in children with a history of ankle bleeds. Increased knee flexion ROM in swing was also shown in hemophilia adults with ankle arthropathy.[24] In general, solitary ankle arthropathy was not well detected with the current set-up. However, patients with ankle arthropathy in our study in whom gait was not affected based on current GaitSmart™ parameters, did not straighten their legs at the end of stance as was seen on the gait plot. This parameter is currently not used in GaitSmart™ as some healthy people also habitually do not straighten their legs at the end of stance. Alternatively, a functional exercise test could be added to the protocol, for example single leg squats (SLS). SLS puts full loading on joints and would likely identify an ankle problem on one or both sides. This has been evaluated with GaitSmart™ in football players and is currently being investigated in patients with hip replacements[25], and should also be evaluated in hemophilia patients.

Our study is the first to evaluate gait analysis with inertial measurement units in the hemophilia population. To date, only Lobet et al have evaluated gait in adult hemophilia patients and they used three-dimensional gait analysis.[26] We performed a large prospective study in both adults and children to study its potential value. Because of inclusion criteria we did not include patients with severest arthropathy as they were unable to walk 20 meters. Therefore, a relatively small number of patients with

confirmed lower limb arthropathy were included and consequently a ceiling effect of the HJHS was observed. We did not exclude patients with degenerative arthropathy next to hemophilic arthropathy as this reflects the actual clinical situation. However, this could explain why not all patients with arthropathy were identified by the HJHS, as the score only focusses on joints prone to bleeds and degeneration often presents in hips. We did not correlate GaitSmart™ outcomes to conventional gait analysis or imaging techniques as the system has already been compared and validated to an optical system.[10] Correlation between GaitSmart™ and MRI should be studied to evaluate whether gait alterations reflect joint damage and vice versa. Due to the cross-sectional design we could only answer the question whether or not GaitSmart™ is able to detect gait abnormalities in hemophilia patients. Future studies with long-term follow-up will provide insight on the usefulness of evaluating progression of gait alterations in patients. In that case, GaitSmart™ could function as an objective measure to monitor recovery after a muscle or joint bleed, to evaluate prophylactic treatment and to substantiate physical therapist exercises, something thus far impossible.

In contrast to static imaging evaluation or passively active clinical examination, gait analysis evaluates joint function in a dynamic, weight-bearing situation[9] which is more clinically relevant.[20] Conventional gait analysis is often optical-based which is time-consuming and therefore expensive. Pressure sensitive mats, such as GAITRite®[8] can be used but still require a gait laboratory and do not give information on joint ROM and causes of altered performance, only temporal data.[27] GaitSmart™ has been developed to monitor gait objectively and accurately without the need of an optical gait lab. It enables rapid and efficient, extensive gait analysis which can be performed during regular outpatient visits to Hemophilia Treatment Centers.

We conclude that GaitSmart™ is able to detect joint-specific arthropathy, specifically of the knee and hip in hemophilia patients.

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Supplemental table 1. Definition of GaitSmart™ parameters

RANGE OF MOTION SCORE	Evaluates sagittal movement
ROM sag Knee	Average value of left and right knee flexion of the selected stride
ROM sag Tibia	Average value of left and right tibia movement of the selected stride
ROM sag Femur	Average value of left and right femur swinging of the selected stride
ROM sag Hip	Average value of left and right hip flexion of the selected stride
ROM sag Pelvis	Average value of left and right pelvis swinging of the selected stride
Stride duration	The time it takes to complete a stride (a stride is the time to take two steps, i.e. the left leg and then the right leg)
QUALITY OF GAIT SCORE	Evaluates coronal movement and flexion of knee during stance phase
ROM cor Femur	Coronal movement divided by sagittal movement (%) for the left and right femur
ROM cor Tibia	Coronal movement divided by sagittal movement (%) for the left and right tibia
Stance Flexion	Flexion in stance from knee angle plot for both the left and right knee
SYMMETRY SCORE	Negative values indicate less movement on left than right, positive values mean less movement on right than left
Symmetry Knee	Left knee range minus right knee range divided by the average knee range (%)
Symmetry Femur	Left thigh range minus right femur range divided by the average femur range (%)
Symmetry Tibia	Left calf range minus right tibia range divided by the average tibia range (%)

ROM, Range of motion; sag, sagittal; cor, coronal.

Supplemental table 2. Gait parameters in patients with arthropathy categorized per affected joint(s)

	Controls	Lower limb arthropathy	Knee L	Knee R	Ankle L	Ankle R	Both ankles, Knee L	Both ankles, both hips	Both knees, both hips	Both knees, both ankles	Knee L, ankle L	Knee L, ankle R
	N=46	N=21	N=1	N=4	N=3	N=7	N=1	N=1	N=1	N=1	N=1	N=1
HJHS lower limbs	-	7 (6)	1	8 (4)	5 (3)	5 (2)	-	8	-	28	5	10
ROM SCORE	98 (4)	87 (17)	96	78 (22)	94 (10)	98 (4)	86	86	57	68	100	50
ROM sag Knee Left °	66.49 (5.37)	60.52 (6.75)	56.09	61.29 (6.27)	62.84 (10.50)	62.25 (3.81)	62.36	70.16	50.24	53.19	61.88	47.50
ROM sag Knee Right °	65.28 (4.38)	60.72 (9.64)	69.98	49.00 (4.99)	63.62 (5.52)	66.15 (6.49)	59.58	74.13	53.78	44.73	69.21	53.84
ROM sag Tibia Left °	78.75 (5.02)	72.98 (6.71)	70.17	73.85 (6.45)	76.23 (9.78)	75.40 (3.51)	74.92	78.36	61.63	64.06	72.11	59.36
ROM sag Tibia Right °	78.22 (3.68)	73.76 (7.10)	73.91	69.79 (5.64)	74.17 (7.18)	78.58 (6.20)	76.01	79.32	66.85	62.72	76.51	61.93
ROM sag Femur Left °	43.69 (5.19)	37.86 (7.00)	40.54	39.87 (5.41)	42.45 (6.52)	40.45 (5.16)	38.77	24.22	23.77	29.04	37.90	30.79
ROM sag Femur Right °	43.76 (4.68)	40.24 (6.95)	38.24	42.50 (7.77)	43.81 (6.51)	43.21 (4.90)	42.84	26.04	27.95	35.54	36.17	34.37
ROM sag Hip Left °	42.70 (6.06)	36.06 (8.99)	41.66	39.48 (4.16)	41.74 (5.45)	39.16 (6.03)	37.29	12.80	16.91	26.84	35.36	29.07
ROM sag Hip Right °	42.87 (4.77)	38.87 (5.89)	37.94	37.67 (5.78)	44.13 (5.39)	41.41 (4.91)	42.37	34.75	26.66	34.07	34.85	32.69
ROM sag Pelvis Left °	3.87 (1.43)	4.44 (2.40)	3.05	4.22 (1.39)	3.78 (0.93)	3.64 (1.42)	3.38	12.95	7.35	4.42	5.87	2.53
ROM sag Pelvis Right °	4.03 (1.42)	4.92 (2.44)	3.81	7.16 (2.18)	3.74 (1.10)	4.04 (1.09)	6.77	11.84	3.60	3.36	3.71	2.14
Stride duration s	1.07 (0.08)	1.11 (0.11)	1.11	1.19 (0.16)	1.07 (0.05)	1.08 (0.04)	1.21	1.03	0.96	1.07	1.02	1.37
QUALITY OF GAIT												
ROM cor Femur Left %	30.21 (13.70)	27.92 (8.72)	26.10	23.08 (7.18)	21.32 (5.25)	27.38 (6.29)	15.34	45.49	38.55	41.23	29.89	41.76
ROM cor Femur Right %	28.36 (12.69)	26.73 (12.47)	24.28	22.11 (7.18)	21.71 (1.13)	26.70 (5.88)	14.78	74.66	27.90	18.14	34.74	26.42
ROM cor Tibia Left %	19.52 (9.20)	24.30 (8.60)	26.87	26.00 (10.24)	20.14 (5.00)	28.33 (10.07)	15.92	27.91	28.88	15.86	18.66	13.56
ROM cor Tibia Right %	20.00 (10.82)	16.92 (8.10)	38.90	18.72 (9.44)	14.45 (7.51)	14.20 (6.79)	14.26	16.40	11.14	20.69	14.18	22.19
Stance Flexion Left °	20.19 (5.13)	17.73 (5.67)	12.66	19.84 (3.27)	22.13 (6.91)	19.16 (3.79)	18.60	23.11	8.29	10.03	12.30	7.47
Stance Flexion Right °	19.97 (4.89)	18.02 (7.15)	25.20	9.94 (4.64)	20.33 (8.51)	20.35 (4.69)	19.59	30.25	13.26	10.33	24.69	11.94
SYMMETRY SCORE*												
Knee sym %	1.74 (7.79)	0.31 (15.45)	-22.04	22.21 (14.02)	-1.88 (12.97)	-5.80 (8.83)	4.55	-5.51	-6.81	17.29	-11.18	-12.51
Femur sym %	-0.29 (6.95)	-6.37 (7.40)	5.84	-5.70 (8.40)	-3.25 (8.43)	-6.76 (4.44)	-9.97	-7.24	-16.14	-20.13	4.67	-10.99
Thigh sym %	0.58 (4.47)	-1.03 (6.13)	-5.20	5.60 (4.61)	2.51 (3.70)	-3.94 (6.78)	-1.44	-1.22	-8.12	2.12	-5.91	-4.22

ROM, Range of motion; sag, sagittal; cor, coronal.

Data are presented as mean (standard deviation); grey reflects parameters outside range of normal.

*negative values mean less movement on left than right; positive values mean less movement on right than left.

CHAPTER 9

General discussion

GENERAL DISCUSSION

The mainstay of treatment of inherited bleedings disorders is the infusion of coagulation factor concentrates in order to prevent bleeding and to treat bleeding episodes. This treatment results in a temporary increase of coagulation factor levels. A permanent solution to restore hemostasis in patients with bleeding disorders is not yet available. Recently, the first hemophilia B patients have successfully been treated with gene therapy, resulting in an increase of factor IX levels to 2-10% of normal, thereby reducing the rate of spontaneous bleeding.[1,2] However, it will take time before such a therapy is generally available and even longer before it is available for other bleeding disorders, such as hemophilia A or Von Willebrand Disease (VWD)[3]. In the meantime we have to rely on treatment options that can only temporarily improve hemostasis including coagulation factor concentrates and desmopressin.[4] Desmopressin increases Von Willebrand factor (VWF) and factor VIII (FVIII) in the circulation and may also improve primary hemostasis in platelet disorders. Although desmopressin is not suitable for every patient, it has several advantages over coagulation factor concentrates as it is inexpensive and carries no risk of viral transmission or the development of inhibitory antibodies against coagulation factors.[5] For that reason it is of value to increase its applicability as is reflected in the fact that desmopressin was recently added to the World Health Organization's Model Lists of Essential Medicines.[6] The aim of the performed studies described in this thesis was to improve knowledge on the value of desmopressin as a hemostatic agent with the overall aim to increase its use in order to be able to prevent bleeding in patients with inherited or acquired bleeding disorders. Second, the outcome of current treatment strategies, including desmopressin was evaluated in patients with bleeding disorders.

Effects of hemostatic treatment

Variation in desmopressin response

The well-known inter-individual variation in FVIII:C increase after desmopressin, the so called desmopressin response, requires patients with mild or moderate hemophilia A to undergo a desmopressin test-dose prior to treatment. It is expected that a higher baseline FVIII level is associated with a better response to desmopressin, but whether a certain FVIII:C baseline level guarantees a certain FVIII:C peak level is still debatable.[7-9] In our large cohort of 97 mild and moderate hemophilia A patients, we observed an association between type of *F8* gene mutation and type of desmopressin response, which was also found by others.[8-12] The low response seen in patients with Arg2169His amino acid change in the C1 domain is explained by the reduced binding capacity between FVIII and VWF. Yee and Chiu et al recently showed with electron

microscopy that the C1 domain is the major binding site on FVIII for VWF[13,14]. So the reduced binding of FVIII to VWF results in a faster clearance of FVIII and therefore a lower desmopressin response. In contrast to earlier studies, we did not find consistent desmopressin responses within families and within subjects with the same mutation.[15] The RISE study, an international cohort of hemophilia A patients who have received desmopressin, consists of more patients with similar *F8* gene mutations and larger families and will likely be able to further elucidate the role of the *F8* gene mutations and other determinants of the response to desmopressin.[16]

Currently, a desmopressin test-dose consists of blood sampling prior to and one hour after infusion, sometimes also four hours. In general, a FVIII:C level ≥0.50 IU/ml is considered a good response. Ideally, the response to desmopressin can be predicted based on specific determinants and a desmopressin test-dose will become unnecessary or at least, will require as limited blood sampling as possible. By means of population based pharmacokinetic modeling this could be realized, which is currently used to optimize perioperative FVIII:C and FIX:C concentrate administration in hemophilia patients.[17] The pharmacokinetics and pharmacodynamics of FVIII after desmopressin administration have been studied previously both in healthy volunteers as well as in hemophilia A patients.[18,19] Argenti et al showed that a wide range of FVIII:C plasma concentrations are observed after a fixed desmopressin dose which is in line with the observed inter-individual variation in FVIII:C response.[18] This underlines the need for a population pharmacokinetic-pharmacodynamic model of desmopressin in hemophilia to individualize treatment. Until then, every patient should receive a desmopressin test-dose prior to its use as a treatment option. Many blood sampling protocols for a test-dose of desmopressin already limit blood sampling to only before and directly after infusion.[10,20] However, we have shown that based on FVIII:C directly (one hour) after infusion no accurate estimation of the duration of the response can be made in many patients, thus requiring a follow-up blood sample.[21]

Although the source of FVIII has long been unclear, it was recently shown that liver sinusoid endothelial cells and not hepatocytes, are the main cellular source of hepatic FVIII.[22-24] Some studies have suggested that FVIII and Von Willebrand Factor (VWF) are synthesized and stored within the same cell that functions as a desmopressin-releasable storage pool (e.g. Weibel-Palade body) and that FVIII storage is VWF-dependent.[25,26] However, other studies reported that sinusoidal endothelial cells do not express VWF and do not contain Weibel-Palade Bodies[27,28], whereas a study by Harrison et al indicated the contrary.[29] Alternatively, lung endothelial cells have been identified as a co-storage site for both FVIII and VWF[30] that express endogenous vasopressin-2 receptors.[31] Overall, the mechanism behind the desmopressin induced FVIII increase still remains to be elucidated.

Side effects of desmopressin

Although many side effects of desmopressin have been reported in the literature, including headache, flushing and nausea, it is considered a safe treatment option. Nonetheless, this opinion is based only on case reports, small case series, or studies not primarily focused on side effects.[8,32] We systematically evaluated side effects of desmopressin in a large cohort of patients with bleeding disorders. Our study only included a small number of children, in whom it has been suggested that they experience more severe side effects, such as severe hyponatremia and seizures.[33] Others have argued against this as these side effects may occur both in adults and older children and are not specifically related to age but more to uncontrolled fluid intake.[20] Several studies have investigated the response to desmopressin in children, however the side effects were not or scarcely reported.[8,11,20] Hence, future studies are needed to specifically address the issue of desmopressin side effects in children. In the Netherlands only the intravenous and intranasal route of desmopressin administration are registered, whereas in other countries subcutaneous administration is widely used.[7,19,34] The FVIII response after subcutaneous administration is similar to intravenous administration, only peak levels are achieved later.[19] As administration is easier and gives less side effects[34], subcutaneous administration seems preferable. We showed that when adequate fluid restriction is applied, desmopressin causes few temporary side effects and that it is a safe treatment option in children and adult patients with bleeding disorders.[35] This justifies increasing the clinical applicability.

Desmopressin response definition and implication for clinical use

As there is no agreed definition of an adequate desmopressin response in hemophilia A[8,11,20,36], we suggested a uniform desmopressin response definition based on clinically relevant FVIII activity (FVIII:C) cut-offs (0.30 IU/ml and 0.50 IU/ml). Hereby distinguishing between patients without response (<0.30 IU/ml), with a partial response (≥0.30-0.49 IU/ml) or a complete response (≥0.50 IU/ml) to desmopressin. As not all surgical interventions require coagulation factor levels ≥0.50 IU/ml to ensure adequate hemostasis[37], patients with only partial responses can benefit from desmopressin. In our cohort, 47% of moderate hemophilia A patients (FVIII:C 0.01-0.05 IU/ml) achieved a FVIII:C level ≥0.30 IU/ml one hour after desmopressin, i.e. sufficient for treatment in case of minor bleeding or surgery.[38] This is consistent with a study by de la Fuente et al in which 38% of patients achieved this FVIII:C level.[39] However, the RISE study recently reported that in their cohort of 81 moderate hemophilia A patients, only 21% showed FVIII:C ≥0.30 IU/ml after desmopressin.[16] An explanation for this discrepancy could be the classification of severity. The RISE study patients were identified as moderate based on their FVIII:C baseline on the day of desmopressin administration. In our study we found baseline FVIII:C prior to administration to be consistently higher than

historically lowest FVIII:C levels. As a consequence, the FVIII:C baseline level could have been overestimated in the RISE study. In the future, combined treatment with desmopressin and FVIII concentrates could result in less FVIII concentrate consumption and more desmopressin use. In theory, this could already lead to less FVIII concentrate consumption in patients with only a small FVIII:C increase after desmopressin, supporting the use of desmopressin in patients with low FVIII:C levels at baseline. The currently ongoing DAVID trial aims to study combined treatment (trial registration: NTR5383).

Desmopressin in other bleeding disorders

Desmopressin can also be used outside the scope of hemophilia A and VWD, for example in platelet disorders. Recent studies showed that although desmopressin has no direct effect on platelets, it may enhance platelet activation by the transient increase of high-molecular-weight VWF multimers.[40-42] In patients with cirrhosis, desmopressin is used in case of bleeding or as prophylactic treatment prior to invasive procedures. Traditionally, cirrhosis was considered a bleeding disorder but the existence of rebalanced hemostasis is now generally accepted. However, this seems less stable compared to healthy individuals and consequently can lead to both bleeding and thrombotic complications.[43] The rebalanced state is not well reflected by the routinely performed hemostatic tests, which complicates identifying the underlying hemostatic defect.[44] As a result, when hemostatic tests, such as an increased INR, suggest impaired coagulation and treatment is given, there may be no clinical benefit or even an opposite effect.[45] Studies reported transfusion-free liver transplantation supporting the hypothesis that patients with liver disease do not necessarily have a bleeding tendency.[46]

Evidence for the use of desmopressin in cirrhosis is inconclusive. Several studies have reported shortening of the bleeding time and an increase in different hemostatic parameters, including VWF[47-49], whereas others report a lack of efficacy.[50-52] We did not observe a beneficial hemostatic effect of desmopressin in cirrhosis, as only VWF propeptide (VWFpp) and FVIII levels increased, whereas VWF, FVIII, VWFpp, high molecular weight VWF multimers and VWF-dependent platelet adhesion increased in a control group of hemophilia A patients. We hypothesized that the lack of VWF increase may be related to the severity of liver disease. It is well known that in patients with liver disease, VWF levels are increased and that this increase is highest in the most severely affected.[53] In patients with milder disease, with less increased VWF levels, an increase of VWF after desmopressin can be expected whereas in patients with severe liver disease, no additional effect is achieved. Baseline VWF levels were indeed lower in studies in cirrhosis in which desmopressin resulted in VWF increase.[48,49]

Surprisingly, in patients with cirrhosis in our study, only VWFpp increased after desmopressin but not VWF antigen (VWF:Ag) itself. It could be that released VWF:Ag is instantly consumed, either systemically or in the liver. Alternatively, the released VWF:Ag may remain attached to the activated endothelial cells, which are known to have the capacity to bind VWF.[54] Nonetheless, the limited increased VWF:Ag may exert a pro-hemostatic effect via the enhancement of platelet adhesion in cirrhosis, as has been suggested previously.[53] This is in line with the fact that we found a non-significant improvement in platelet adhesion which corresponds to shortening of the skin bleeding time, also observed in other studies.[47-49,55] Subcutaneous administration of desmopressin in patients with cirrhosis resulted in shortening of the skin bleeding time but a lack of VWF increase[55], somewhat similar to our study.

Outcome of hemostatic treatment

Postpartum hemorrhage

Women with bleeding disorders require specialized care during pregnancy and labor to prevent bleeding complications. International guidelines recommended prophylactic hemostatic treatment before delivery if FVIII, FIX or VWF are below 0.50 IU/ml in the third trimester of pregnancy.[56,57] The aim of coagulation factor concentrate administration is to normalize coagulation factor levels and prevent postpartum hemorrhage (PPH). We studied the outcome of this current treatment strategy in a large series of women with bleeding disorders. In our study we found an increased risk of PPH in women who received prophylactic treatment. This seems counterintuitive as prophylactic treatment was administered to establish adequate hemostasis. Also others reported greater blood loss in women receiving prophylactic treatment compared to untreated women with bleeding disorders.[58] Apparently, these women need higher coagulation factor levels during labor and delivery than we currently aim for. High levels are also observed in healthy women, in whom VWF and FVIII levels of >2.00 IU/ml are found. This suggests that women with bleeding disorders are undertreated and that treatment strategies could (or should) be improved.

Therefore, we propose to increase coagulation factor target levels to 1.50-2.00 IU/ml instead of 1.00 IU/ml in patients receiving prophylactic treatment. What advocates against this proposal is the observation in the study by James *et al* that blood loss was similar in untreated women with VWD type 1 (VWF ≥0.50 IU/ml) and women without VWD.[58] The authors therefore recommended refraining from prophylactic treatment in type 1 patients when VWF activity level (VWF:Act) was ≥0.50 IU/ml. This suggests that a more differential approach is needed as treatment strategy. Also in our study, women with VWD type 1 and carriers of hemophilia A seemed to have a PPH risk comparable to the general population, which may be due to an additional physi-

ological stress increase in VWF and FVIII during delivery.[59] Hence, they could be treated according to the existing guideline, which recommends to infuse coagulation factor concentrate in women with VWF:Act or FVIII:C <0.50 IU/ml in the third trimester, aiming at a levels of 1.00 IU/ml before delivery.[60] However, carriers of hemophilia B and women with VWD type 2 lack such a physiological increase or have an increase of only dysfunctional VWF and remain at increased risk of PPH. We suggest prophylactic treatment in carriers of hemophilia B with <1.00 IU/ml FIX in the third trimester, aiming at FIX levels of 1.50 IU/ml before delivery and prophylactic treatment in all VWD type 2 patients with VWF:Act target levels of 2.00 IU/ml. This should however first be evaluated in a prospective study. General measures to be taken in all patients consist of fibrinolysis inhibitors as tranexamic acid peripartum and active labor management, including the use of (double) uterotonics.

A randomized controlled trial evaluating current treatment versus treatment with the suggested increased target levels and/or the use of tranexamic acid and/or intensifying obstetric management in relation to PPH, would be ideal but seems unfeasible. Meanwhile, international prospective registries could be initiated in which information is collected on the currently used treatment strategy and clinical outcome. Our study only evaluated primary PPH (onset within 24 hours postpartum) whereas secondary PPH (onset after 24 hours – 6 weeks postpartum) is also a serious complication, especially in hemophilia carriers with low factor VIII or IX levels.[56] This should also be incorporated in the proposed registries.

Tranexamic acid was not consistently used in our population but was recently recommended for the treatment of PPH.[61] This was based on a randomized controlled trial which showed that high-dose tranexamic acid can reduce blood loss and maternal morbidity in women with PPH.[62] The ongoing WOMAN trial (World Maternal Antifibrinolytic Trial) is an international, randomized controlled trial that aims to evaluate the effect of early administration of tranexamic acid for the treatment of postpartum hemorrhage.[63]

The use of desmopressin during pregnancy and delivery remains controversial. The main concerns are inducing maternal and fetal hyponatremia as well as inducing preterm labor by mimicking an oxytocin effect.[64] Nonetheless, it is suggested to be safe[65,66] but no randomized controlled trials have been performed to support this and no trial will be initiated based on ethical considerations as pointed out in a recent review.[67] It is recommended that the outcome of desmopressin treatment during pregnancy and delivery should also be included in the proposed registry.

Hemophilic arthropathy

The major long-term complication of severe hemophilia is the development of hemophilic arthropathy due to recurrent joint bleeds which can be seriously invalidating.

Despite regular prophylactic treatment two or three times a week with coagulation factor concentrates bleeding still occurs, especially in severe hemophilia patients.[68] Different strategies can be used to evaluate the outcome of prophylactic treatment on the musculoskeletal system, such as the Hemophilia Joint Health Score (HJHS). The HJHS is currently considered the gold standard for clinical joint health evaluation, but alternatively, imaging can be performed by X-ray, MRI or ultrasound.[69] Although this provides information on the patient's musculoskeletal status, it is a static evaluation or in case of the HJHS, based on passively active movement. Gait analysis is considered a very useful tool to monitor joint function[70], however is time-consuming and necessitates the use of expensive tools. Gait analysis has been performed previously in hemophilia but most studies were only in children[71-74] and all required a gait laboratory and/or used systems that were laborious and expensive. Although other equipment, such as three-dimensional gait analysis, may render more extensive data compared to our used inertial-measurement units, it can be questioned whether this is always essential and besides it requires specialist facilities. We have performed gait analysis with a sensor-based device that enables rapid gait assessment during dynamic, weight-bearing conditions, when generally functional limitations and pain become evident. The advantage of GaitSmart™ is that it is inexpensive, accurate and portable and consequently could be used in the outpatient department as a diagnostic screening tool as was shown recently in patients who underwent a total knee arthroplasty, in whom gait was assessed as a functional outcome measure.[75] Furthermore, the system does not use a magnetometer to measure rotation on sagittal and coronal angles and is therefore insensitive to distortion by local magnetic fields.[76] This enables its use around force plates. The use of GaitSmart™ is not only limited to the hemophilia population but can have a much broader application, i.e. all situations where gait can be affected. It is already used in patients with knee osteoarthritis and as a functional outcome measure in total knee and hip arthroplasty[75,77] and in several different sports to monitor injured players during training.[78]

An important limitation in our study was the relatively small number of patients with confirmed lower limb arthropathy. Although GaitSmart™ seemed able to identify joint-specific arthropathy in hemophilia patients, a larger group of patients in whom similar joints are affected by arthropathy, would provide further evidence for this finding. We performed a cross-sectional pilot study and consequently could not evaluate alterations over time within patients. Future studies should be conducted including follow-up to monitor the outcome of prophylactic treatment or on-demand treatment in case of a bleeding episode. In this case, also the effect of physical therapy on the musculoskeletal system could be monitored objectively. Additionally, the system could be used to evaluate the effect of orthoses on gait as many hemophilia patients have orthoses due to hemophilic arthropathy.[79]

To conclude, the studies described in this thesis provide new insights and findings regarding hemostatic treatment, in particular of desmopressin, which may change current diagnostic strategies and therapy in patients with bleeding disorders. In general, future research should be aimed at individualization of treatment as it is clear that personalized treatment is especially important in this vulnerable patient group.

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CHAPTER 10

Summary
Samenvatting

SUMMARY

In this thesis, the results of several studies focusing on the effects and outcome of hemostatic treatment in bleeding disorders, are described.

In **chapter 1**, we introduce the use of desmopressin, which causes an increase in von Willebrand factor (VWF) and coagulation factor VIII (FVIII) and is therefore a treatment option in patients with Von Willebrand disease (VWD) or hemophilia A. In general, the response to desmopressin is assessed in each patient prior to treatment as it is known that not all patients have similar responses, so called inter-individual variation. Desmopressin is usually administered in hemophilia A patients with FVIII levels ≥0.05 IU/ml or even ≥0.10 IU/ml as is stated in international guidelines. However this recommendation is based on only a limited number of studies. As desmopressin is an inexpensive drug that carries no risk of viral transmission and has no risk of the development of inhibitory antibodies against FVIII, in contrast to coagulation factor replacement therapy, further evidence to support its use is very relevant.

In **chapter 2**, we investigated the desmopressin responses in hemophilia A patients with FVIII levels <0.10 IU/ml. In 37 of 48 patients (77%), FVIII levels ≥0.30 IU/ml were observed one hour after desmopressin infusion, 17 of whom even had FVIII ≥0.50 IU/ml. Three hours after infusion, 50% still had FVIII levels ≥0.30 IU/ml. After 6 hours the majority of patients had FVIII levels <0.30 IU/ml. FVIII ≥0.30 IU/ml is considered sufficient for hemostasis in case of minor trauma or surgery. In this study we showed that the majority of non-severe hemophilia A patients with FVIII <0.10 IU/ml reaches this target level up to at least 3 hours after infusion. Although the response is short-lived, desmopressin is a treatment option for small surgery or minor injury in these patients and should be used more frequently.

To further elucidate the inter-individual variation in response, we studied the relationship between the causative *F8* gene mutation in 97 hemophilia A patients and the desmopressin response (**chapter 3**). Patients with an amino acid change Arg2169His or Pro149Arg had significantly lower FVIII levels before and after desmopressin compared to other mutations and consequently showed less response to desmopressin. In case of Arg2169His this is probably due to a reduced binding capacity between VWF and FVIII, leading to a shorter half-life of FVIII. For Pro149Arg it is unclear but may be related to less efficient secretion of the protein. The results of this chapter indicate that desmopressin response can be dependent on the type of *F8* gene mutation. Nonetheless, variable responses were observed between patients with similar *F8* gene mutations indicating that not only *F8* gene mutations but multiple factors influence the desmopressin response.

Several side effects of desmopressin have been reported previously. Desmopressin is a synthetic analogue of the antidiuretic hormone vasopressin and therefore causes water retention that can lead to (severe) hyponatremia when fluid intake is not restricted. Desmopressin selectively stimulates the vasopressin-2 receptor and this leads to a decrease in blood pressure, increase in heart rate and flushing. In most patients side effects are mild and transient, however several case-reports have been published describing severe side effects, sometimes even lethal. We performed a prospective study in 108 patients with VWD or hemophilia A who underwent a desmopressin test-dose to evaluate side effects in a large, unselected population (**chapter 4**). A change in water balance parameters was observed in a minority of patients and after 24 hours, 4 patients (4%) had hyponatremia (≤135mmol/L) but no severe hyponatremia (≤125mmol/L) occurred. After desmopressin infusion, 38% of patients were hypotensive (≤90mmHg systolic blood pressure and/or ≤60mmHg diastolic blood pressure) but this did not sustain at 24 hours. In only one patient infusion was discontinued because of nausea, tachycardia and malaise. Self-reported side effects included: headache, fatigue, flush and dizziness. The observed side effects correspond with the known effects of desmopressin. Side effects were temporary and not clinically relevant when adequate fluid restriction was applied and this supports the use of desmopressin as a safe treatment option.

In **chapter 5**, we investigated the need for a clinical desmopressin response definition as well as an individualized test-dose regimen in hemophilia. Currently, no standard definition of an adequate desmopressin response in hemophilia patients is used and definitions vary from clinical FVIII cut-offs (≥0.30 IU/ml for minor and ≥0.50 IU/ml for major bleeds or surgery) to FVIII cut-offs combined with FVIII fold increase over baseline. We evaluated the different response definitions used in the literature and found that FVIII fold increase over baseline is not associated with the peak and duration of desmopressin response. Therefore, it seems most relevant to use a response definition based solely on clinical FVIII cut-offs. In addition, we evaluated the optimal blood sampling protocol during a desmopressin test-dose and found that 40% of patients suffice with blood sampling prior to and one hour after desmopressin administration. The FVIII level one hour after desmopressin can discriminate between patients in whom additional sampling is needed in order to make a good estimation of the duration of the response, and patients in whom this can already be done based on the FVIII level after one hour.

In **chapter 6**, the results of a study on the response to desmopressin in patients with cirrhosis are described. Cirrhosis is associated with multiple changes in the hemostatic system including thrombocytopenia, platelet function defects and increased VWF and FVIII levels. Reversal of the coagulopathy is required in case of invasive procedures and desmopressin is frequently used to improve hemostasis. The mechanism by which

desmopressin improves hemostasis in cirrhosis is unclear. In this study the potential pro-hemostatic effects of desmopressin in cirrhosis were studied to provide a rationale for its use. Following desmopressin administration, only VWF propeptide and FVIII levels increased in patients with cirrhosis, whereas VWF, FVIII, VWF propeptide, high molecular weight VWF multimers and VWF-dependent platelet adhesion increased in a control group of hemophilia A patients. ADAMTS13 levels were unchanged in both groups. These results imply that desmopressin has no relevant effect in patients with cirrhosis and argue against its clinical use.

In **chapters 7** and **8**, the outcome of hemostatic treatment in bleeding disorders is studied. As discussed previously, coagulation factor replacement therapy is the most commonly used option to improve hemostasis. In women with VWD or carriers of hemophilia, coagulation factor concentrates are infused before delivery to prevent postpartum hemorrhage (PPH). During pregnancy, VWF and FVIII show a physiological increase of 150-200% leading to levels of 2.00-3.00 IU/ml, whereas factor IX only shows a minimal increase. In women with VWD or carriership of hemophilia, coagulation factors are also increased but usually not to the same extent as in healthy women. Therefore, when coagulation factor levels are ≤0.50 IU/ml in the third trimester of pregnancy, current treatment guidelines recommend coagulation factor replacement therapy prior to delivery to prevent bleeding.

In hemophilia patients, the most frequently occurring and invalidating bleeds are joint- and muscle bleeds, which can ultimately lead to hemophilic arthropathy. Prophylactic treatment with coagulation factor concentrates once to thrice weekly aims to prevent this. Whether prophylactic treatment is successful in preventing joint arthropathy can be evaluated by monitoring the musculoskeletal status in hemophilia patients. This can lead to alterations in treatment, such as dosage adjustments for prophylactic treatment, physical therapy exercises or referral to a rehabilitation specialist or orthopedic surgeon.

In **chapter 7**, the results of a retrospective study, performed to investigate the outcome of current guidelines in pregnant women with inherited bleeding disorders in three Dutch Hemophilia Treatment Centers, are described. We included 185 deliveries over a ten-year period. In 62 deliveries (34%), primary PPH (≥500 ml blood loss within 24 hours postpartum) was documented, 14 of which concerned severe PPH (≥1000 ml). In 26 deliveries, coagulation factor replacement therapy was administered, 14 (54%) of which were complicated by PPH. We found an increased PPH risk in deliveries given coagulation factor replacement therapy (OR 2.7, 95% CI 1.2-6.3). It is apparent from our results that delivery outcome in women with bleeding disorders is currently unsatisfactory. Future research and improvements are needed to reduce this unacceptably high PPH risk in these women.

In chapter 8, the MOVE-study is reported in which we performed gait analysis in 106 hemophilia patients with a sensor-based system (GaitSmart™). Monitoring of musculoskeletal function in hemophilia patients is important as this is affected by (repeated) joint- and muscle bleeds. Especially dynamic function evaluation is clinically relevant as pain and functional limitations often become clear during these situations. Gait analysis is such an example, however in general is laborious and requires extensive equipment which makes it expensive. GaitSmart™ has been developed for rapid gait analysis without the need for a gait laboratory. The system was able to identify knee- and hip- but not ankle arthropathy in hemophilia patients. Some patients without signs of arthropathy had significantly different gait parameters compared to healthy controls. The results presented in this chapter suggest that GaitSmart™ provides complimentary information to the current international gold standard for clinical joint health evaluation (Hemophilia Joint Health Score). Future studies with follow-up are needed to assess the value of GaitSmart™ in substantiating physical therapy exercises and in monitoring the recovery of a bleed and progression of arthropathy.

In chapter 9 and 10, the results of the studies described in this thesis are summarized and discussed in the view of other recent studies on desmopressin use in bleeding disorders. Also suggestions for future studies are presented.

SAMENVATTING

In dit proefschrift staan de resultaten beschreven van verschillende studies, die zich richten op de effecten en gevolgen van de behandeling van bloedingsziekten.

In **hoofdstuk 1** introduceren we het gebruik van desmopressine dat een stijging veroorzaakt van von Willebrand factor (VWF) en stollingsfactor VIII (FVIII) en zodoende een behandeloptie is voor patiënten met de ziekte van Von Willebrand (VWZ) of hemofilie A. In het algemeen wordt bij iedere patiënt voorafgaand aan behandeling de respons op desmopressine bepaald, aangezien bekend is dat niet iedereen dezelfde respons heeft, de zogenaamde interindividuele variatie. Desmopressine wordt conform huidige richtlijnen alleen gegeven aan hemofilie A patiënten met een FVIII gehalte ≥0.05 IU/ml of zelfs alleen ≥0.10 IU/ml, omdat bij lagere uitgangswaarden onvoldoende respons wordt verondersteld. Echter, deze aanbeveling is gebaseerd op een beperkt aantal studies. Aangezien desmopressine een effectief, goedkoop en veilig medicijn is waarbij er geen risico is op virusoverdracht of de ontwikkeling van remmende antistoffen tegen FVIII, is verder bewijs om het gebruik ervan te ondersteunen klinisch zeer relevant.

In **hoofdstuk 2** hebben we de respons op desmopressine bij hemofilie A patiënten met FVIII waardes <0.10 IU/ml onderzocht. Bij 37 van 48 patiënten (77%) werden FVIII waardes ≥0.30 IU/ml gezien één uur na infusie, waarvan 17 zelfs FVIII ≥0.50 IU/ml hadden. Drie uur na toediening had 50% van de patiënten nog steeds FVIII waardes ≥0.30 IU/ml. Na 6 uur had de meerderheid van de patiënten FVIII waardes <0.30 IU/ml. FVIII ≥0.30 IU/ml wordt als voldoende beschouwd voor adequate hemostase in geval van kleine ingrepen of traumata. In deze studie hebben we laten zien dat de meerderheid van niet-ernstige hemofilie A patiënten met FVIII waardes <0.10 IU/ml deze streefwaarde behaald tot ten minste 3 uur na infusie. Ondanks dat het een kortdurende respons is, is desmopressine een behandeloptie voor kleine ingrepen of verwondingen bij deze patiënten en zou vaker gebruikt kunnen worden.

Om de interindividuele variatie in respons verder op te helderen, hebben we de relatie tussen de *F8* gen mutatie en desmopressine respons bestudeerd bij 97 hemofilie A patiënten (**hoofdstuk 3**). Patiënten met een mutatie leidend tot een aminozuurverandering Arg2169His of Pro149Arg hadden significant lagere FVIII waardes voor en na desmopressine toediening in vergelijking met andere mutaties en hadden zodoende minder respons op desmopressine. In het geval van Arg2169His komt dit waarschijnlijk door een verminderde bindingscapaciteit tussen VWF en FVIII, resulterend in een kortere halfwaardetijd van FVIII. Voor Pro149Arg is het onduidelijk, maar er zou een verminderde secretie van het eiwit kunnen zijn. De resultaten in dit hoofdstuk wijzen erop dat de respons op desmopressine mede afhankelijk is van het type *F8* gen muta-

tie. Niettemin werden er verschillende responsen gezien bij patiënten met dezelfde *F8* gen mutatie, wat suggereert dat niet alleen *F8* gen mutatie maar meerdere factoren de desmopressine respons beïnvloeden.

Er zijn diverse bijwerkingen van desmopressine gerapporteerd. Desmopressine is een synthetisch analoog van het antidiuretisch hormoon vasopressine en zorgt zodoende voor water retentie wat kan leiden tot (ernstige) hyponatriëmie als er geen vochtbeperking wordt aangehouden. Desmopressine stimuleert selectief de vasopressine-2 receptoren wat leidt tot een daling van de bloeddruk, stijging van de hartslag en een rode verkleuring van het gelaat. Bij de meeste patiënten zijn de bijwerkingen mild en van voorbijgaande aard. Er zijn echter enkele case-reports gepubliceerd waarin ernstige bijwerkingen beschreven zijn, zoals acuut myocardinfarct die soms zelfs fataal waren. Om de bijwerkingen te evalueren in een grote, niet geselecteerde populatie, hebben wij een prospectieve studie uitgevoerd bij 108 patiënten met VWZ of hemofilie A die een desmopressine test ondergingen (**hoofdstuk 4**). Na 24 uur werd bij een minderheid van de patiënten een verandering in waterbalans parameters gezien; 4 patiënten (4%) hadden een hyponatriëmie (≤135 mmol/L), maar niemand ernstige hyponatriëmie, gedefinieerd als ≤125 mmol/L. Na desmopressine infusie had 38% van de patiënten hypotensie (systolische bloeddruk ≤90 mmHg en/of diastolische bloeddruk ≤60 mmHg) maar dit herstelde snel. In één patiënt werd de infusie gestaakt vanwege misselijkheid, tachycardie en malaise. Zelf gerapporteerde bijwerkingen waren: hoofdpijn, moeheid, rode verkleuring van het gelaat en duizeligheid. De opgetreden bijwerkingen corresponderen met de bekende effecten van desmopressine. Bijwerkingen waren van tijdelijke aard en niet klinisch relevant wanneer er een adequate vochtbeperking werd toegepast. Dit onderbouwt het gebruik van desmopressine als veilige behandeloptie bij patiënten met bloedingsziekten.

In **hoofdstuk 5** hebben we de noodzaak voor een klinische desmopressine respons definitie en een geïndividualiseerd bloedafname protocol bij een desmopressine test bestudeerd bij hemofilie. Op dit moment is er geen standaard definitie wat een adequate desmopressine respons bij hemofilie patiënten is. Definities variëren van klinische FVIII afkapwaardes (≥0.30 IU/ml voor kleine en ≥0.50 IU/ml voor grote bloedingen of ingrepen) tot een combinatie van deze afkapwaardes met de relatieve toename van FVIII na desmopressine. We hebben de verschillende respons definities die gebruikt worden in de literatuur geëvalueerd en gevonden dat de relatieve FVIII toename niet geassocieerd is met de piek en de duur van de desmopressine respons. Daarom lijkt een respons definitie gebaseerd op klinische FVIII afkapwaardes het meest relevant. Daarnaast hebben we het optimale bloedafname protocol bij een desmopressine test geëvalueerd en gevonden dat 40% van de patiënten kan volstaan met een bloedafname voor en één uur na desmopressine infusie. Met de FVIII waarde

één uur na desmopressine kan in deze groep een goede inschatting van de duur van de respons gemaakt worden

In **hoofdstuk 6** worden de resultaten beschreven van een studie naar desmopressine respons bij patiënten met cirrhose. Cirrhose is een ernstige leverziekte, die is geassocieerd met meerdere veranderingen in de hemostase waaronder trombocytopenie, trombocytopathie en sterk verhoogde VWF en FVIII waardes. In geval van invasieve ingrepen is behandeling noodzakelijk en desmopressine wordt vaak gebruikt om de hemostase te verbeteren. Het mechanisme waardoor desmopressine de hemostase verbetert bij cirrhose is onduidelijk. In deze studie zijn de potentiële pro-hemostatische effecten van desmopressine bij cirrhose bestudeerd om het gebruik ervan bij cirrhose te onderbouwen. Bij patiënten met cirrhose stegen na desmopressine toediening alleen VWF propeptide en FVIII. Bij de controle groep bestaande uit hemofilie A patiënten stegen VWF, FVIII, VWF propeptide en hoog moleculair VWF gewicht multimeren en nam VWF afhankelijke bloedplaatjes adhesie gemeten met een ristocetine co-factor assay toe. Waardes van ADAMTS13 bleven onveranderd in beide groepen. Deze resultaten impliceren dat desmopressine geen relevant effect op stollingsparameters heeft bij patiënten met cirrhose, wat pleit tegen het klinisch gebruik.

Hoofdstuk 7 en **8** bespreken de gevolgen van hemostatische behandeling bij bloedingsziekten. Zoals eerder besproken, is toediening van stollingsfactorconcentraat de meest gebruikte optie om de hemostase te verbeteren. Bij vrouwen met VWZ of draagsters van hemofilie wordt vaak stollingsfactorconcentraat toegediend voor de bevalling om postpartum bloedingen (postpartum hemorrhagie, PPH) te voorkomen. Gedurende de zwangerschap is er een sterke fysiologische stijging van VWF en FVIII resulterend in waardes van 2.00-3.00 IU/ml (normaal 0.60-1.40 IU/ml), terwijl factor IX slechts een minimale stijging laat zien. Bij vrouwen met VWZ of draagster van hemofilie A stijgen stollingsfactoren ook, maar niet in dezelfde mate als bij gezonde vrouwen. Om bloedingen te voorkomen is het advies in de huidige internationale richtlijnen om stollingsfactorconcentraat voor de bevalling toe te dienen, wanneer stollingsfactor waardes <0.50 IU/ml zijn in het 3^{e} trimester van de zwangerschap.

In **hoofdstuk 7** worden de resultaten beschreven van een retrospectieve studie die als doel had de uitkomst van het huidige peripartum beleid bij zwangere vrouwen met bloedingsziekten te onderzoeken in drie Nederlandse hemofiliebehandelcentra. We hebben 185 bevallingen over een periode van tien jaar geïncludeerd. Bij 62 bevallingen (34%) was primaire PPH (≥500 ml bloedverlies binnen 24 uur postpartum) gedocumenteerd, waarvan 14 ernstige PPH (≥1000 ml) betrof. Bij 26 bevallingen werd stollingsfactorconcentraat toegediend, waarvan 14 (54%) werden gecompliceerd door PPH. We hebben een verhoogd risico op PPH gevonden in bevallingen waarbij stollingsfactorconcentraat is toegediend (OR 2.7, 95% BI 1.2-6.3). Uit onze resultaten blijkt dat de uitkomst van bevallingen bij vrouwen met bloedingsziekten

op dit moment onvoldoende is. Verder onderzoek en verbeteringen zijn nodig om dit onacceptabele hoge risico op PPH bij deze vrouwen te verminderen.

De meest voorkomende en invaliderende bloedingen bij hemofiliepatiënten zijn gewricht- en spierbloedingen die uiteindelijk kunnen leiden tot hemofilie artropathie. Profylactische behandeling met stollingsfactorconcentraat één tot drie keer per week heeft als doel dit te voorkomen. Of profylactische behandeling succesvol is in het voorkomen van artropathie kan geëvalueerd worden door het controleren van de musculoskeletale status bij hemofiliepatiënten. Dit kan leiden tot veranderingen van behandeling, zoals bijvoorbeeld dosis aanpassingen van profylaxe, fysiotherapie oefeningen of verwijzing naar een revalidatiearts of orthopeed.

In hoofdstuk 8 wordt de MOVE-studie besproken waarin we ganganalyse hebben uitgevoerd met een systeem bestaande uit sensors (GaitSmart™) bij 106 hemofiliepatiënten. Het controleren van de musculoskeletale functie bij hemofiliepatiënten is belangrijk omdat dit wordt aangetast door (herhaalde) gewricht- en spierbloedingen. Met name dynamische functie evaluatie is klinisch relevant omdat pijn en functionele beperkingen doorgaans zichtbaar worden in deze situaties. Het bestuderen van het looppatroon door middel van ganganalyse is daar een voorbeeld van, maar is doorgaans echter tijdrovend en vereist uitgebreide apparatuur wat het duur maakt. GaitSmart™ is ontwikkeld om op snelle wijze ganganalyse uit te voeren zonder het gebruik van een looplaboratorium. Het systeem bleek in staat om knie- en heup-, maar niet enkelartropathie te identificeren bij hemofiliepatiënten. Sommige patiënten zonder tekenen van artropathie hadden significant andere gang parameters in vergelijking met gezonde controles. De resultaten in dit hoofdstuk suggereren dat GaitSmart™ aanvullende informatie geeft naast de huidige internationale gouden standaard voor klinische gewrichtsevaluatie (Hemophilia Joint Health Score). Verdere studies waarin patiënten vervolgd worden in de tijd, zijn nodig om de meerwaarde vast te stellen van GaitSmart™ in het onderbouwen van fysiotherapie oefeningen en in het controleren van het herstel na een bloeding en de verergering van artropathie.

In hoofdstuk 9 en 10 worden de resultaten van de beschreven studies samengevat en bediscussieerd in het licht van andere, recente studies naar desmopressine gebruik bij bloedingsziekten. Daarnaast worden ook suggesties gedaan voor toekomstig onderzoek.

List of publications

LIST OF PUBLICATIONS

Stoof SCM, Leebeek FWG,Cnossen MH, Kruip MJHA.
Desmopressin in hemophilia: a proposal for a clinical response definition and individualized test-dose regimen. Submitted.

Stoof SCM, Hodgins D, Horemans HLD, Cnossen MH, Leebeek FWG, Praet SFE, Kruip MJHA.
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Side effects of desmopressin in patients with bleeding disorders. *Haemophilia.* 2015;June online. doi: 10.1111/hae.12732.

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Infusion of DDAVP does not improve primary hemostasis in patients with cirrhosis. *Liver Int.* 2015;35:1809-15.

Stoof SCM, Sanders YV,Cnossen MH, de Maat MPM, Leebeek FWG, Kruip MJHA.
Desmopressin response in Hemophilia A patients with FVIII:C <0.10 IU/ml. *J Thromb Haemost.* 2014;12:110-2.

Stoof SCM, Sanders YV, Petrij F, Cnossen MH, de Maat MH, Leebeek FWG, Kruip MJHA.
Response to desmopressin is strongly dependent on *F8* gene mutation type in mild and moderate Hemophilia A. *Thromb and Haemost.* 2013;109(3):440-449.

Scientific sessions/ Awards and prizes

SCIENTIFIC SESSIONS

2015 **XXV Congress of the International Society on Thrombosis and Haemostasis (ISTH), Toronto, Canada**

§ Poster presentation: *"Measurement of gait variability in patients with and without hemophilic arthropathy; MOVE-study"*

2015 **Musculoskeletal Conference, World Federation of Hemophilia, Belfast, United Kingdom**

§ Oral presentation: *"Measurement of gait variability in patients with and without hemophilia arthropathy; MOVE-study"*

2013 **XXIV Congress of the International Society on Thrombosis and Haemostastis (ISTH), Amsterdam, the Netherlands**

§ Oral presentation: *"High rate of postpartum hemorrhage in women with Von Willebrand Disease and carriers of hemophilia, despite specialized care"*

§ Oral presentation: *"Prediction of the extent and duration of desmopressin response in moderate and mild hemophilia A"*

2013 **Symposium Dutch Society of Thrombosis and Haemostasis (NVTH), Koudekerke, the Netherlands**

§ Oral presentation: *"High rate of postpartum hemorrhage in women with Von Willebrand Disease and carriers of hemophilia, despite specialized care"*

2012 **Bayer Hematology Conference, Dresden, Germany**

§ Poster presentation: *"Response to desmopressin in hemophilia A patients with low FVIII levels"*

§ Poster presentation: *"Response to desmopressin is strongly dependent on F8 gene mutation type in mild and moderate hemophilia A"*

2012 **World Federation of Hemophilia (WFH) 2012 World Congress, Paris, France**

§ Poster presentation: *"A prospective study on side effects of desmopressin in patients with bleeding disorders"*

2012 **European Association of Haemophilia and Allied Disorders (EAHAD), the 5th Annual Congress, Rome, Italy**

§ Poster presentation: *"Response to desmopressin in hemophilia A patients with low FVIII levels"*

2012 **Sixth Dutch Hematology Conference, Papendal, the Netherlands**

§ Oral presentation: *"Response to desmopressin in hemophilia A patients with low FVIII levels"*

2011 **American Society of Hematology (ASH), 53rd Annual Congress, San Diego, United States**

§ Oral presentation: *"Relation between mutations in F8 gene and response to desmopressin in patients with hemophilia A"*

AWARDS AND PRIZES

2014 **Unrestricted Research Grant**
Investigator Initiated Research, Pfizer B.V.

2013 **Young Investigator Award**
XXIV Congress of the International Society on Thrombosis and Haemostastis

2013 **Scientific Excellence Award**
Abstract award, annual symposium Dutch Society of Thrombosis and Haemostasis (NVTH)

2012 **CSL Behring Prof. Heimburger Award 2012**

2012 **First abstract prize**
European Association of Haemophilia and Allied Disorders (EAHAD), the 5th Annual Congress

Dankwoord

DANKWOORD

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Marc, als student belandde ik aan jouw oude bureau op L-4 en jij bij het raam. Ik heb een leuke tijd gehad met jou, Fazil en later Annemiek. Ik luisterde graag mee met de klinische vraagstukken die langskwamen!
Ook dank aan de andere collega's van de afdeling voor de kletspraatjes in de gangen en de gezelligheid en in het bijzonder de secretaresses voor de hulp bij het oplossen van allerhande problemen!

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Margot, inmiddels geen huisgenoot meer, maar nog steeds goede vriendin, dank voor je interesse!
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Lieve TJ, AM en TJP, jullie vinden het vanzelfsprekend, maar ik zeg het toch: dank voor alles!
Lieve Maarten, wie had dat gedacht? Dank voor je aandacht en rust, met jou is het leuker!

Curriculum vitae

CURRICULUM VITAE

S. Carina M. Stoof werd geboren op 17 oktober 1989 te Heemstede. Zij heeft in 2007 haar gymnasium diploma behaald aan de Katholieke Scholengemeenschap Hoofddorp. In september van dat jaar is zij via de decentrale selectie gestart met de studie geneeskunde aan de Erasmus Universiteit Rotterdam. Haar afstudeeronderzoek deed zij gedurende 2011 op de afdeling hematologie van het Erasmus Universitair Medisch Centrum onder leiding van dr. M.J.H.A Kruip waarop zij in november van dat jaar haar doctoraal diploma behaalde. In navolging op haar afstudeeronderzoek begon zij in januari 2012 haar promotieonderzoek op de afdeling hematologie, onder supervisie van dr. M.J.H.A. Kruip en prof.dr. F.W.G. Leebeek. Het promotieonderzoek resulteerde in het proefschrift dat nu voor u ligt. In november 2015 is zij begonnen met de coschappen en in 2017 hoopt zij haar artsexamen te behalen.

PhD portfolio

PHD PORTFOLIO SUMMARY

Name PhD student: S.C.M. Stoof
Erasmus MC Department: Hematology
Research School: COEUR

PhD period: January 2012 to June 2015
Promotor: Prof. Dr. F.W.G. Leebeek
Copromotor: Dr. M.J.H.A. Kruip

1. PhD training	Year	Workload (Hours/ECTS)
General academic skills		
Presentation training	2013	1.5
Course Research Integrity	2014	0.3
Research skills		
Introduction to clinical research (NIHES)	2012	0.9
Biostatistics for clinicians (NIHES)	2012	1.0
Regression analysis for clinicians (NIHES)	2013	1.9
In-depth courses (e.g. Research school, Medical Training)		
3x NVTH annual AIO course on hemostasis and thrombosis	2012-2014	3.0
COEUR course on Cardiovascular Medicine	2013	1.5
Presentations		
Oral presentation American Society of Hematology	2011	0.5
Oral presentation Dutch Hematology Congress	2012	0.8
Oral presentation COEUR PhD day	2012	0.8
2x Oral presentation Prof. Heimburger award conference	2012,2014	1.6
Oral presentation NVTH symposium	2013	0.8
2x Oral presentation International Society on Thrombosis and Haemostasis	2013	1.6
Invited speaker International AFFIRM meeting, haemophilia society	2014	0.8
Oral presentation WFH Musculoskeletal Congress	2015	0.8
5x Poster presentation EAHAD 2012, WFH 2012, Bayer Hematology Conference 2012, ISTH 2015	2012-2015	1.5
International conferences		
Dutch Hematology Congress	2012	0.3
European Association of Hemophilia and Allied Disorders Congress	2012	0.9
4x NVTH symposium	2012-2015	2.1
Prof. Heimburger award conference, Marburg	2012	0.3
2x World Federation of Hemophilia Congress	2012,2014	3.0
Bayer Hematology Conference, Dresden	2012	0.6
2x International Society on Thrombosis and Haemostasis congress	2013,2015	3.6
Van Creveld symposium	2014	0.3
Maastricht Consensus Conference on Thrombosis	2015	0.3
WFH Musculoskeletal Congress	2015	1.2

	Year	Workload (Hours/ECTS)
Seminars and workshops		
3x NVTH PhD day	2012-2014	1.2
8x COEUR research seminars and lectures	2012-2015	2.9
2x COEUR PhD day	2012-2013	0.8
2x Local training for hematologists	2012-2013	0.6
3x Local seminar on hematological cases	2012-2014	0.3
2. Teaching activities		
Lecturing		
10x Coagulation lecture for nurses	2012-2014	1.0
Supervising practicals and excursions		
2x review 2nd year medical students (coagulation course)	2013-2014	1.0
4x 2nd year medical students (coagulation course)	2013-2015	0.4
Supervising Master's thesis		
Supervising final year medical student (1x 27 weeks)	2014	1.0
Other		
Developing teaching material for coagulation course	2012	0.5
Total		41.6