Variation in FVIII / FIX activity in haemophilia: classification and clinical implications

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Variation in FVIII / FIX activity in haemophilia: classification and clinical implications

Variatie in FVIII / FIX activiteit in hemofilie: classificatie en klinische implicaties

(met een samenvatting in het Nederlands)

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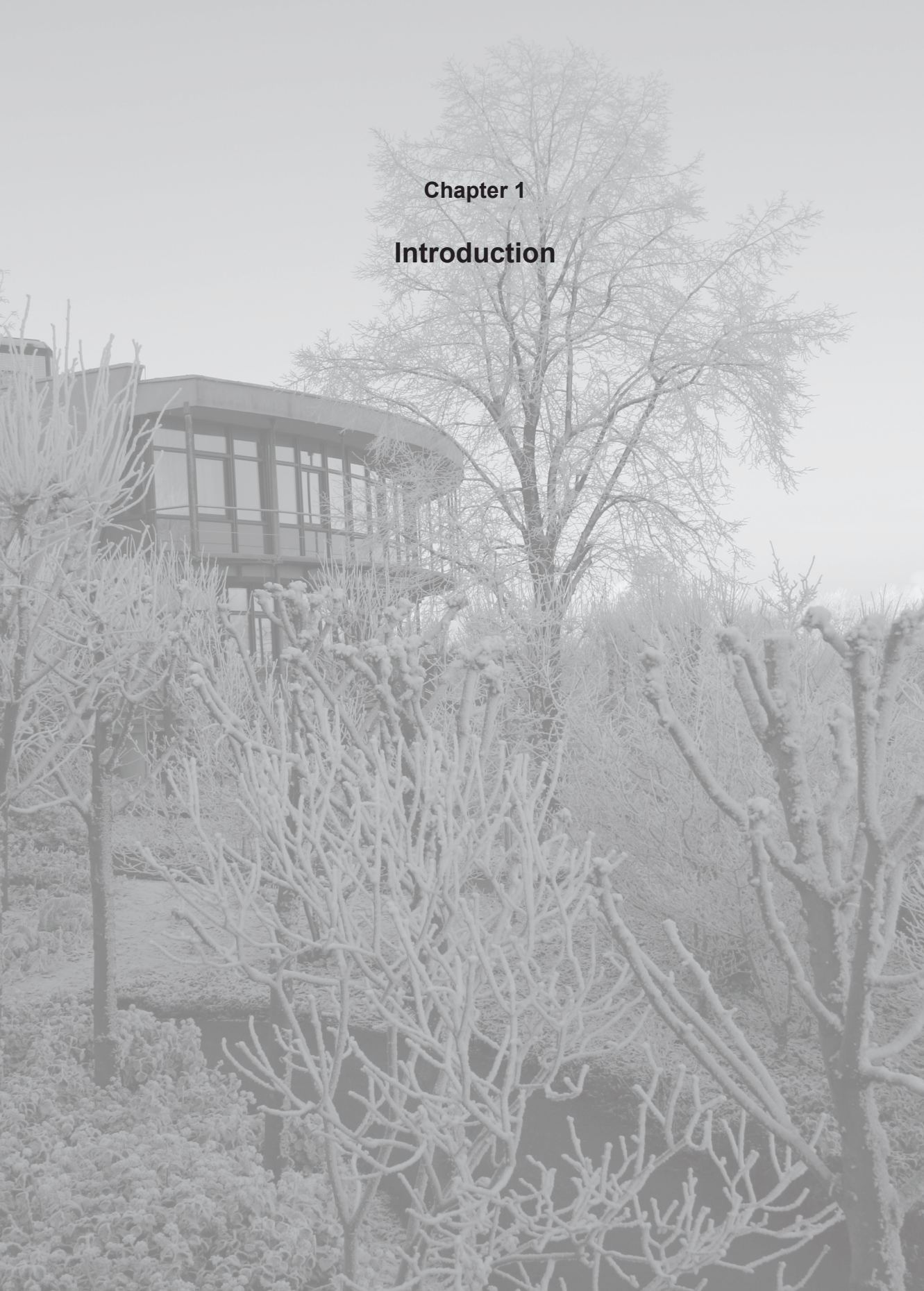
Contents



Chapter 1

Introduction

Haemophilia is an inherited, x-linked bleeding disorder. Patients with haemophilia lack factor VIII (FVIII) or factor IX (FIX) causing spontaneous or prolonged bleeding. The hallmark of severe haemophilia is recurrent joint bleeding, eventually leading to severe arthropathy. Functional arthropathy-related limitations and the awareness of an incurable disease needing lifelong treatment have a large impact on a patient's daily life.

Classification of haemophilia

Haemophilia is classified into two types and three severities. About 85% of patients lack FVIII, resulting in haemophilia A. The prevalence of haemophilia A is estimated to be around 1 in 10.000 males[1]. Patients with haemophilia B lack FIX, which has a prevalence of 1 in every 35.000 males[1]. Haemophilia B is also known as Christmas disease, after the first patient ever diagnosed[2]. Until the 1950s, both types were perceived as different disease, rather than two types of the same disease[3].

The second system of classification, according to severity, is based on laboratory characteristics. This classification was first described by Biggs and MacFarlane in 1958 when they compared clinical outcome and laboratory assays of 187 patients with haemophilia A or B[4]. In 2001, this classification was accepted as standard by the scientific subcommittee of the International Society of Thrombosis and Haemostasis[5]. Three severities are distinguished: approximately 40% of patients have severe, 20% have moderate and 40% have mild haemophilia[6,7]. As reference, patient without haemophilia have 100% or 100 IU/dl FVIII/FIX activity. Patients with severe haemophilia have less than 1% or <1IU/dl FVIII/FIX activity. Severe haemophilia is characterized by spontaneous bleeding in joints and other tissues. Patients with moderate haemophilia have 1-5 IU/dl FVIII/FIX activity, 1-5% of normal. These patients have few spontaneous joint bleeds, but can bleed extensively after minor trauma. Patients with mild haemophilia have 6-40 IU/dl FVIII/FIX activity, 6-40% of normal. Mild haemophilia patients rarely suffer from bleeding complications, only after trauma they show prolonged bleeding. Above 40 IU/dl FVIII/FIX activity, bleeding risk is minimal and these levels are not considered abnormal[5,8].

Moderate haemophilia

Haemophilia can be treated with intravenous replacement of FVIII (in haemophilia A) or FIX (in haemophilia B). Replacement therapy with clotting factor concentrates has been readily available since the early 1960s[9]. At first, patients were only treated on demand, i.e. treatment after a bleeding episode. In 1970s, Nilsson started to treat patients with severe haemophilia prophylactically, i.e. replacement factor to prevent (joint) bleeding[10]. Nilsson provided prophylaxis at regular intervals, increasing the baseline factor activity level from <1 IU/dl to, currently, at least the baseline factor activity level of moderate haemophilia, >1 IU/dl. The rationale was that with increased factor activity, severity of bleeding decreased to the milder bleeding pattern of moderate haemophilia[11]. Moderate haemophilia is the rarest form of haemophilia[7]. In 1965, Ahlberg et al[12] published a comparison of outcome in severe and moderate haemophilia. In that study, 20% of patients with moderate haemophilia had complications and joint dysfunction comparable to severe haemophilia patients. Since then, most studies have been performed on treatment and orthopaedic improvement of severe haemophilia patients[13]. Some studies have addressed moderate hae-

mophilia patients, but usually combined with mild haemophilia. Although moderate haemophilia is the target for prophylaxis in severe haemophilia, little is known about long term outcome in patients with moderate haemophilia.

Hypotheses

This thesis addresses the following hypotheses. First, the classification of haemophilia remained unchallenged for 60 years. Is it justified to associate increasing factor activity with a decrease in clinical severity? Second, some patients with moderate haemophilia show symptoms similar to severe haemophilia, yet moderate haemophilia often is classified as mild haemophilia and treated accordingly. Are these patients treated adequately? Third, prophylaxis aims to turn severe haemophilia into moderate haemophilia, but is this possible?

Research questions

- Does the classification of haemophilia from the 1950s still stand?
- Are patients with moderate haemophilia undertreated?
- Is severe haemophilia turned into moderate haemophilia with long-term prophylaxis?

Outline of this thesis

Part 1. Classification in haemophilia: right or wrong?

The classification of haemophilia has been unchallenged since the 1950s. In *Chapter 2*, we compared self-reported outcome across severities, according to the current classification, to determine whether severe, moderate and mild haemophilia have in fact different outcomes.

Bleeding frequency decreases with increasing factor activity level. In *Chapter 3* the association of self-reported bleeding frequencies with baseline factor activity levels was modelled to quantify this decrease.

Severity of haemophilia is not only determined by the number of bleeds, but also by the onset of bleeding. In *Chapter 4*, the current classification is compared to the age at diagnosis of haemophilia, onset of treatment and joint bleeding. Additionally, the association between joint bleeding frequency and factor activity level is revisited with more objective data, extracted from patient files.

Clinical opinion is that haemophilia B has a milder bleeding phenotype than haemophilia A. In *Chapter 5*, joint bleeding and age at onset of joint bleeding was compared between both haemophilia types.

Inhibitors are a negative side-effect of replacement therapy. In severe haemophilia peak treatment is a well documented risk factor for inhibitor development. *Chapter 6* describes inhibitor status and the association with peak treatment in moderate and mild haemophilia.

Part 2. Moderate haemophilia

Little is known about long-term outcome in moderate haemophilia. *Chapter 7* provides an insight in bleeding frequency, joint status and quality of life of patients with moderate haemophilia.

The goal of prophylaxis is to turn a severe bleeding pattern into a more moderate one, essentially turning severe haemophilia into moderate haemophilia. *Chapter 8* determines whether this goal is reached by comparing outcome in moderate haemophilia to outcome in prophylactically treated patients with severe haemophilia.

Arthropathy is the hallmark of haemophilia. Recently, MRI has become available for monitoring joint disease. *Chapter 9* describes the results of a pilot study to assess the value of MRI diagnostics for assessment of joint damage in teenagers and adolescents with moderate or severe haemophilia.

Professor van Creveld used to let healthy donors run up and down the stairs in order to increase the level of FVIII in donor plasma (Els Haan, personal communication). In *Chapter 10*, we tested the capability of patients with moderate or mild haemophilia A to increase their FVIII activity levels after physical activity.

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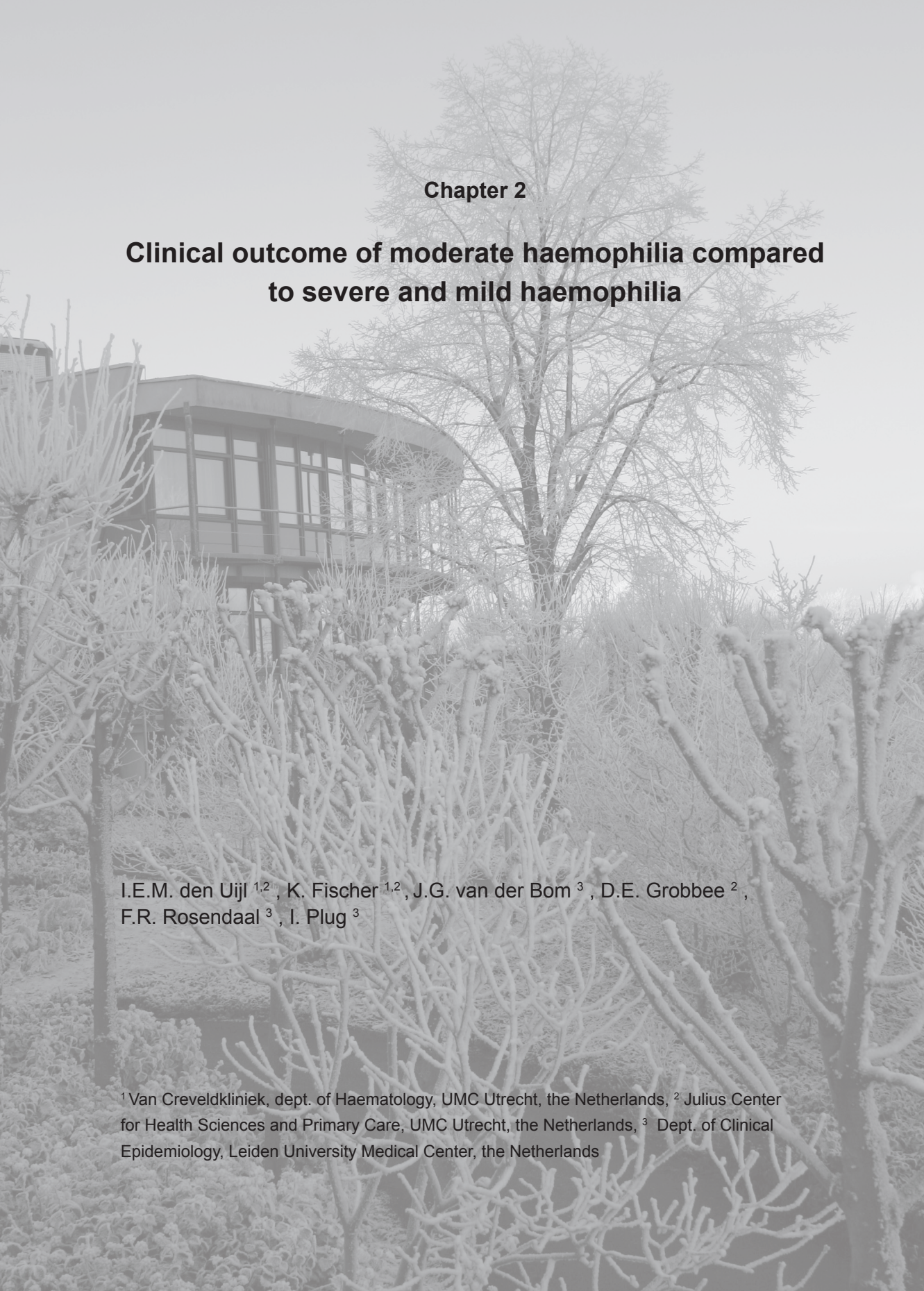

Chapter 2

Clinical outcome of moderate haemophilia compared to severe and mild haemophilia

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Abstract

Information on outcome in moderate haemophilia is scarce, little is known about outcome and treatment of moderate haemophilia. In this study we compared self reported burden of disease in moderate haemophilia to severe and mild haemophilia.

A nationwide questionnaire on bleeding pattern, treatment, impairment and quality of life was sent to 1567 Dutch patients with haemophilia. Their disease severity was cross-checked with laboratory results from the patient files of the participating clinics.

Out of 1066 respondents (response rate: 68%), 16% had moderate, 44% severe and 39% mild haemophilia. Median age was 36 years. Although overall outcome in moderate haemophilia was in between severe and mild haemophilia, moderate haemophilia patients did report a substantial burden of disease. The majority of patients with moderate haemophilia (73%) reported bleeds in the previous year, and a considerable proportion of moderate patients reported joint impairment (43%), chronic pain (15%), needed orthopaedic aids (24%) or were unemployed because of disability (27%).

Within the group of moderate haemophilia patients, a large variation in bleeding pattern and outcome was observed. A subgroup (26%) reported a more severe phenotype and intermittent use of prophylaxis. They experienced frequent bleeding, with a median of 8 bleeds per year, including 2 joint bleeds, and 68% reported joint impairment.

Although outcome in moderate haemophilia is generally in between severe and mild haemophilia, moderate haemophilia patients reported a substantial burden of disease, and for more than a quarter of patients with moderate haemophilia long-term prophylaxis was implemented because of frequent bleeds.

Introduction

Haemophilia is a hereditary, sex-linked bleeding disorder. Patients are deficient in either coagulation factor VIII (haemophilia A) or coagulation factor IX (haemophilia B). Further classification of haemophilia patients is based on residual levels of factor VIII or IX, first mentioned by Biggs and MacFarlane in 1958[1] and has been adopted as standard since 2001[2]. This classification is not based on the clinical expression of the disease, but on FVIII/ IX activity levels measured in a patient's blood. Normal factor VIII/IX activity levels are defined at 1 IU/dl or 100%[2].

Patients with severe haemophilia have less than 1 IU/dl factor VIII or IX. Severe haemophilia patients are characterized by a high bleeding tendency with frequent spontaneous joint and muscle bleeds. Repeated joint bleeds and subsequent arthropathy will eventually lead to functional impairment[3]. In the Netherlands, and many other Northern European countries, severe haemophilia patients are treated with prophylaxis; a regular infusion of factor concentrates administrated at home at 1-3 days' interval, in order to reduce bleeds and joint damage[4]. Prophylaxis is preferably introduced after one or two joint bleeds[5]. In contrast, patients with mild haemophilia, who have 6-40 IU/dl residual factor VIII or IX, rarely bleed spontaneously. Bleeds in mild haemophilia patients occur only after trauma or surgery; therefore patients with mild haemophilia are treated on demand. Usually, mild haemophilia patients have no functional limitations.

Moderate haemophilia is diagnosed in 20% of haemophilia patients. Except for two scientific articles[6,7], little is known about the outcome and treatment in moderate haemophilia. By classification, it would be expected that bleeding rate, joint function and limitations in daily life of patients with moderate haemophilia are in between those of severe and mild haemophilia. This assumption is widely adopted, but formal descriptions of outcome in moderate haemophilia are lacking. Clinical impression is that treatment of haemorrhages in moderate haemophilia is often delayed, possibly due to less prophylactic and home treatment in combination with less experience in recognizing a bleed. This may result in undertreatment of moderate haemophilia patients, leading to joint damage and limitations in daily life.

Assuming outcome in patients with moderate haemophilia is in between outcome in mild and severe haemophilia patients, what disease burden do patients with moderate haemophilia actually experience? The aim of this study was to describe bleeding pattern, joint impairment, daily functioning and quality of life in moderate haemophilia compared to severe and mild haemophilia.

Patients and methods

This study was based on data from the 5th 'Haemophilia In the Netherlands' (HIN) questionnaire, administered nationwide in 2001. The questionnaire was sent to 1567 patients with haemophilia listed with the Dutch Haemophilia Society and all haemophilia centres in the Netherlands, as well as to patients participating in earlier surveys[8]. The Haemophilia In the Netherlands questionnaire included items on treatment, annual number of bleeding episodes in 2000, degree of joint impairment, disability, employment, absence from school or work, sports and daily living. The generic SF-36 on Health-Related Quality of Life questionnaire (HRQoL) was also included. Self-reported severity and type of haemophilia were cross-checked with laboratory results in patient files from the participating clinics[8].

Disablement Process

Variables were classified according to the domains of the Disablement Process, reporting on the domains of pathology, impairment, functional limitations, and disability[9,10]. In this study the domain 'pathology' (diagnosis and disease) contained haemophilia severity. A previous study on quality of life showed no distinction between haemophilia A and B, therefore haemophilia type was not considered. 'Impairment' (dysfunctions and structural abnormalities in specific body systems) consisted of self-reported bleeding frequency in 2000, impairment in knee, ankle or elbow due to haemophilia, pain and use of orthopaedic aids. 'Disability' (difficulty performing activities in daily life) comprised employment or school, occupational disability and participation in sports. 'Functional limitations' (restrictions in basic physical and mental actions) were assessed by the physical domains of the SF-36 questionnaire; physical functioning, physical role and bodily pain.

Data analysis

Since data were not normally distributed, medians with 10-90 percentiles and percentages were reported. To allow for better interpretation of the data, confidence intervals for all proportions/percentages were calculated by STATA 9.0. Treatment and outcomes are presented according to severity. Additionally, outcome in moderate haemophilia patients is presented according to treatment regimen.

Home treatment was defined as treatment by patient, parent, guardian or partner outside a hospital setting. On demand treatment was defined as treatment in case of a bleeding episode. Continuous prophylaxis was defined as regular infusions of factor concentrate, with a minimum of twice a week. Intermittent prophylaxis comprised treatment on demand with prophylaxis on days with special activities.

Questions about bleeding and bleeding frequency were asked over the year previous to the administration of the questionnaire (2000). As the SF-36 questionnaire is only validated for patients aged 17 and onwards, data on SF-36 were analysed for patients aged over 17 years only. Participants were asked per joint to what extent it was impaired due to haemophilia: no impairment, some impairment without impact on daily functioning, some impairment with impact on daily functioning, or severe impairment with complete loss of function. For this analysis, impairment was defined as any impairment in a joint. Work and occupational disability were analysed for patients who answered questions about employment only, excluding participants who had never held a regular job. In the questionnaire, weekly time spent at sports was registered categorically. For purpose of analysis these categories were converted to a scale variable. Categories 0- ½, ½-1, 1-2, 2-3, 3-4, 4-6, 6-8 and more than 8 hours/week were recoded as mean of the category: 0.25, 0.75, 1.5, 2.5, 3.5, 5, 7 and 8 hours/week respectively.

Results

A total of 1066 Dutch haemophilia patients returned the questionnaire, a response rate of 68%. The characteristics of this group are presented in Table 1. A total of 16% of participants had moderate haemophilia, 44% severe and 39% mild. Type A haemophilia was reported in 85% of all respondents. The youngest participant for whom the questionnaire was completed was less

than one year old, the oldest patient was 90 years of age, with a median age of 36 years. Most moderate and mild haemophilia patients reported a positive family history, while 52% of patients with severe haemophilia were the first in the family with haemophilia. The number of moderate haemophilia patients infected with HIV (Human Immunodeficiency Virus) or HCV (Hepatitis C) was, with 3% and 34% respectively, in between frequencies for severe and mild haemophilia.
In contrast to those with severe haemophilia, patients with moderate haemophilia used less home treatment (32%) or prophylactic treatment (26%). As expected in mild haemophilia, few patients received prophylaxis (9%) or used home treatment (7%).

Table 1. Patient characteristics and treatment of patients with haemophilia, n=1066

	mild	moderate	severe
n	470	176	420
Median age (years)	39 (8-64)	32 (6-64)	32 (5-58)
Haemophilia A	88% (85-91)	88% (83-93)	85% (82-89)
Positive family history	72% (68-76)	77% (71-83)	48% (44-53)
HIV infected	2% (-1-1)	3% (0-4)	9% (3-8)
Current HCV infection	18% (13-20)	34% (27-41)	49% (44-54)
Home treatment	9% (6-12)	32% (25-39)	82% (79-86)
Any prophylactic treatment	7% (5-9)	26% (20-33)	84% (81-88)

Values presented are percentages (95% confidence interval), or medians (10 - 90 percentiles)

Bleeding

The total number of bleeds, as well as the total number of joint bleeds of patients with moderate haemophilia were in between those with severe and mild haemophilia (Table 2). The median number of all bleeds was only one per year, but 73% (95%CI 66-79%) of moderate haemophilia patients did report bleeding during the previous year. The median number of joint bleeds was low in patients with moderate haemophilia; the majority (61%) had had no joint bleeds.

Table 2. Outcome in haemophilia patients of all 1066 haemophilia patients in the year 2000

	mild	moderate	severe
Annual no. of bleeds	0 (0-3)	1 (0-13)	6 (0-25)
Annual no. of joint bleeds	0 (0-0)	0 (0-6)	3 (0-15)
Without bleeds	61% (57-64)	27% (21-34)	8% (5-10)
Without joint bleeds	91% (88-93)	61% (54-68)	34% (30-39)
Without any impaired joints	72% (68-76)	57% (50-64)	39% (34-44)
Pain	7% (4-9)	15% (10-21)	29% (24-33)
Use of orthopaedic aids	11% (9-15)	24% (17-30)	49% (43-53)

Values presented are percentages (95% confidence interval), or medians (10 - 90 percentiles)

Physical impairment

In general, physical impairment followed the classification of haemophilia. Nevertheless, a significant proportion (43%; 95%CI 36-50%) of patients with moderate haemophilia reported joint impairment, of which 32% had two or more impaired joints (Table 2).
Use of orthopaedic aids, like crutches and walking sticks, was reported by 24% of moderate hae-

mophilia patients, compared to 49% in severe and 11% in mild haemophilia. Complaints of pain were also less frequent in patients with moderate compared to patients with severe haemophilia. However, 15% of moderate haemophilia patients reported pain, in contrast to 7% of mild haemophilic patients.

Table 3. Socio-economic characteristics of haemophilia patients in the year 2000

	mild	moderate	severe
Fulltime/part-time job	84% (80-88)	78% (70-86)	75% (69-80)
Occupational disability	24% (19-28)	27% (18-35)	41% (35-48)
Sick leave from work (days)	2 (0-26)	2 (0-33)	4 (0-49)
Absence in school (days)	2 (0-17)	3 (0-14)	5 (0-20)
Sports participation	56% (52-61)	54% (47-61)	52% (48-57)
Participation in sports (hours/week)	2.5 (0-5)	1.5 (0-5)	1.5 (0-7)

Values presented are percentages (95% confidence interval) or medians (10 - 90 percentiles)

Disability

Almost half of patients with moderate haemophilia (46%; 95%CI 39-53%) considered their haemophilia a serious disease. Although most moderate haemophilia patients kept a fulltime or part-time paid job (78%), occupational disability, either partly or fully, was mentioned by 27% (95%CI 18-35%). Most severe haemophilia patients also kept a paid job, but the proportion of patients reporting occupational disability was a little higher (41%) than in moderate haemophilia. As expected, mild haemophilia patients reported the highest participation in paid work and least occupational disability. The median number of days with sick leave or absence from school due to haemophilia was 2-3 days in moderate haemophilia, again in between reports by severe and mild haemophilia patients. Participation in sports was around 50% across severities, although both moderate and severe haemophilia patients spent less time at sports than mild patients.

Table 4. Outcome in patients with moderate haemophilia according to treatment regimen

	on demand	any prophylaxis
n	125	46
Age	32 (4-65)	31(9-62)
Annual no. of bleeds	1 (0-8)	8 (0-24)
Annual no. of joint bleeds	0 (0-3)	2 (0-17)
More than 9 bleeds/ yr incl. at least 3 joint bleeds	4% (1-7)	35% (21-49)
Without any bleeds	35% (27-44)	2% (-2-6)
Without joint bleeds	72% (64-80)	28% (15-41)
Without impaired joints	66% (58-75)	32% 19-46

Values presented are percentages (95% confidence interval) or medians (10 - 90 percentiles)

HRQoL

The scores for the different severities from the SF-36 questionnaire are shown in Figure 1. SF-36 was only analysed for participants over 17 years, resulting in scores for 125 moderate, 280 severe and 363 mild haemophilia patients. Mental health scores were similar in all participants. The physical domains appeared to be more sensitive to severity of haemophilia. In the domains

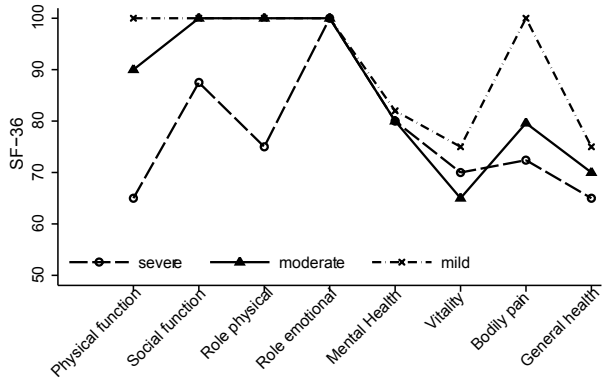
of 'physical functioning' , 'role limitations due to physical functioning' and 'bodily pain' , patients with moderate haemophilia scored on average 16 points higher than those with severe, but 13 points lower than those with mild haemophilia. Although these differences are considered clinically relevant[11], variation within the severities was large and SF-36 was not significantly different across severities.

Outcome in patients with moderate haemophilia

In order to provide more detail on moderate haemophilia, outcome in moderate haemophilia patients is presented according to treatment regimen in Table 4. Patients with moderate haemophilia treated with prophylaxis reported worse outcome compared to patients treated on demand. Out of all 46 moderate haemophilia patients on prophylaxis (26%; 95%CI 20-33%), 19 patients received prophylactic treatment continuously, while the remaining 27 used intermittent prophylaxis. Despite prophylactic treatment, 35% reported more than 9 bleeds and at least 3 joint bleeds per year.

Patients on prophylaxis suffered a median of 4 bleeds per year, including 2 joint bleeds, while patients treated on demand experienced a median of 1 soft tissue bleed per year and no joint bleeds. Moderate haemophilia patients on prophylaxis also reported more impairment (78% reported having at least one impaired joint) than moderate haemophilia patients treated on demand, of whom 44% reported having one or more impaired joints (Table 4). From these data, it was clear that patients with more severe bleeding patterns were prescribed prophylaxis, while those with only infrequent bleeds were treated on demand.

Figure 1. Results from SF-36 questionnaire from all 768 patients aged over 17 years, according to severity of haemophilia



Discussion

This study provides an elaborate description of self-reported outcomes in moderate haemophilia compared to severe and mild haemophilia. In accordance with the classification, outcome in moderate haemophilia was in between outcome of severe and mild haemophilia. Accordingly, a considerable proportion of moderate haemophilia patients reported joint impairment (27%), chronic pain (15%) and needed orthopaedic aids (24%), which was reflected in a significant decrease in

quality of life.

This study presents self-reported outcomes. These are less objective than standardized measurements performed by trained professionals. However, self-reported burden of disease in daily life is certainly a valid measurement of quality of life, since it is the patient who is the expert on his own perceived quality of life. Haemophilia is a lifelong disease with very demanding treatment. As a consequence patients are generally well informed about treatment possibilities and outcomes, especially severe and moderate patients[12]. Therefore, it may be assumed that this study provides a reliable insight into the burden of disease of haemophilia patients.

Some moderate haemophilia patients showed unfavourable outcomes, a high number of bleeds and joint impairment, despite treatment with prophylaxis. Most likely, these patients presented with a high bleeding tendency and were treated similar to severe haemophilia patients. Despite prophylactic treatment, this higher tendency to bleed resulted in worse outcomes for this group of patients than for the group treated on demand. Patients with moderate haemophilia are usually on secondary prophylaxis; prophylaxis started in case of synovitis or target joints[13]. The bleeds previous to prophylactic treatment might have already initiated irreversible joint damage. This suggests that 'confounding by indication' is present in the analysis of treatment and outcome in moderate haemophilia: patients with a more severe bleeding pattern have suffered more bleeds and therefore more joint damage prior to treatment with prophylaxis.

Information about outcome and clinical status for moderate haemophilia patients is scarce. Since the report by Ahlberg et al[7] in 1965, only one study has confirmed the bleeding pattern of moderate haemophilia[6]. The results of the current study compare well with the report by Ahlberg et al[7], although some surprising differences were observed. Ahlberg also reported outcomes in moderate haemophilia to be in between those of mild and severe disease. However, compared to this study, Ahlberg reported less disability in moderate haemophilia patients. In 1965, 12 out of 59 (20%) patients were markedly or severely disabled and 10 out of 59 (17%) used orthopaedic aids. In the current study 43% of patients with moderate haemophilia reported joint impairment of one or more joints and 24% reported using orthopaedic aids. These differences are unexpected, because since 1968 replacement therapy became available. The age distribution in both study populations was similar (median around 30 years old) and disability was assessed in both studies using questionnaires, although the exact definition of outcomes may well have been different. Lack of sensitivity for severities in the mental domains of SF-36 for haemophilia patients was also reported by Szucs et al[14], Miners et al[15] and Solovieva[16]. The scores in physical functioning, physical role and bodily pain reported by Solovieva were similar to the scores reported in the present study. In general, 3-5 points difference in a domain of the SF-36 is considered clinically important[11]. The average observed difference between moderate and mild patients was much larger with 13 points (Fig. 2).

Unlike this study, Szucs et al.[14] and Miners et al.[15] did not report results of moderate haemophilia patients separately, which made comparison difficult; Szucs et al.[14] combined severe and moderate haemophilia and Miners et al.[15] categorized moderate and mild into one group.

Because moderate haemophilia is rare, studies on moderate patients are inevitably hampered by low numbers. Unfortunately, clinical outcomes and associations between treatment and outcomes could not be studied with the current self-reported data. Future studies, possibly multicen-

tre with larger numbers of participants, can confirm these results by using objectively measured outcomes like joint function, joint damage and cumulative number of bleeds.

In moderate haemophilia, one source of variation is factor level, between 1 IU/dl and 5 IU/dl. As this study suggests, baseline activity of 1%-5% does not preclude bleeding and consequential arthropathy. The severity of haemophilia patients was classified following the laboratory results extracted from patient files of the participating centres. The results of this study suggest that a classification of haemophilia patients merely according to laboratory criteria might not be sufficient, but that clinical expression of the haemophilia severity also needs to be taken into account. Investigating the variability of outcomes in these patients may lead to improved care and quality of life in all patients with moderate haemophilia.

In accordance with the classification of haemophilia according to laboratory results, burden of disease in moderate haemophilia was in between that of severe and mild haemophilia. However, the burden of moderate haemophilia may be considerable: about 25% of patients with moderate haemophilia reported frequent bleeding and joint disease. These patients may benefit from more intensive treatment.

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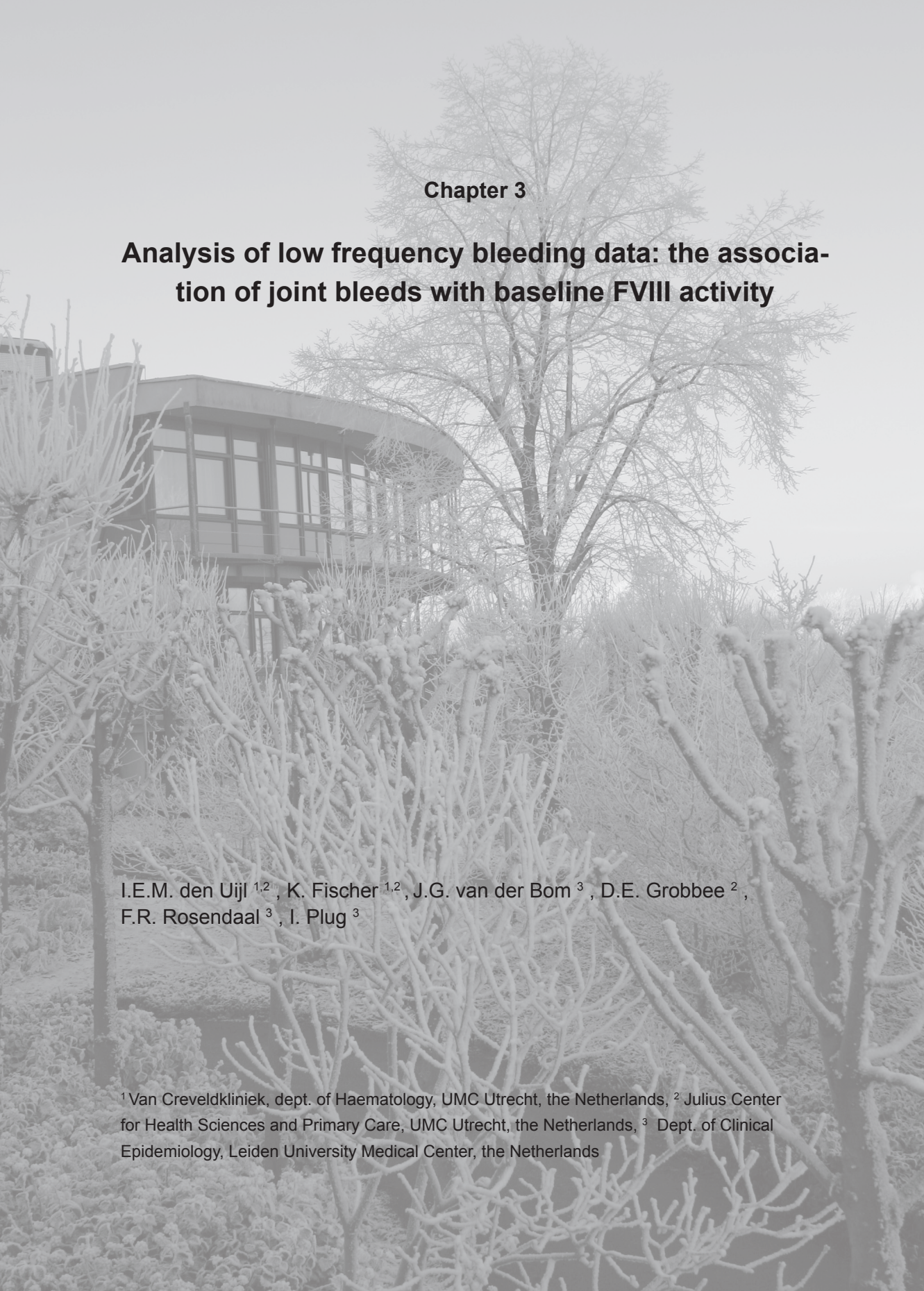

Chapter 3

Analysis of low frequency bleeding data: the association of joint bleeds with baseline FVIII activity

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Abstract

Many studies in the field of haemophilia and other coagulation deficiencies require analyses of bleeding frequencies. In haemophilia, the association of bleeding frequency with FVIII activity is known from experience, but actual data are lacking. Bleeding frequencies in haemophilia are highly skewed count data, with large proportions of zero's. Both the skewness and a high amount of zero's pose problems for standard (linear) modelling techniques.

This study investigated the optimal analyzing strategy for bleeding data by using the association of residual factor activity level and number of joint bleeds in moderate and mild patients treated on demand as an example.

In total 433 patients with moderate (27%) and mild (73%) haemophilia A, treated on demand, were included in this study. One year of self-reported data on joint bleed frequency and baseline clotting factor activity were analyzed using Poisson, negative binomial, zero-inflated Poisson, and zero-inflated negative binomial distributions.

Multivariate regression analysis using negative binomial distribution provided the optimum data analytic strategy. This model showed 18% reduction (Rate Ratio 0.82; 95%CI 0.77-0.86) of bleeding frequency with every IU/dl increase in residual FVIII activity. The actual association is expected to be higher due to exclusion (30 out of 463 pts) of patients on prophylaxis (baseline FVIII levels 1-6 IU dl).

The best way to analyze low frequency bleeding data is using a negative binomial distribution.

Introduction

Repeated bleeding, especially into joints, is the hallmark of haemophilia. Many studies in haemophilia as well as in other fields of coagulation research require analysis of bleeding frequencies. Bleeding frequencies are count data with right-skewed distributions and a high proportion of zero's, which creates problems for regular linear modelling.

Patients with less than 1 IU/dl (<1%) factor activity were classified as having severe haemophilia, patients with 1-5 IU/dl (1-5%) factor activity as moderate and those with 6-40 IU/dl (6-40%) factor activity were classified as having mild haemophilia[1]. Patients with severe haemophilia suffer from frequent spontaneous joint bleeds, while those with moderate haemophilia only very rarely show spontaneous joint bleeds. Patients with mild haemophilia only bleed after trauma. The distinction between patients who have no detectable FVIII levels and those who have is clear. However, the distinction between moderate and mild haemophilia is more problematic.

The association of bleeding pattern and baseline FVIII levels is very important[2]. Nevertheless, data are virtually lacking, largely because the association is not linear. In any analysis of skewed data such as bleeding frequencies, choosing the right modelling distribution is paramount to get meaningful results. This may explain why a recent linear model of the association between bleeding and time below one percent factor activity of patients on prophylaxis showed the counter-intuitive result of no association[3].

The aim of the present study was to determine the optimal statistical strategy for analyzing low frequency joint bleed data, using data from a nationwide Dutch study.

Patients and methods

In 2001, as part of a series of surveys, a nationwide questionnaire was administered to 1587 Dutch haemophilia patients[4]. Patients answered questions on several topics including bleeding frequency and treatment in 2000. Self-reported factor activity levels of participating patients were cross-referenced with their haemophilia treatment centre.

Factor activity levels for 908 (out of 1066 participants) patients were supplied by the haemophilia treatment centres. Using these data, all 463 haemophilia A patients with factor levels between 1 IU/dl and 40 IU/dl were included in this study. Thirty patients (factor activity levels 1-6 IU/dl) were excluded because of prophylactic treatment, leaving 433 patients for analysis.

Data analysis

The analysis aimed at estimating the association between number of joint bleeds and baseline factor levels. As number of joint bleeds is a count outcome with a skewed distribution and a high proportion of zero's, several distributions designed for count outcomes were fitted. Adjustments were made for difference in age in all analyses.

Rate Ratios (RR) were reported with their 95% confidence intervals (95%CI). Coefficients of the count distributions were converted into RR, to ease the interpretation of the results from the different distributions.

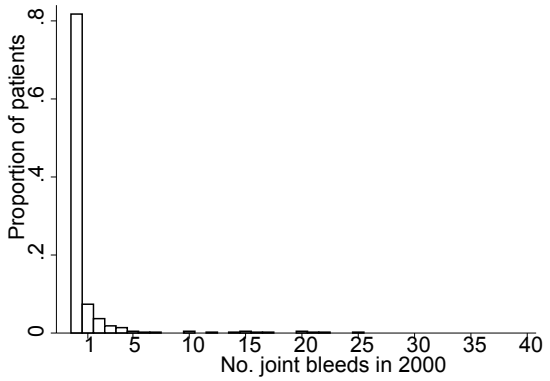
To determine which model described the association between joint bleeds and factor activity level best, Akaike's Information Criterion (AIC)[5] was used. A model was considered more appropriate,

when AIC was two points per degree of freedom (df) lower than for the comparison model.
To visually check model fit, the difference between the observed and predicted probabilities according to bleeding frequency was plotted. Points above the null on the y-axis indicate higher observed counts than predicted, points below zero indicate lower observed than predicted counts[6].
The Vuong statistic was computed to determine whether the zero-inflated model achieved a better fit than the regular Poisson or negative binomial model. A significant ($p<0.05$) Vuong statistic was used as a criterion to indicate preference for the zero-inflated model[7].
All analyses were performed using Stata SE9.0 (StataCorp, College Station, TX).

Results

The study population included 119 patients (27%) with moderate haemophilia and 314 patients (73%) with mild haemophilia. Age was similar across levels of severity: median age of patients with moderate haemophilia was 34 years (range 0-83 years), median age of patients with mild haemophilia was 37 years (range 0-87 years). Factor VIII activity levels of 1% were infrequently reported.

Figure 1. Highly right skewed distribution of joint bleeds, with high proportion of zero's



The distribution of joint bleeds was highly skewed to the right (Fig. 1). Annual number of joint bleeds decreased with increasing baseline factor activity levels. Mean bleeding frequency was 1 joint bleed/year (standard deviation 2.98, median 0 joint bleeds/year). Bleeding frequency varied between groups: 40% (48 patients) of patients with moderate haemophilia reported joint bleeding, while 11% (33 patients) of patients with mild haemophilia reported joint bleeds in 2000.

Analysis of bleeding frequency according to factor activity level

First, the performance of the statistical models was compared. The rate ratios (RR) describing the age-adjusted effect of factor activity levels varied between 0.82 and 0.91 (Table 1). Although linear regression showed a significant decrease in joint bleeding with increasing factor activity (linear coefficient; -0.09; 95%CI -0.12;-0.06), it proved to be a very bad fit. Compared to the count models, the linear model had a very high AIC of 2171, while the worst fitted count model, the Poisson model had an AIC of 1308. Additionally linear modelling showed large deviations from 0 in the

observed- predicted probability plot (Fig. 2).

Both the negative binomial and zero-inflated negative binomial had low AIC's (683 and 684 respectively), and good fits in the observed minus predicted probabilities plot; both hardly deviated from zero on the y-axis (Fig. 2). Like the AIC, the Vuong statistic indicated a preference for the negative binomial over the zero-inflated negative binomial (p=0.09). The negative binomial distribution was the optimal distribution to analyze this kind of data.

Table 1. Association between number of joint bleeds and baseline factor activity for different distributions compared to linear modelling

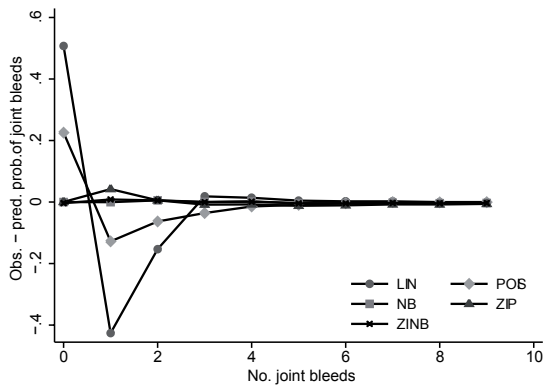
	Factor activity (per IU/dl) RR (95% CI)	AIC	df	p-Vuong
LIN (coefficient)	-0.09 (-0.12;-0.06)	2171	3	-
POIS	0.83 (0.80;0.85)	1308	3	-
NB	0.82 (0.77;0.86)	683	4	-
ZIP 1. POIS	0.91 (0.88;0.94)	912	6	<0.01
2. LOG (OR)	0.14 (0.08;0.20)	-	-	-
ZINB 1. NB	0.88 (0.80;0.97)	684	7	0.09
2. LOG (OR)	0.15 (0.01;0.21)	-	-	-

All models used annual number of joint bleeds as dependent variable and were adjusted for age.

RR = rate ratio, OR = odds ratio, CI = confidence interval, AIC = Akaike's information criterion, df = degrees of freedom. LIN = linear, POIS = Poisson, ZIP = zero-inflated Poisson, NB = negative binomial, ZINB = zero-inflated negative binomial, LOG = logistic

The RR for the association between number of joint bleeds and baseline factor activity in the negative binomial model was 0,82 (95%CI 0,77-0,86), which may be interpreted as an 18% reduction of bleeding frequency with every IU/dl increase in baseline FVIII activity level.

Figure 2. Plot of the difference between observed and predicted probabilities of having a joint bleed

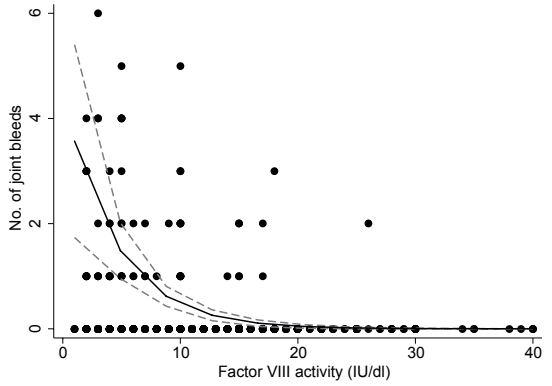


LIN = linear, POIS = Poisson, NB = negative binomial, ZIP = zero-inflated Poisson, ZINB = zero-inflated negative binomial

Figure 3 shows the expected association of joint bleed frequency with baseline FVIII activity on basis of the fitted negative binomial model. Patients with low baseline factor activity levels (<5%) had the highest risk for joint bleeds, and patients with factor activity levels of 10% or higher had very low risks, which approximated zero in patients with baseline factor activity of 15% or higher.

The confidence interval was widest around low factor activity levels, due to low patient numbers in that range.

Figure 3. Scatterplot and predicted association between annual number of joint bleeds and factor activity



Based on the multivariate negative binomial model adjusted for age. Dotted lines represent 95% confidence interval of the predicted values

Discussion

The present study describes the statistical strategy used in the analysis of the association of joint bleed frequency with baseline clotting factor activity in patients with moderate or mild haemophilia A. In 433 patients baseline factor activity levels showed an asymptotic association with self reported number of joint bleeds. The asymptotic association was best predicted by the negative binomial distribution: 18% reduction of bleeding frequency with every percent increase in baseline factor activity level.

Poisson and zero-inflated Poisson distributions were not suited for this analysis. Bleeding frequencies vary considerably between patients[8]. In this study, the variance (8.9 joint bleeds/yr) exceeded the mean (1 joint bleed/yr), violating an important assumption for the Poisson model; the variance should equal the mean[9]. The negative binomial model was designed for count data with more heterogeneity[6], and was therefore more appropriate for this kind of data. The zero-inflated models were specifically designed to analyze count data with a high proportion of zeroes. Zero-inflated models consist of two models: first, a binary component modelling the probability of being a true zero (i.e. a person who does not experience any joint bleeds at all), and second, a negative binomial or Poisson component modelling the number of joint bleeds. Although the performance of both the negative binomial and the zero-inflated negative binomial model was similar, the negative binomial model was preferred, not only because of the Vuong test, but also because it is easier to interpret for clinicians.

The results of the present study are influenced by patient selection; only Dutch patients treated on demand. In Western Europe, patients with severe bleeding phenotypes are treated with prophylaxis, a regular infusion of FVIII. In our study, 30 patients with low factor activities (1-6 IU/dl) were therefore excluded. As a result, in the lower baseline factor activity levels, the association is expected to be higher, resulting in a steeper left tail than shown in Figure 3. The risk for

patients with >10% baseline factor activity level will probably be similar to the curve shown here, as a large number of patients with mild haemophilia was analyzed in this study and the number of joint bleeds in patients with factor activity levels between 10% and 40% appeared to have very little variation.

By analyzing joint bleeds, misclassification of bleeds was minimized, since joint bleeds have fewer problems with definitions, misclassification and recall bias than soft tissue bleeds. However, follow up in this study was only one year, and analysis over such a short period introduces more variation compared to studies over several years. Consequently, confidence intervals were wide, especially around the low levels, as only few patients had moderate haemophilia.

These considerations on modelling are important to the field of haemophilia, as bleeding frequencies are key parameters in haemophilia research and patient numbers are mostly limited[2]. The analysis of bleeding data is complicated, so complex modelling techniques must be applied with caution, to avoid overfitting the data and difficult interpretations, whereas simple (linear) modelling does not always suffice (Fig. 2).

In the study by Ahnstrom et al[3], the association of bleeding frequency with time below 1 IU/dl FVIII activity was analyzed in patients on prophylaxis. The highly skewed data, analysed with a linear model, showed only a very limited, non-significant association. These results may be very different if such a study would be analyzed using a model with a better fit such as the negative binomial model. Indeed, this was clearly demonstrated in a recent report of Collins et al[10], showing a significant association of time below 1 IU/dl FVIII activity and bleeding frequencies in patients with severe haemophilia A.

The negative binomial distribution is appropriate for analysing skewed count data when dealing with low bleeding frequencies, such as in severe patients treated with prophylaxis, or bleeding in mild or moderate patients. In contrast, joint bleeding frequencies in populations of patients with haemophilia treated on demand, for instance in areas where prophylaxis is not available, are more normally distributed. This is illustrated very well in the recent study of Manco-Johnson et al.[11]: number of joint bleeds in the group of boys treated on demand was reasonably normally distributed with a mean of 4.89 (sd=3.57) and a median of 4.35 joint bleeds. As expected, the distribution of number of joint bleeds in the group of boys on prophylaxis was more skewed with mean 0.63 (sd 1.35) and median 0.20 joint bleeds.

In conclusion, the highly right skewed association between bleeding frequency and baseline factor activity in these patients may best be analyzed using multivariate analysis with a negative binomial distribution. The present study shows an 18% reduction of joint bleed frequency with every percent increase in residual factor activity in moderate and mild patients with haemophilia A treated on demand. The association is, however, expected to be higher in patients with low baseline levels due to the treatment characteristics of this population.

Analyzing bleeding data is very important, since it is one of the most important outcome parameters in haemophilia. Fitting the right model to the data ensures the best possible results.

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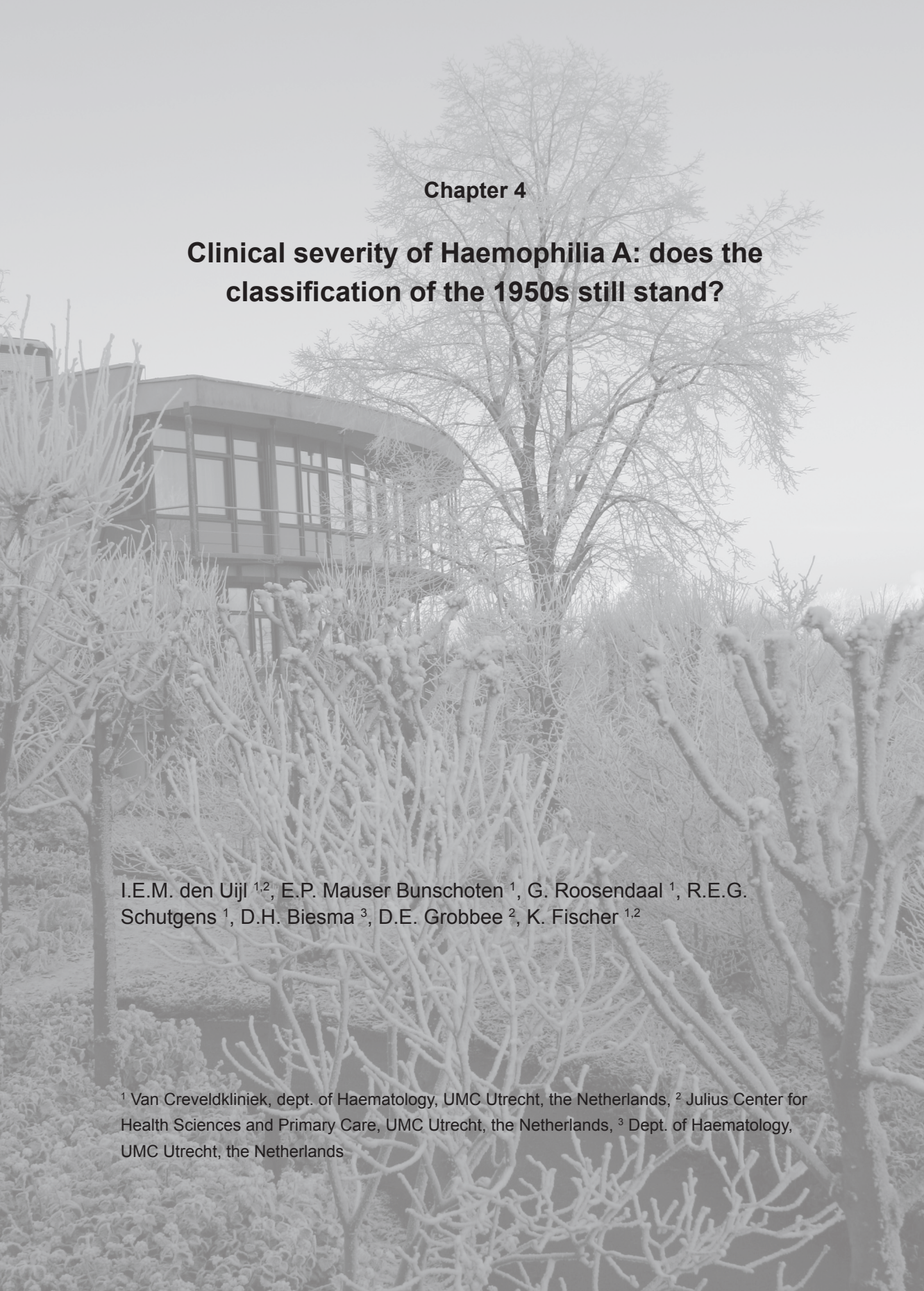

Chapter 4

Clinical severity of Haemophilia A: does the classification of the 1950s still stand?

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Abstract

The classification of severity of haemophilia dates back to the 1950s. Does this classification still stand today?

Data on age and reason of diagnosis, onset of treatment, onset of bleeding, and bleeding frequency from 411 patients with haemophilia A born after 1970 were collected. Data were analysed according to baseline FVIII activity levels. Age at diagnosis, onset of bleeding and start of treatment according to FVIII activity levels were compared to the current classification.

Overall, the distinction between severe and non-severe haemophilia was clear. The distinction between mild and moderate haemophilia was more difficult, mostly due to the wide variability in patients with moderate haemophilia.

Patients with severe haemophilia experienced their milestones like diagnosis, first treatment and joint bleed earliest, mostly as infants, ranging from 0 to 3 years, while patients with moderate haemophilia reached these milestones around toddler age, ranging 2-7 years, and patients with mild haemophilia reached them when they were in elementary school, around the ages of 5-14 years.

This study confirms the clinical distinction between severe and non severe haemophilia A. However, the group of moderate haemophilia patients showed a wide variability, warranting close follow-up and individualised treatment.

Introduction

Classification of a disease is very important in research. It enables comparison of studies and standardisation of results.

The classification of haemophilia was first described in 1958 by Biggs and MacFarlane[1]. They described the relation between bleeding and residual factor VIII activity (FVIII) in haemophilia A patients, resulting in a classification which is used to this day. Since 1958, treatment and impact of the disease have changed tremendously and laboratory assays have become more accurate. Nevertheless, the standard for current classification, adopted in 2001 by the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis[2], is the same one developed almost fifty years earlier. The validity of this classification has not been studied since.

Current treatment is predominantly guided by haemophilia severity. Severe haemophilia patients (FVIII <1 IU/dl) are closely monitored and receive early prophylaxis, while moderate (FVIII 1-5 IU/dl) and mild patients (FVIII 6-40 IU/dl) are monitored less closely and receive mostly on demand treatment. The difference in approach between severe and non-severe haemophilia is very distinctive, but is this difference justified?

This study aims to repeat the clinical observation of the 1950s in a large single centre cohort. Does the current classification compare to onset of bleeding, age at first treatment and annual joint bleeding frequency according to baseline FVIII activity?

Patients and methods

From the cohort treated at the Van Creveldkliniek, UMC Utrecht, the Netherlands, data on 411 patients with haemophilia A born after 1970 were selected. From 1970 onwards, annual data on bleeding and treatment were routinely collected for all patients. These data were extracted from patient files. In total 34 patients, who visited the clinic only once or had insufficient data available due to late referral to the clinic, were excluded (2 patients with severe and 32 with mild haemophilia). Eventually, data were available for 377 patients.

Factor VIII activity was measured by one-stage assay[3] in a single laboratory. If residual factor levels were measured more than once (median number of measurements was 4 times, IQR 2-5 times), the lowest factor level was used for the classification.

A positive family history was defined as one or more previous cases of haemophilia recognized before entering the clinic. Joint bleeds were defined as complaints in ankles, knees, elbows, hips, wrists, or shoulders requiring treatment with FVIII at least once.

Statistics

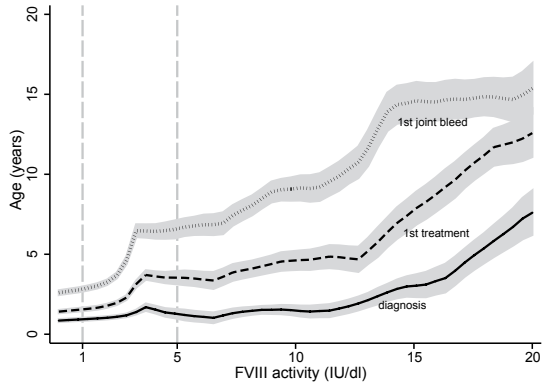
Local polynomials, which are smooth lines through scatter plots, were fitted to describe the trend of onset of bleeding and treatment as well as annual joint bleeding frequency according to baseline FVIII activity. The results were compared to the current standard classification into severe (FVIII <1 IU/dl), moderate (1-5 IU/dl) and mild (6-40 IU/dl) haemophilia[2]. Kaplan Meier cumulative incidence distributions were calculated for age at diagnosis, onset of treatment and joint bleeding. Differences in Kaplan Meier curves were analysed using the Log Rank test.

Statistical analyses were performed using Stata 10 (Statacorp, TX, USA).

Results

Complete data on treatment and onset of bleeding were available for 377 patients, including 182 (48%) patients with severe, 73 (20%) with moderate and 122 (32%) with mild haemophilia A.

Figure 1. Age at diagnosis, onset of treatment and joint bleeding according to FVIII activity

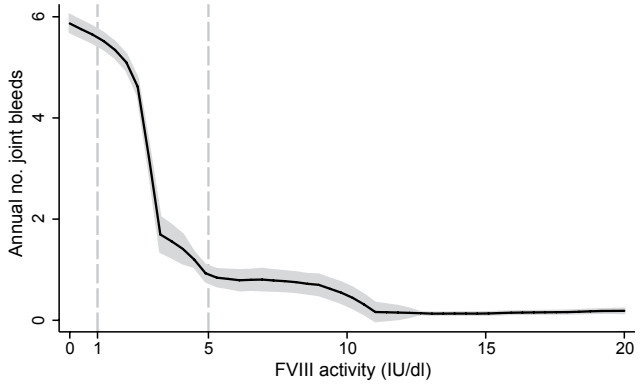


Black lines are medians, shaded areas interquartile ranges

Phenotype according to baseline FVIII activity level

The current classification compared well with parameters age of diagnosis, first treatment and first joint bleed according to FVIII activity (Fig. 1). This observation was confirmed by the joint bleeding frequency according to baseline FVIIII activity (Fig. 2). Above 5 IU/dl age at diagnosis, onset of treatment and joint bleeding kept increasing steadily, while the number of joint bleeds decreased to approximately zero in patients with more than 12 IU/dl FVIII.

Figure 2. Annual number of joint bleeds according to FVIII activity



Black lines are medians, shaded areas interquartile ranges

Overall, the distinction between severe and non-severe haemophilia was clear. The distinction between mild and moderate haemophilia was more difficult. Age at diagnosis was dependent on

both family history (Table 1) and haemophilia severity (Fig. 1 and 3). Patients with a positive family history tended to be diagnosed significantly earlier, especially in non-severe haemophilia. Most patients had a positive family history for haemophilia: 93 patients (51%) in severe, 45 (62%) in moderate and 85 (69%) in mild haemophilia (Table 1).

Significant differences in bleeding pattern were observed, which corresponded well with the current classification. Joint bleeding started at a much younger age in severe haemophilia patients than in moderate haemophilia (moderate vs. severe: $p<0.001$), while mild patients were oldest at onset of joint bleeding (mild vs. severe: $p<0.001$ and moderate vs. mild: $p<0.01$). Consequently, patients with severe haemophilia needed treatment sooner than patients with moderate or mild haemophilia (severe vs. non-severe: $p<0.001$) (Fig. 2). However, the distinction between moderate and mild haemophilia was not significant for overall age at diagnosis (moderate vs. mild: $p=0.82$), nor for age at first treatment (moderate vs. mild: $p=0.07$). When adjusted for family history, patients with moderate haemophilia were diagnosed and treated earlier than those with mild haemophilia ($p=0.02$) (Table 1).

Table 1. Patient characteristics and cumulative incidence of age at diagnosis, onset of treatment and joint bleeding, according to the current classification

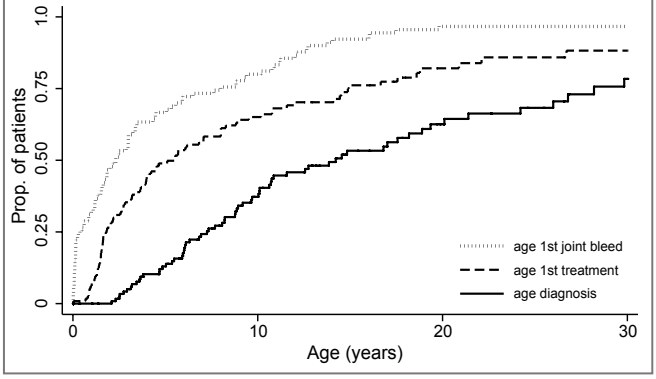
	mild	moderate	severe
n	122 (32%)	73 (20%)	182 (48%)
Current age (years)	17 (9-25)	22 (15-29)	22 (11-31)
Positive family history	85 (69%)	45 (62%)	93 (51%)
Age at diagnosis (years)			
positive family history	1.2 (0.1-5.0)	1.0 (0.2-2.0)	0.5 (0.0-0.8)
negative family history	3.4 (1.8-11.2)	4.0 (1.1-8.1)	1.0 (0.8-1.3)
Age 1st treatment (years)	5.5 (1.8-15.0)	2.9 (1.1-12.2)	1.1 (0.8-1.4)
Age 1st joint bleed (years)	14.2 (7.3-28.2)	6.7 (3.7-23.9)	1.9 (1.2-3.0)

Values presented are medians (25-75 percentiles) or numbers (%)

Patient and treatment characteristics according to severity

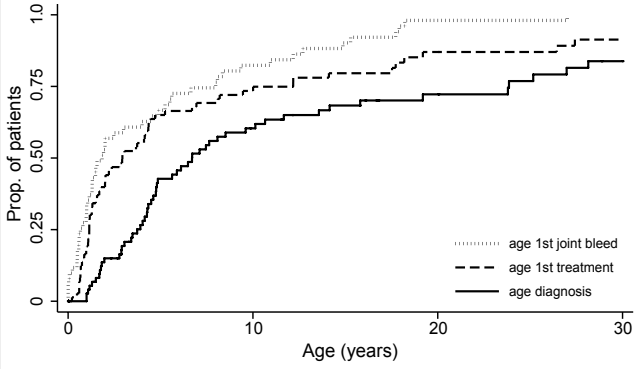
Mild haemophilia. In mild haemophilia, the majority of patients (69%) reported a previous case of haemophilia in their family. At their first visit to the clinic, 25% presented with a bleed. Patients with mild haemophilia were diagnosed at median 2.3 years (range 0-36 years). Patients with mild haemophilia received their first replacement therapy at median 5.5 years (range 0.7-36 years). Their first joint bleed occurred at a median of 14.2 years (range 1.4-36 years) (Table1). At the age of 20 years, 54% of mild patients had never suffered a joint bleed (Fig. 3a).

Figure 3a. Milestones of mild haemophilia according to the current classification



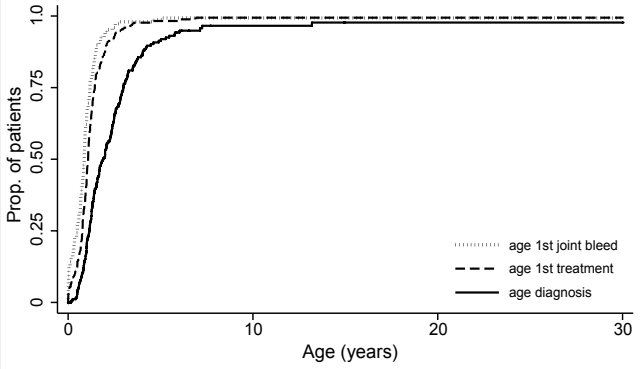
Moderate haemophilia. Of patients with moderate haemophilia, 62% reported a previous case of haemophilia in their family. Patients with moderate haemophilia were diagnosed at a median of 1.8 years (range 0-27 years). First replacement therapy in moderate haemophilia was administered at median 2.9 years (range 0.2-33 years), while most moderate patients suffered their first joint bleed at median 6.7 years (range 1-34 years) (Table 1). At the age of 20 years, 20% of moderate patients had never suffered a joint bleed (Fig. 3b).

Figure 3b. Milestones of moderate haemophilia according to the current classification



Severe haemophilia. Half of the patients with severe haemophilia (51%) reported a previous case in the family. Severe haemophilia was diagnosed at median 0.8 years (range 0-5 years). First treatment was administered at median of 1.1 years (range 0-7 years) and the median age of onset of joint bleeding was 1.9 years (range 0-15 years) in severe haemophilia (Table1). Only 10 patients with severe haemophilia (5.6%) had not suffered a joint bleed prior to age 20 years, while all but one of these patients started primary prophylaxis before their first joint bleed (Fig. 3c).

Figure 3c. Milestones of severe haemophilia according to the current classification



Discussion

These data confirm the validity of the original haemophilia classification by Biggs and MacFarlane from 1958[1], which was formalized as worldwide standard in 2001[2]. Patients with severe haemophilia experience their milestones, including diagnosis, first treatment with clotting factor concentrates and joint bleed, mostly as infants. Patients with moderate haemophilia reach these milestones around toddler age and patients with mild haemophilia when they are in elementary school. Although the distinction between severe and non-severe haemophilia was clear, moderate and mild haemophilia were much less divergent.

The group of moderate haemophilia patients was very heterogeneous; age at diagnosis and age at first treatment were similar to mild haemophilia, while age at first joint bleed was significantly lower. A sharp bend in the curves was observed around 3 IU/dl. Several factors may explain this

large variation in moderate haemophilia. First, it may be due to the low number of patients: it is the rarest from of haemophilia. Second, laboratory variation could add to this heterogeneity: patients with moderate haemophilia increase their FVIII activity under stressful conditions[4]. In this study, misclassification was minimized by taking the lowest FVIII activity ever measured. Another explanation could be that some patients have more severe bleeding phenotypes than others, or had repetitive bleeding into the same joint, especially those with <3 IU/dl. In severe haemophilia, these differences in phenotypes are well known[5]. This variation might have consequences for treatment of moderate haemophilia patients and warrants close monitoring.

The early milestones of haemophilia, age of diagnosis and onset of joint bleeding, are mostly independent of treatment[5]. Because of the different approaches to treatment between severities and centres, these parameters are very suitable for comparison. Two things need to be considered for this comparison. First, some patients received prophylaxis before their first joint bleed, either for surgery or intracranial or major bleeding complications. This was reflected by a few patients with severe haemophilia who had not yet experienced a joint bleed due to intensive early prophylaxis. Second, although time of diagnosis and onset of bleeding are good indicators for clinical phenotype, time of diagnosis can be influenced by a previous family history of haemophilia. Moderate and mild haemophilia could not be distinguished from each other based on general onset of treatment. However, when corrected for family history, patients with mild haemophilia were diagnosed and received their first treatment later than patients with moderate haemophilia.

Treatment and monitoring of haemophilia have changed enormously after the introduction of replacement therapy. Therefore, we only included patients born after 1970, who had access to replacement therapy from a young age. The age distributions across severities were similar, therefore the effect of changes in haemophilia treatment will not influence these results.

Bleeding was deliberately plotted without correction for prophylaxis to determine whether the classification of haemophilia would stand even though treatment and disease management have changed. Despite the availability and use of prophylaxis, severe haemophilia patients have a higher bleeding rates than non-severe haemophilia patients suggesting that irrespective of current treatment, bleeding rates seem to be dependent on factor activity.

In this study, treatment was defined as treatment with clotting factor concentrate. With respect to treatment, it can be expected that mild haemophilia patients were treated with DDAVP prior to clotting factor concentrate. The first treatment of mild haemophilia patients may therefore be earlier than is reported by this study. First joint bleeds were, however, registered even if they were treated with DDAVP and thus independent of treatment.

To compare FVIII activity and clinical phenotype, Biggs and MacFarlane[1] used total bleeding frequency; the distinction between joint bleeds and soft tissue bleeds was not made. In this study we used joint bleeding frequency, because misclassification is lower for joint bleeds than for muscle and soft-tissue bleeds.

The distribution of severities in patients who had complete data available differed slightly from the distribution of haemophilia in the Netherlands[6]. The underrepresentation of mild haemophilia can be explained in two ways. Missing data were most prevalent in patients with mild haemophilia. Because of few problems and less intensive treatment in this group of patients, documentation has been less comprehensive than in moderate or severe haemophilia patients. Second, the Van

Creveldkliniek is a haemophilia referral centre. Patients with disease related problems are more likely to travel the extra distance to a specialized centre[7]. In the current study this was reflected by the high proportion of patients with mild haemophilia with a history of joint bleeding.
Haemophilia B was excluded from this study, because of the low incidence and clinical impression that haemophilia B has a milder phenotype[8]. The original classification of Biggs and MacFarlane from the 1950s was also based on clinical bleeding pattern and clotting factor activity tests in haemophilia A patients only.
In 1990, Ljung et al[9] published that in only 17% of severe and moderate haemophilia patients diagnosis was made because of a positive family history. Four years later, Conway and Hilgartner[10] reported that 34% of patients with severe haemophilia were diagnosed because of family history. Conway et al[10] reported all severe patients and 28% of mild and moderate patients to be diagnosed before the age of 1 year. In contrast, 74% of severe, 57% of moderate and 53% of mild patients were diagnosed before the age of 1 year in the current study. In a recent study from the American CDC by Kulkarni et al[11], patients aged 0-2 years were earlier diagnosed if they had a positive family history. In their study, 56% of severe, 65% of moderate and 79% of mild haemophilia patients had a positive family history. This compares well with the current study where 51% of severe, 62% of moderate and 69% of mild patients with haemophilia were diagnosed because of a positive family history. The early diagnosis across severities may be a result of this trend towards family testing and intensive genetic counselling, and age at diagnosis is therefore lower than in older reports.
Data from this study confirm the current accepted classification of haemophilia. Treatment differences between severities can be justified by the results. Onset of bleeding is earlier and bleeding frequencies iare higher in severe compared to non-severe haemophilia. A wide variation in clinical phenotype can be seen within the group of moderate haemophilia patients. Treatment decisions, such as starting prophylaxis, should therefore be tailored according to bleeding pattern, rather than based on the residual clotting factor activity levels.

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Chapter 5

Comparison of haemophilia types: slightly less bleeding in haemophilia B

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Abstract

Clinical impression is that haemophilia B has a milder phenotype than haemophilia A. The aim of the present study was to compare the bleeding phenotypes between haemophilia A and B.

All patients treated for at least 5 consecutive years at the Van Creveldkliniek were included in this study; their data on (onset of) bleeding and treatment were available from 1970 onwards.

In total 7964 years of 507 haemophilia A and 800 years of 51 haemophilia B patients were analysed. Severities were unequally distributed across haemophilia types. All univariate analysis showed similar results for haemophilia A and B. Onset of joint bleeding was similar across severities, even after adjustment for age at entry of the clinic and baseline factor activity. After adjustment for age, baseline factor activity, prophylaxis and repeated measurements, joint bleeding frequency was reduced by 17% (95%CI; 4-29%) in haemophilia Bcomapred to haemophilia A.

In unadjusted, univariate analyses, no differences were found between haemophilia A and B. Only after adjusting for treatment and patient characteristics, a slightly lower joint bleed frequency in haemophilia B patients could be detected. Therefore, future studies on outcome in haemophilia should analyse data for haemophilia A and B separately.

Introduction

Two types of haemophilia can be distinguished. Patients with haemophilia A lack clotting factor VIII (FVIII) and patients with haemophilia B lack clotting factor IX (FIX). Haemophilia B, previously known as Christmas disease after the first patient diagnosed[1], was first considered a different disease from haemophilia[2]. Only since 1952, haemophilia A and B are perceived as two types of the same disease.

According to experienced physicians, haemophilia B patients have a milder clinical phenotype[3,4]. However, this observation has only been supported by few studies, predominantly comparing secondary outcome parameters. Compared to haemophilia A, patients with haemophilia B appeared to have less arthropathy[5], less need for hospitalisation[6] and used less prophylaxis[7]. Another crude comparison of bleeding, a primary outcome in haemophilia, suggested no difference between the two haemophilia types[8]. Bleeding is the most important primary endpoint in haemophilia[9], and thus most appropriate to compare both haemophilia types. However, bleeding is difficult to analyze, because bleeding frequencies are greatly influenced by treatment and residual factor activity.

The aim of the present study was to compare bleeding phenotypes between haemophilia A and B, independent of treatment and differences in severity in the cohort.

Patients and methods

The Van Creveldkliniek cohort is a very well documented group of more than 600 patients with haemophilia A or B of all severities. Most patients have been treated at the clinic from early childhood onwards. However, some were only referred for surgeries or hepatitis treatment, or visited the clinic only once for carrier determination and were excluded from the study.

Data of all patients treated at the Van Creveldkliniek for at least 5 consecutive years were included in this study. Patients with severe haemophilia visited the clinic routinely every 3-4 months, while those with moderate or mild haemophilia visited once or twice a year. At each visit, data on number and type of joint and soft-tissue bleeds were collected, as well as treatment regimen, factor VIII/IX consumption, hospital admissions and/or surgical procedures. Bleeding and treatment data were extracted from patient files containing at least 5 years of follow-up, in yearly evaluations from 1970 onwards.

Joint bleeds were defined as complaints in ankles, knees, elbows, hips, wrists, or shoulders requiring treatment with clotting factor concentrate at least once. Prophylaxis was defined as at least one regular infusion per week for at least 45 weeks per year. Residual factor VIII/IX activity was defined by the lowest measured factor activity, median number of measurements was 4 times (IQR 2-5 times). As joint bleeds are less sensitive to misclassification, all analyses concerning bleeding frequencies were performed using the annual number of joint bleeds as outcome parameter.

Data analysis

Baseline characteristics across haemophilia types were compared using a Mann-Whitney test for continuous variables and a chi-square test for categorical variables.

The association of age at first joint bleed with haemophilia type was analyzed using a linear model. The association of haemophilia type with age at first joint bleed was adjusted for residual factor VIII/IX activity and age at first visit to the clinic.

To assess the true effect of haemophilia type on joint bleeding, the association was assessed independently of severity, age and treatment in a negative binomial multi-level model, adjusted for repeated measurements per patient. Because evaluations comprised not 1 year exactly, time between visits was added as offset to correct for differences in time between evaluations. Predicted values were calculated to show the association of haemophilia type with joint bleeding independent of age, severity, treatment and repeated measurements.

Analyses were performed using Stata 11.0 (Statacorp LP, TX, USA).

Results

Data on 8764 patient years from 558 patients were available: 7964 years from 507 (91%) patients with haemophilia A and 800 years from 51 (9%) patients with haemophilia B. Per patient, median 15 annual evaluations (IQR 6-24 evaluations) were available; a median follow up of 14.8 years per patient.

Table 1. Patient characteristics according to haemophilia type

	Haemophilia A	Haemophilia B	p-values
Severe haemophilia			
n	260 (51%)	33 (65%)	0.09
Age (years)	35.1 (21.7-48.9)	39.8 (24.0-43.9)	0.69
Factor activity (IU/dl)	<1	<1	-
Patient years	19.4 (12.0-26.8)	20.8 (8.6-28.3)	0.82
History of prophylaxis	215 (83%)	28 (84%)	0.76
Age first joint bleed (years)	1.8 (1.2-2.8)	2.2 (1.0-3.0)	0.65
Annual no. joint bleeds	3.7 (1.6-8.7)	4.2 (1.9-6.9)	0.81
Moderate haemophilia			
n	65 (13%)	8 (16%)	0.72
Age (years)	40.2 (24.6-54.3)	35.0 (27.3-38.8)	0.57
Factor activity (IU/dl)	3 (2-4)	2 (2-3)	0.03
Patient years (years)	15.3 (10.2-22.8)	10.2 (5.5-22.9)	0.32
History of prophylaxis	22 (34%)	2 (25%)	0.62
Age first joint bleed (years)	5.4 (4.0-9.4)	6.4 (4.6-10.1)	0.65
Annual no. joint bleeds	0.8 (0.2-1.9)	0.21 (0-0.45)	0.02
Mild haemophilia			
n	182 (36%)	10 (19%)	0.03
Age (years)	28-7 (15.1-51.2)	47.3 (12.8-63.4)	0..47
Factor activity (IU/dl)	10 (15-19)	14.5 (6-22)	0.85
Patient years (years)	9.0 (5.0-15-2)	6.3 (4.8-10.1	0.19
History of prophylaxis	8 (4%)	0	0.50
Age first joint bleed (years)	9.7 (5.9-17.5)	8.5 (6.0-23.8)	0.98
Annual no. joint bleeds	0.06 (0-0.22)	0.15 (0-0.4)	0.32

Values are median (IQR) or n (%).

Table 1 shows patient, treatment and bleeding characteristics according to haemophilia type and severity. Haemophilia severity was not equally distributed across both haemophilia types (p= 0.02), therefore all models were corrected for baseline factor level. Mild haemophilia was more frequent (36% vs. 19%, p=0.03), and severe haemophilia was slightly less frequent (51 % vs. 65%, p=0.09) in haemophilia A compared to haemophilia B. All other parameters were similar for both haemophilia types. Haemophilia B patients were somewhat (median 4 years), but not significantly older than Haemophilia A patients. Age at first joint bleed was comparable at a median of 3 years (range 0-22 years) for both types (p=0.47). Mean annual number of joint bleeds was equal in mild and severe haemophilia A and B (p=0.32 and 0.81), however in moderate haemophilia A, a slightly higher bleeding rate than in haemophilia B patients (median 0.8 vs. 0.21 joint bleeds/ year, p=0.02) was observed.

Table 2 shows the crude (univariate) and adjusted (multivariate) risk ratios for the associations between haemophilia type and age at first joint bleed or annual bleeding frequency.

Table 2. The association of age at first joint bleed with haemophilia type

	Univariate RR (95%CI)	Adjusted RR (95%CI)
Age first joint bleed haemophilia B	0.88 (0.58-1.32)	1.05[1] (0.76-1.4)
Annual no. joint bleeds haemophilia B	1.0 (1.0-1.3)	0.83[2] (0.71-0.96)

RR = Incidence Risk Ratio; CI = Confidence Interval; [1] adjusted for factor level and age at first visit to haemophilia treatment centre; [2] adjusted for severity (i.e. factor level), treatment, age and repeated measurements

Age at first joint bleed was similar for haemophilia A and B in the crude comparison (RR 0.88; 95%CI 0.58-1.32), as well as independent of the effects of factor level and age (RR 1.05; 95%CI 0.76-1.4). Factor level was a more important determinant of age first joint bleed; RR 1.1; 95%CI 1.1-2.3 per percent increase in baseline factor VIII/IX activity.

The crude association between annual number of joint bleeds and haemophilia type was borderline significant (p=0.05). After adjustment, haemophilia B patients showed 17% less joint bleeds per year than haemophilia A patients (RR 0.83; 95%CI 0.71-0.96). As expected, an additional association between joint bleeding and factor VIII/IX activity was also observed: the number of joint bleeds decreased with 12% (95%CI 11-13%) for every percent increase in factor VIII/IX activity.

Discussion

The present study presents a comparison of haemophilia types in the largest dataset containing bleeding data until now. After adjustment for the effects of age, factor VIII/IX activity, treatment and repeated patient evaluations, haemophilia B showed a 17% lower bleeding frequency than haemophilia A. However, onset of joint bleeding, an indicator of severity of haemophilia, was similar for haemophilia A and B.

The distribution of severities varied across the two haemophilia types in this cohort. It contained fewer patients with mild haemophilia B than with mild haemophilia A. Therefore, all analyses were adjusted by including baseline factor activity in the model, resulting in the effect of the different

haemophilia types independent of these differences in severities. Haemophilia B is possibly under diagnosed, which might lead to an even lower bleeding frequency if all patients with haemophilia B could be included.

The present findings appear in contrast with a previous report from our group, suggesting similar phenotypes in haemophilia A and B[8]. In the descriptive and unadjusted data of the current study, haemophilia B also seemed to be similar to haemophilia A (Table 1). However, when adjusted for age, severity and treatment, haemophilia B did show a lower bleeding risk. This emphasizes the importance of a correct modelling technique[10] and sufficient data to perform the analysis[9]. The multi-level structure of the model, which included all individual evaluation periods, allowed the distinction of bleeding frequencies within the same patient according to treatment, while taking into account the effects of increasing age.

This study confirms the current clinical opinion that haemophilia B has a milder bleeding phenotype than haemophilia A[3-5,9,11]. In classification studies, haemophilia B patients were mostly classified as moderate or mild phenotype[12,13]. Using data of 77 patients with haemophilia A and 23 with haemophilia B, Schulman et al[15] designed and validated a haemophilic severity score, consisting of joint bleeding frequency, factor consumption and orthopaedic outcome. Haemophilia B scores were consistently lower than in haemophilia A. Santavirta et al[16] derived their classification of phenotype by principal factor analysis. In 20 patients with haemophilia B (7 severe, 13 moderate) and 82 with haemophilia A (60 severe, 22 moderate), about 2/3 of the severe patients had a severe phenotype in both haemophilias, but only moderate patients with haemophilia A (7/22) also had severe phenotypes, while all moderate haemophilia B patients had moderate or mild phenotypes.

Besides haemophilia type, treatment, age, and factor VIII/IX activity, joint bleeding may also be influenced by physical activity. Unfortunately, information on physical activity was not available in this dataset. Physical activity was expected not to be related to haemophilia type. Therefore, lack of this information will not confound the association of haemophilia type with joint bleeding.

The lower bleeding tendency in haemophilia B may be explained by the different plasma concentrations of FVIII and FIX. At 90nM, the plasma concentration of FIX is almost 130 times higher than that of FVIII (0.7nM), but both form coagulation complexes using single FVIII/FIX molecules[14]. Consequently, a patient with 5% FIX activity has considerably more molecules per litre plasma than a patient with 5% FVIII activity. FVIII and FIX also have different influences on thrombin generation, due to different interactions with platelets[15]. Differences in thrombin generation might explain the different clinical pictures in both haemophilia types. Additionally, in vitro experiments suggested that thrombin generation may be higher at low FIX activity levels: maximum thrombin generation rates were achieved in the presence of less than 1% FIX activity, but required almost 100% FVIII activity to achieve the same rate[16].

Another explanation could be the lower proportion of patients with null mutations in haemophilia B, resulting in a higher proportion of patients who may be able to produce a trace of FIX activity. This trace FIX, though less than 1%, could perhaps reduce the risk of joint bleeding[5].

Although the risk of joint bleeding seems to be reduced in haemophilia B, these patients still suffer from joint bleeding and subsequent joint damage[17]. Some authors have suggested that primary prophylaxis should be avoided in haemophilia B[5,18]. In our opinion, prophylaxis is still indicated in all patients with spontaneous joint bleeding, including primary prophylaxis.

Future comparisons on even larger, multi-centre cohorts are needed to confirm these results. However, univariate analyses should be interpreted with caution. This study showed that correction for patient and treatment characteristics was needed to confirm clinical opinion.

This study confirmed the clinical opinion that haemophilia B has a lower joint bleeding frequency than haemophilia A. Haemophilia B patients had, on average, slightly milder phenotypes than haemophilia A patients. In future studies on evaluation of treatment and bleeding, both haemophilia types should be analysed separately to avoid biased outcomes.

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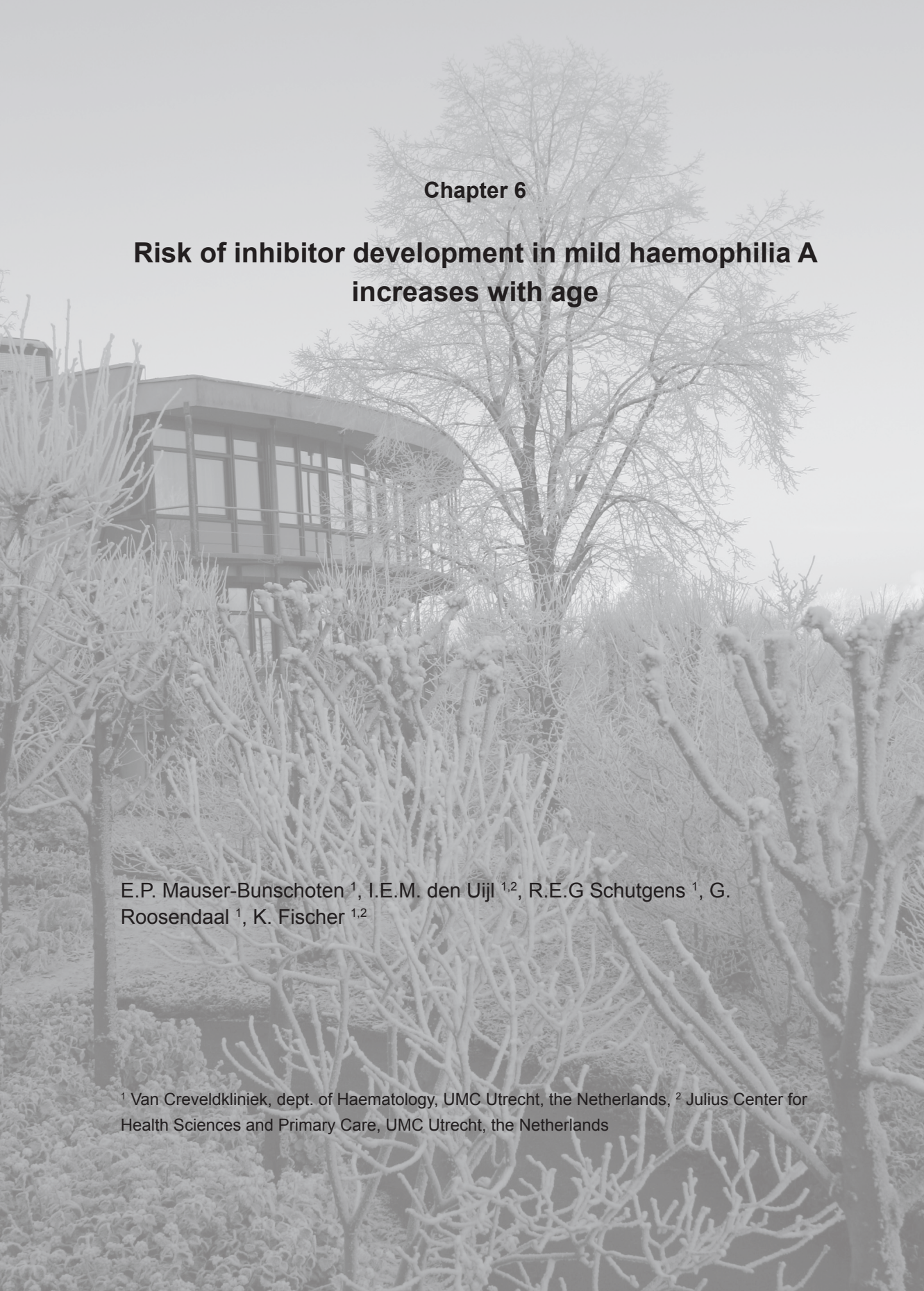

Chapter 6

Risk of inhibitor development in mild haemophilia A increases with age

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Abstract

Mild haemophilia A is a rare disease with a relatively mild phenotype. Treatment with factor VIII (FVIII) is indicated after trauma or for surgery only. FVIII infusion may result in the development of inhibiting antibodies against FVIII. This study describes the association between age and other risk factors for inhibitor development in mild haemophilia.

A retrospective cohort study was conducted among all patients with mild hemophilia registered at the Van Creveldkliniek, UMC Utrecht, The Netherlands. Data on peak treatment with FVIII, gene mutation and history of inhibitor development were obtained from patient files from the period between 1-1-1970 and 31-12-2009.

231 out of 297 (78%) patients had at least one exposure to FVIII, of whom 14 (6,1%) developed inhibitors to FVIII, at a median age of 66 years. Age at first exposure, age at peak treatment, number of peak treatments and Arg593Cys mutation were significantly associated with the development of inhibitors, while continuous infusion with FVIII was not.

Although the incidence of inhibitors in mild haemophilia is low, it increases with age and peak treatments. With increasing age, patients with mild haemophilia will suffer from co-morbidity more frequently, requiring surgical interventions and exposing them to an increased risk of inhibitor development. Especially patients with a change of arginine to basepair cysteine at 593 in the FVIII gene are at risk for inhibitor development.

Introduction

Mild haemophilia A is a rare disease, which is usually caused by missense mutations in the factor VIII gene, mainly in the A domains, resulting in a decreased clotting factor VIII (FVIII) activity of 6-40 IU/dl[1,2]. Novel splicing errors, small rearrangements and promoter mutations have also been described[3]. Mild haemophilia is mostly diagnosed early in life in case of a positive family history, or later after (repeated) post-operative or traumatic bleeding. Median age at diagnosis in children with mild haemophilia is 28.6 months, whereas severe haemophilia is diagnosed at a median age of 5.8 months[4]. However, as its phenotype is mild and bleedings only occur after trauma or after surgery, diagnosis is often made during adulthood[5,6]. Treatment of mild haemophilia A consists of desmopressin (1-desamino-8-D-arginine vasopressin, DDAVP), which may result in a 2-6 fold increase of baseline factor VIII levels[7,8]. Due to tachyphylaxia, DDAVP can only be used for short periods at a time. Replacement FVIII is only indicated in mild haemophilia patients when factor correction for a longer period is indicated. A risk factor for inhibitor development in haemophilia is a period of intensive FVIII infusion, a so called peak treatment[9]. Additional risk factors for inhibitor development in mild haemophilia are certain missense mutations[10]. The exact incidence of inhibitor development in mild haemophilia is unknown, but is reported to be between 3-13%[11-13]. Inhibitors may cross react with endogenous FVIII, resulting in a decrease of endogenous factor VIII to <1 IU/dl, converting mild into moderate or severe haemophilia[14,15]. At present, life expectancy of patients with mild haemophilia is equal to that of non-haemophilic men[16]. With increasing age, it may be expected that patients with mild haemophilia will develop age-related co-morbidities, which may require surgery and subsequent peak treatment moments. Especially older patients with mild haemophilia who have never received clotting factor concentrate may be at risk of inhibitor development. Moreover, it is not yet determined whether age itself is a risk factor.

To study the association between age and other risk factors for inhibitor development in mild haemophilia, a retrospective cohort study was conducted among all patients with mild haemophilia treated at a large haemophilia treatment centre; the Van Creveldkliniek, University Medical Centre Utrecht, the Netherlands.

Patients and methods

All patients with mild haemophilia A (FVIII 6-40 IU/dl) registered at the Van Creveldkliniek between 1-1-1970 and 31-12-2009 were included in this study. Registration of bleeds and treatment was available for all patients treated at the Van Creveldkliniek from 1-1-1970 onwards. Data on peak treatment with factor VIII for surgery, trauma or severe bleeding, as well as history of inhibitor development were obtained from patient files from the period between 1-1-1970 and 31-12-2009. Peak treatment was defined as treatment with FVIII for at least 5 consecutive days, either by bolus injection or by continuous infusion. For patients born before 1970, years risk for inhibitor development was calculated from 1970 onwards, as only then treatment with clotting factor VIII became available for this patient group.

Laboratory assays

Factor VIII was measured using the one stage assay. Mild haemophilia was defined as FVIII levels of 6-40 IU/dl.

Screening for inhibitors was performed during yearly check-ups, after peak treatment moments or at any time an inhibitory antibody was clinically suspected. For inhibitor screening, samples of 3.8% sodium citrate anticoagulated venous blood (0.5-4.5 ml) were drawn. Before December 1st 1996 inhibitor testing was performed by the Bethesda method, and subsequently by the Bethesda assay using the Nijmegen modification[17,18]. A positive inhibitor titer was defined as more than 1 BU/ml by the Bethesda method and more than 0.3 BU/ml by the modified Bethesda method. The factor VIII recovery was considered to be decreased when it was less than 66% of the expected level. The expected level of factor VIII activity was calculated according to Lee et al.[19].

DNA analysis

The F8 gene mutation was determined in the patient or in a family member with haemophilia. We assumed that the defect causing mild haemophilia in the patient was identical to that of all of his family members with mild haemophilia. DNA analysis in EDTA blood was performed as described previously[20]. The sequences were compared to the reported gene sequence using the SeqScape program (Applied Biosystems).

Novel missense mutations were considered detrimental when 1) other substitutions at the same position were reported, 2) the nature of the substitution was non-conservative, 3) the degree of conservation between species was high, and/or 4) no other mutation was identified in the entire coding region of the F8 gene of the patient.

Statistical analysis

All parameters were compared between inhibitor positive and negative patients, p-values were calculated with a z-test for proportions or Mann-Whitney U test for continuous variables. With 14 cases, power was insufficient to perform multivariate regression. Stata10.0 (Statacorp LP, TX, USA) was used for all analyses.

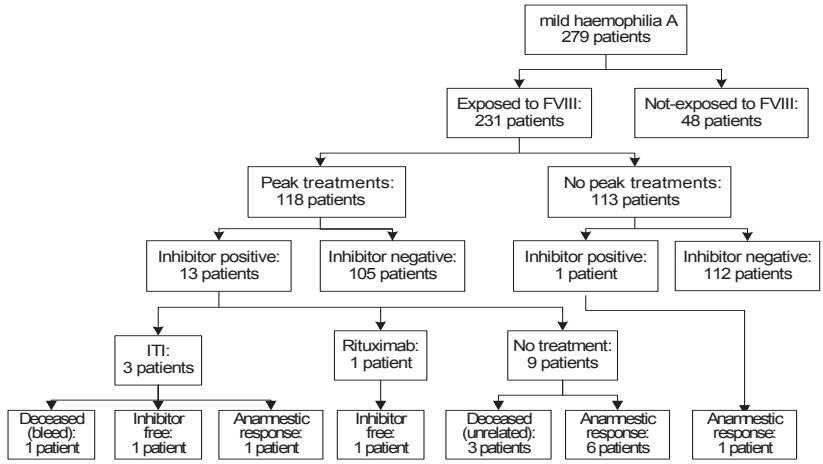
Results

Forty-eight out of 279 patients with mild haemophilia (17.2%) were not exposed to FVIII at the time of the study (median age 35 years; range 0-90 years). The remaining 231 patients with histories of factor VIII treatment had a median age of 38 years (range 1-87 years). This group consisted of 228 patients with Caucasian and three with African ethnicities. Fourteen out of 231 patients developed an inhibitor; a cumulative incidence of 6.1%. All were Caucasian.

Patient characteristics for both groups are summarized in Table 1. One-hundred-eighteen patients received a total of 223 peak treatments, 13 (5.8%) resulted in the development of inhibitors (Figure 1). One patient developed an inhibitor without a peak treatment, five after their first peak treatment.

Endogenous FVIII activity was similar between those with and without inhibitors, but patients with inhibitors received their first treatment with FVIII at a much later age: median of 43 years

Figure 1. Clinical cause and inhibitor development of mild haemophilia patients



*ITI = Immune Tolerance Therapy which comprised 50 U/kg FVIII, 3 times weekly

(IQR 15-60 years) compared to a median of 6.7 years (IQR 2-19 years) in non-inhibitor patients. Median age at inhibitor development was 66 years (IQR 4-49 years). Initial inhibitor titres ranged from 0.4-13 BU/ml, with a median of 2.3 BU/ml. Maximum inhibitor titres ranged from 0.6-55 BU/ml (median 4.7 BU/ml). In 13 out of 14 patients with inhibitors, a decrease in endogenous FVIII activity was found. Only in one patient with low titre inhibitor (max titre 0.6 BU/ml), FVIII activity remained stable.

Table 1. Characteristics and peak treatments of patients with mild haemophilia A, ever treated with FVIII, according to inhibitor status

	Inhibitor positive	No inhibitor	p-value
Patients (n)	14	217	
Age at inhibitor or last test (years)	61 (42-84)	38 (18-56)	<0.01
Endogenous FVIII activity (IU/dl)	14 (12-17)	15 (10-20)	0.06
Mutation Arg593Cys	5 (36%)	20 (9%)	<0.01
Age at first exposure (years)	43 (15-60)	6.7 (2.0-19)	<0.001
<50 exposures	10 (71%)	167 (77%)	0.88
Age at peak treatment (years)	53 (30-66)	21 (10-64)	<0.01
Age first peak (years)	59 (25-69)	21 (7-49)	<0.01
Age at peak leading to inhibitor or last peak (years)	66 (49-74)	27 (12-52)	<0.01
Number of patients with peak treatment	13 (93%)	105 (48%)	<0.01
Patient years at risk			
Total	242	2360	
Per patient	19 (5.5-25.0)	14 (4.3-29)	0.66
Number of peak treatments			
Sum	49	174	
Per patient	3 (3-5)	1(1-2)	<0.01
Continuous infusion at peaks	18 (37%)	55 (32%)	0.62

Values are medians (IQR) or numbers (%)

Six patients had FVIII levels between 2 and 8 IU/dl, and 7 patients ≤1 IU/dl in the presence of inhibitors. These 7 patients also showed increased bleeding tendencies, for which they were treated with bypassing agents (5 cases) or high dosages of FVIII (2 cases).
The clinical cause of inhibitor patients is shown in Figure 1. Three patients with persistent low titre inhibitors had anamnestic responses after renewed exposure to FVIII. A total of four patients with inhibitors died during follow-up at ages between 70-86 years. Three patients died from unrelated causes (one malignancy, one malignancy with myocardial infarction and one pneumonia) and one from a spontaneous massive retroperitoneal bleed.

Table 2. Number of patients with different mutations according to inhibitor presence

Mutation	Inhibitor positive	No inhibitor	p-value
Arg593Cys	5	20	<0.01
Asn618Ser	2	68	0.3
Arg531Cys	2	11	0.4
Phe1175Val	2	0	0.07
Ile2262Thr	1	0	<0.01
Other mutation	0	63	0.14
Mutation unknown	2	42	0.91
Total	14	204	

Risk factors for inhibitor development
Age at first exposure, age at peak treatment, number of peak treatments and Arg593Cys mutations were all significantly associated with the development of inhibitors. The association between mutation and inhibitor development is shown in Table 2.
Almost half of the patients (47%) had one of three mutations. The Arg593Cys mutation was found in 25 patients (11%), of which 5 (20%) developed inhibitora; a significant risk factor. Asn618Ser was found in 70 patients of which 2 (2%) developed inhibitors. Arg531Cys was found in 13 patients of which also 2 (15%) developed inhibitors. Three other patients with inhibitors had novel mutations, not found in patients without inhibitors: Phe1175Val (2 patients, brothers) and Ile2262Thr (1 patient).
Patients who developed inhibitors tended to spend more days in hospital and used more FVIII/kg than patients without inhibitor development. However, these factors were not significant (data not shown). Five inhibitor patients had a positive family history in the second degree, six a negative history and three had no family members with haemophilia. No conclusions on the effect of type of FVIII concentrate on inhibitor development could be drawn as patients were treated with various FVIII concentrates. Continuous infusion with FVIII did not significantly increase inhibitor risk.

Discussion

In this retrospective, single centre cohort study in patients with mild haemophilia A, 14 out of 231 (6.1%) patients who were ever exposed to FVIII developed inhibitors. The major risk factors for inhibitor development were age at first exposure, peak treatment, age and number of peak treatments, and the presence of the Arg593Cys mutation. Unfortunately, power was too low to study

the independent effects of these risk factors.

To our knowledge, this is the largest cohort of patients describing the risk of inhibitor development in patients with mild haemophilia. The incidence in mild haemophilia is lower compared to severe haemophilia and in accordance with other published data on mild haemophilia[21].

We found an association between age at first exposure and at peak treatment and inhibitor development in mild haemophilia A. Our data are in accordance with those recently published by Kempton et al[22]. who found an association between risk of inhibitor development, peak treatment and age in 36 inhibitor patients and 62 controls with moderate or mild haemophilia. They described a stronger interaction between age and intensive replacement therapy in patients with mild haemophilia compared to those with moderate haemophilia. A possible explanation may be the coexistence of malignancy or a change in immune status with age. Formation of inhibitors depends on the interaction of T cells[23] with HLA class II molecules, which is followed by B-cell proliferation and differentiation and then production of inhibitors[24,25]. It is thought that polymorphisms of genes involved in the immune response to FVIII play a role in the development of inhibitors. Some HLA class II alleles are associated with a high risks of inhibitor development, while others may be considered protective. The association between inhibitors and polymorphisms in genes coding for interleukin-10 and tumor necrosis factor-alpha has also been considered. Some genotypic variations of these immunoregulatory cytokines might increase the risk of inhibitors[25]. Endogenous signals released by tissue damage, for example during surgery or another inflammatory state, may also trigger the immune response. As life expectancy in patients with mild haemophilia is similar to the general population, development of malignancies and other age-related co-morbidities can be expected, probably increasing the risk of inhibitor development.

We found a significant increased risk of inhibitor development with the Arg593Cys mutation, causing a change of the arginine residue for cysteine. This is in accordance with several other published cases and case series[9,10,15,26]. The risk of inhibitor development is associated with the type and location of mutations[2,25]. The prevalence of inhibitors in patients with missense mutations is 5%. However, mutations causing changes in the A2 domain of the heavy chain or the junction of the C1 and C2 domain in the light chain of the FVIII molecule are associated with fourfold increased risks of inhibitor development compared to mutations outside these regions (10 vs. 3%)[9,10,25-27].

In addition to other studies, Eckhardt et al.[10,11] suggested increased risk of inhibitor development after surgery. In our study, the risk of inhibitor development after peak treatment was increased. The risk of inhibitor development after continuous infusion seemed to be lower, although not significantly, possibly due to low numbers in our study. Peak treatment is known to be associated with inhibitor development, especially in young children with severe haemophilia who have <50 exposures to FVIII[12,13,28]. Like Kempton et al[22], in their study no difference in risk of inhibitor development between patients with less than 50 exposures compared to those with over 50 exposures was seen. We found a cumulative incidence of inhibitors of 6%, which is much lower than the 25% reported in severe haemophilia A[28]. An explanation might be that patients with mild haemophilia are infrequently treated with FVIII between two peaks. This may decrease or postpone inhibitor development. Oldenburg[25] suggested that patients with missense mutations synthesize endogenous but functional altered FVIII moleculed, which are sufficient to induce immune tolerance

and thus decrease the risk of inhibitor development. However, Viel et al[29] suggest that this FVIII molecule is antigenically distinct from the FVIII concentrates used for treatment of haemophilia A. This “wild type” FVIII is subsequently recognized as non-self and may lead to inhibitor formation. Alloimmunization against factor VIII can also occur in patients with F8 missense mutations that change only a single amino acid residue in the factor VIII protein.

The number of patients who developed inhibitors was small. This led to a lack of power and multivariate regression analysis to determine the independent effect of several risk factors on inhibitor development could not be performed. To do this, large multicenter studies containing sufficient numbers of patients with inhibitors will be required. Currently such a study, the so called INSIGHT study, is initiated by the group of Eckhardt et al.[30] In this European study, 1500 patients with mild and moderate haemophilia A will be included.

We found a low cumulative incidence (6%) of inhibitors in mild haemophilia A. Age at first exposure, peak treatments, peaks at older age and the Arg593Cys mutation were risk factors for inhibitor development. As co-morbidity in older patients with mild haemophilia is increasing, surgical interventions, requiring prolonged FVIII replacement, will be performed more often. This, together with immunological changes of older age, may cause an increase in the incidence of inhibitors in mild haemophilia. Therefore, all patients with mild haemophilia should be treated in haemophilia centres, monitored closely and regularly tested for inhibitors, in particular after peak treatments.

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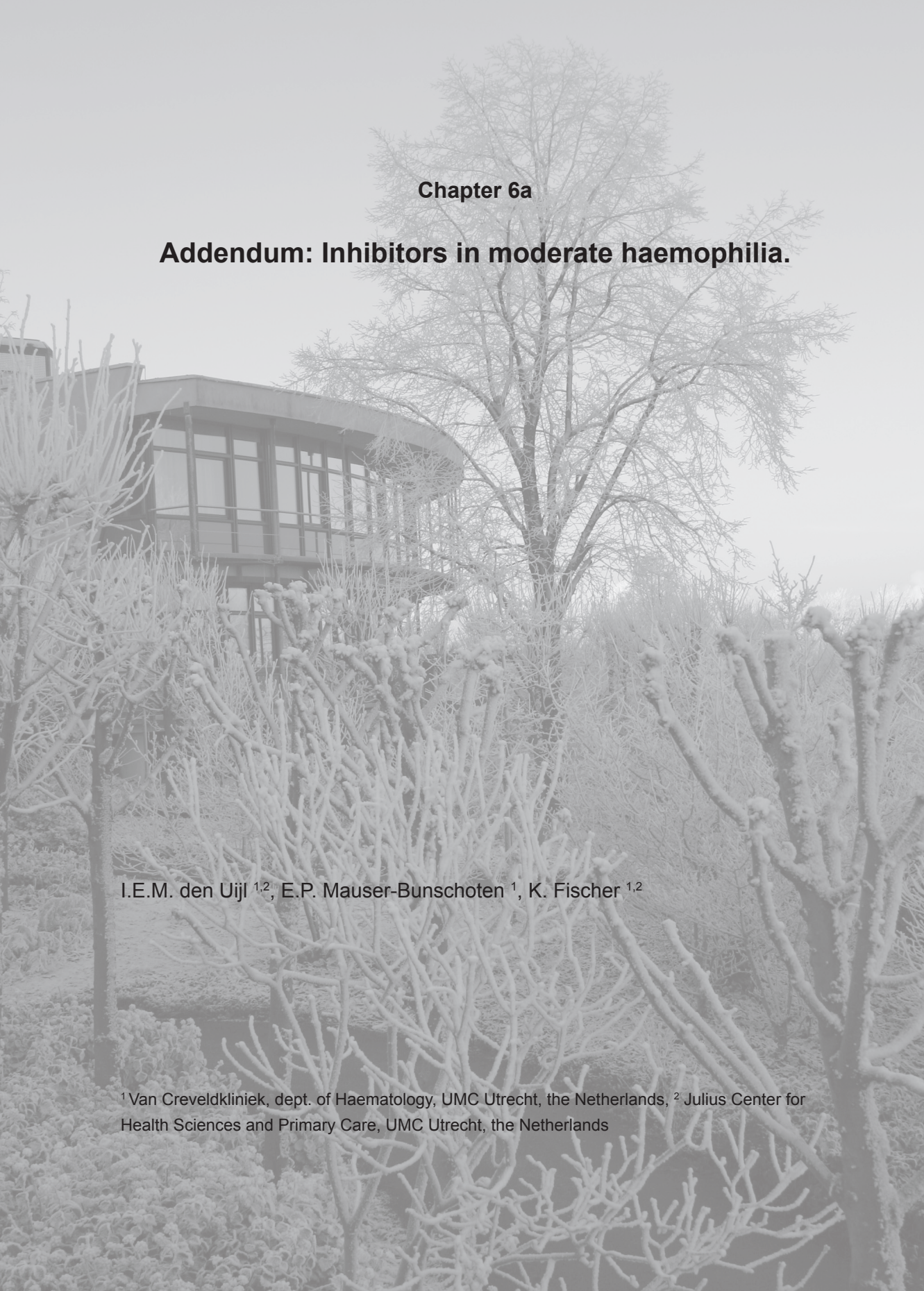

Chapter 6a

Addendum: Inhibitors in moderate haemophilia.

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Introduction

Patients with moderate haemophilia are earlier and more often treated with factor VIII (FVIII) than patients with mild haemophilia[1]. Therefore, these patients also develop inhibitors against FVIII. To our knowledge, there have been no studies on inhibitors in moderate haemophilia. This addendum provides details on inhibitor development in moderate haemophilia and its association with peak treatment.

Patients and methods

Data on peak treatment with FVIII for surgery, trauma or severe bleeding, as well as history of inhibitor development, were obtained from patient files from the period between 1-1-1970 and 31-12-2009. Peak treatment was defined as treatment with FVIII for at least 5 consecutive days either by bolus injection or by continuous infusion. For patients born before 1970, years at risk for inhibitor development were calculated from 1970 onwards, as only then treatment with clotting factor VIII became available for this patient group.

FVIII was measured using the one stage assay. Screening for inhibitors was performed during yearly check-up, after peak treatment moments, or at any time an inhibitory antibody was clinically suspected. Before December 1st 1996, inhibitor testing was performed by the Bethesda method and subsequently by the Bethesda assay using the Nijmegen modification[2,3]. A positive inhibitor titer was defined as more than 1 BU/ml by the Bethesda method, and more than 0.3 BU/ml by the modified Bethesda method.

Statistical analyses could not be performed due to the low numbers of inhibitors in moderate haemophilia.

Results

Complete data was available for 80 patients with moderate haemophilia. One patient was never exposed to FVIII. Five patients developed inhibitors (6.3 %) at a median age of 17 years (range 2-55 years). Table 1 shows patient characteristics according to inhibitor history. Age at first exposure was similar in both groups. Of the five patients who developed inhibitors, two had multiple peak treatments (2 and 5 peak treatments) prior to inhibitor development. The others never had peak treatments. In the other group, 53 patients (71%) had at least one peak treatment, median 2 peak treatments (range 1-8 peak treatments), without inhibitor development. Continuous infusion was similar in both groups, although numbers were too small to draw conclusions.

Inhibitors developed at a median age of 17 years (range 2-55 years). First titre was median 0.7 BU/ml (range 0.5-1.1 BU/ml). Most inhibitor titres remained quite low, with maximum titres of median 1.0 BU/ml (range 0.8-141 BU/ml), while 2 patients had high titre inhibitors (max 55 and 141 BU/ml, respectively).

In all 5 patients, FVIII activity decreased in the presence of the inhibitor. In 2 patients trough levels dropped <1 IU/dl; these patients showed increased bleeding tendencies. Immune tolerance induction was successfully applied in 1 patient, the other 4 patients did not receive any therapy. Two patients had anamnestic responses after rechallenge with FVIII. Currently, 4 patients are

successfully treated with FVIII, although 2 patients need an increased dose, and 1 patient is treated with FVIIa.

Table 1. Patient characteristic according to inhibitor history

	inhibitor positive	No inhibitor
n	5	75
Age at inhibitor or last negative test (years)	17 (16-34)	34 (20-48)
Endogenous FVIII activity (IU/dl)	4	3 (2-4)
Age at first exposure (years)	3.4 (1.3-28)	4.1 (1.3-2.8)
Age first peak (years)	11.4-30	7.6 (1.3-22)
Age at peak leading to inhibitor or last peak (years)	14-54	30 (17-51)
Number of patients with a history of peak treatment	2 (50%)	53 (71%)
Patient years at risk (years)	17 (16-34)	13.5 (5.4-21)
Number of peak treatments		
sum	5	143
per patient	0-3	2 (1-4)
Continuous infusion at peaks	3 (38%)	41 (29%)

Values are median (IQR) or n (%)

Discussion

Since moderate haemophilia is rare, inhibitors in moderate patients are even rarer. This addendum describes an incidence of inhibitors in moderate haemophilia of 6.2%, which is comparable to the incidence of inhibitors found in mild haemophilia[4].

Half of patient with inhibitors had peak treatments; unfortunately numbers are too low to draw conclusions from these data. It seems that patients with moderate haemophilia develop their inhibitors at a younger age (median 17 years; IQR 16-34 years) compared to mild haemophilia patients (median age 61 years; IQR 42-84 years)[4]. This may be associated with earlier treatment in patients with moderate haemophilia, due to their more severe bleeding pattern.

A larger international, multi-centre study on inhibitors in moderate and mild haemophilia is underway which is aiming at including 1500 patients[5]. Hopefully this study will be able to identify whether risk factors for inhibitors in moderate haemophilia are similar to those in mild or severe haemophilia. Peak treatments and age have been identified in both haemophilia severities as risk factors[4,6], so presumably peak treatment will also be a risk factor for moderate haemophilia.

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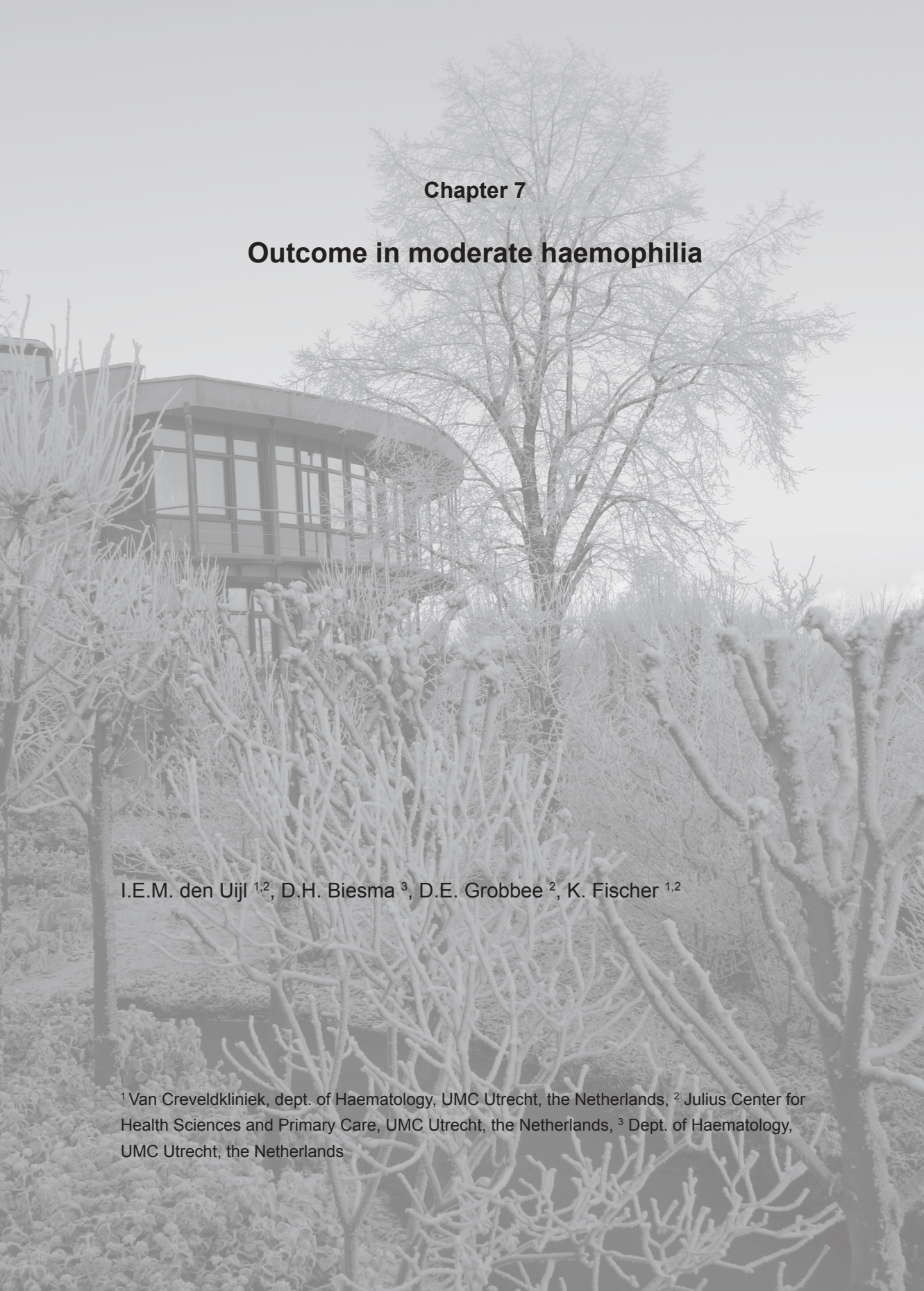

Chapter 7

Outcome in moderate haemophilia

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Abstract

Moderate haemophilia is the rarest form of haemophilia. This study aims to assess short and long-term outcome, including it's association with treatment, in patients with moderate haemophilia.

Seventy-five patients with moderate haemophilia (1-5% FVIII/IX activity), without a history of inhibitors, treated at the Van Creveldkliniek, UMC Utrecht, the Netherlands, were included in the study. Life-long data on bleeding and treatment were collected. Joints were evaluated using the Haemophilia Joint Health Score (HJHS), addionally, adults completed questionnaires on activity (HAL) and quality of life (SF-36, EQ5D).

Haemophilia A was diagnosed in 89%, median age was 37 years (IQR 23-52 years). Bleeding frequency was low; median annual bleeding rate was 2.0 bleeds/year (IQR 0.8-3.7 bleeds/year), including median 0 joint bleeds/year (IQR 0.8-3.7 bleeds/year). Joint function was good: 82% scored <10 out of 126 points with HJHS. Nevertheless, 29% of moderate patients had a history of prophylaxis, because of a high bleeding frequency. Median age at 1st joint bleed was 4.8 years (IQR 3.5-8.5). Use of prophylaxis was better associated with age at first joint bleed ($p<0.01$) than with baseline factor activity ($p=0.12$). Most (52%) patients who suffered their first joint bleed before the age of 5 years required prophylaxis later in life.

The majority of patients with moderate haemophilia have few bleeds and complications; however, a considerable subset of patients with a more severe bleeding pattern need prophylactic treatment. These patients may be identified by the onset of joint bleeding before age 5 years.

Introduction

Haemophilia is an inherited, X-chromosomal bleeding disorder. Moderate haemophilia is the rarest form of haemophilia; globally about 15% of haemophilia patients have residual factor activity levels of 1-5 IU/dl[1]. These patients have milder bleeding patterns than patients with severe haemophilia, who completely lack factor VIII (FVIII) or factor IX (FIX) activity. Patients with moderate haemophilia mostly suffer from traumatic bleeds only and generally do not experience spontaneous bleeds[2]. Consequently, patients with moderate haemophilia are mostly treated on demand[3]. Nevertheless, these patients do report complications due to haemophilia. Results from the 5th HIN (Haemophilia In the Netherlands) nationwide questionnaire suggested severe complaints in moderate haemophilia; 11% of patients aged 19-40 yrs and 33% of patients aged 41-64 years experienced severe hindrance of their daily activities due to haemophilia[4]. Moreover, it has been established that most of these patients are not on home treatment and have limited experience in recognizing a bleed; therefore they are at risk for a delay in treatment[5]. Treatment delay may result in increased and prolonged exposure to intra-articular blood, and eventually increase joint damage. The question is what the bleeding pattern of moderate haemophilia patients is, and whether they are currently being undertreated. Moreover, assessing outcome in moderate haemophilia may be used as a benchmark for the results of life-long prophylaxis in patients with severe haemophilia. This study aims to assess short and long-term outcome, including its association with treatment in patients with moderate haemophilia.

Patients and methods

Patients with moderate haemophilia (1-5% FVIII/IX activity) treated at the Van Creveldkliniek, UMC Utrecht, the Netherlands, born before 2000, were included in the study. Patients who had inhibitors (4 patients), or did not have access to haemophilia care in their childhood (6 patients) were excluded.

Age at entry into the clinic, the onset of bleeding, history of home-treatment, orthopaedic surgery and prophylaxis were collected from medical records. Annual data on bleeding and treatment were collected from 1970 onwards. After signing informed consent, patients underwent a physical examination, using the Haemophilic Joint Health Score (HJHS, version 1.0)[6], those above the age of 18 years were asked to complete questionnaires on quality of life (SF-36[7], EQ5D[8]) and daily activities as measured by the Haemophilia Activities List (HAL[9]).

Onset of bleeding and bleeding frequency, home-treatment, clotting factor consumption, use of prophylaxis, orthopaedic outcomes and quality of life were compared across residual factor activity levels. Clotting factor activity was measured by one-stage assay[10] in a single certified laboratory. If residual factor levels were measured more than once, the lowest factor level was used for classification. Prophylaxis was defined as at least one regular infusion per week for at least 45 weeks per year. Joint bleeds were defined as complaints in ankles, knees, elbows, hips, wrists, or shoulders requiring treatment with FVIII at least once.

Information on bleeding and factor consumption was available from 1970 onwards and had been collected annually from entry at the clinical until 2007.

Statistical testing was performed using Kruskal-Wallis rank-sum test, a non-parametric test for

continuous variables or a Fischer's exact test for categorical variables.

Results

Eventually, 75 patients participated, 60 (80%) underwent a physical examination and 56 (84% of adult patients) returned completed questionnaires. Among the non-responders, 9 were under the age of 18 years, thus not eligible for the questionnaire, 4 were no longer treated at the centre at the time questionnaires and physical examinations were performed, and 6 never responded. Bleeding and treatment data of these 19 patients, extracted from their patient files, were included in the study.

Table 1. Characteristics of patients with moderate haemophilia

	n=75
Age in 2007 (years)	37 (23-52)
Follow-up (years)	14.8 (8.3-22.8)
Age at diagnosis (years)	1.5 (0-5.9)
Baseline factor activity (IU/dl)	3 (2-4)
Haemophilia A	67 (89%)
HCV positive	17 (23%)
HIV positive	1 (1%)
History of CVAD	0
History of prophylaxis	22 (29%)
Prophylaxis at last evaluation	17 (23%)
Age at start prophylaxis (years)	13.7 (6.5-26)
Currently on home treatment	34 (45%)

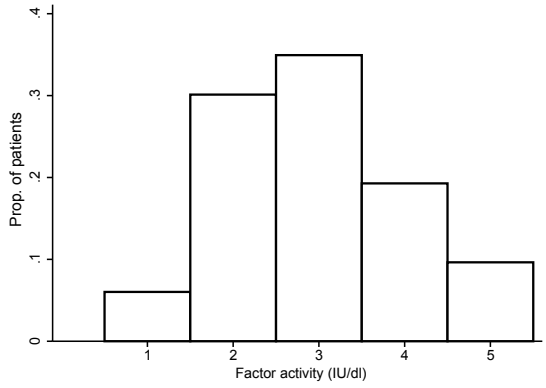
Values are medians (interquartile range) or numbers (%)

Table 1 shows demographics of the moderate haemophilia patients. Haemophilia A was most prevalent (67 patients, 89%). Age at evaluation in 2007 was median 34.3 years and ranged from 5.1 to 73.6 years. Median follow-up was 14.8 years, ranging from 3.1-35.5 years. Patients with moderate haemophilia were diagnosed at a median age of 1.5 years, ranging from 0 to 48 years. The distributions of current age and age at diagnosis were similar across different factor activity levels (p=0.85 and p=0.84, respectively). Chronic HCV infection was present in 17 patients (23%), equally distributed across different factor activity levels, while only one patient was HIV positive. None of the patients with moderate haemophilia had ever needed a central venous access device (CVAD).

Residual factor activity levels ranged from 1-5 IU/dl. Median number of factor measurements was 5 (IQR 3-6), while 8 patients had only 1 measurement of baseline clotting factor activity available. Five patients (6%) had a residual FVIII activity levels of 1 IU/dl, 23 (31%) had 2 IU/dl, 26 (35%) had 3 IU/dl, 13 (17%) had 4 IU/dl and 8 (11%) had 5 IU/dl (Fig. 1).

Twenty-two (29%) patients with moderate haemophilia were treated with prophylaxis at some time in their life. Median age at start of prophylaxis was 14 years, ranging from 1-59 years. All patients were on secondary prophylaxis. They suffered median 4.1 joint bleeds/year (IQR 2.5-6.5 joint bleeds/year) between age at first joint bleed and starting prophylaxis. Seven of these pa

Figure 1. Distribution of residual clotting factor activity of moderate haemophilia patients (n=75



patients (32 %) discontinued prophylaxis after a median of 3.2 years, and 2 restarted prophylaxis due to recurrent bleeding (Table 1). All patients treated with prophylaxis used home treatment; in total 34 patients (45%) used home treatment at last evaluation.

Outcome according to factor activity level

Haemophilia A was similar across factor activity levels (p=0.4). Overall, bleeding frequency was low (Table 2), median annual bleeding frequency was 2.0 bleeds/year (IQR 0.8-3.7 bleeds/year).

Table 2. Outcome according to residual factor activity level

	1 IU/dl n=5	2 IU/dl n=23	3 IU/dl n=26	4 IU/dl n=13	5 IU/dl n=8
Haemophilia A	4 (80%)	20 (87%)	23 (88%)	13 (100%	7 (87%)
Bleeding and treatment					
Age 1st joint bleed (years)	2.8 (2.3-2.8)	4.5 (3.5-8.3)	4.9 (4.1-8.5)	5.7 (4.0-12)	6.1 (4.3-18)
Median annual no. bleeds	2.9 (1.9-7.8)	3.7 (1.8-7.7)	1.9 (0.8-3.6)	1.2 (0.5-3.1)	1.2 (0.2-2.1)
Median annual no. joint bleeds	1.0 (0.3-3.1)	1.3 (0.4-2.5)	0 (0-1.2)	0 (0-0.96)	0 (0-0.73)
Without joint bleeds last 5 years	2 (40%)	7 (32%)	14 (54%)	8 (62%)	3 (38%)
History of prophylaxis	2 (40%)	11 (44%)	5 (17%)	3 (19%)	1 (13%)
Annual factor consumption (IU/kg/year)	119 (48-1215)	264 (119-1044)	217 (34-347)	22 (0-244)	46 (12-277)
Without treatment	0	2 (8%)	0	2 (15%)	2 (25%)
Orthopaedic outcome					
HJHS (max 128 points)	0	5 (2-10)	4 (0-9)	1 (0-4)	2 (0-6)
History of orthopaedic surgery	0	5 (22%)	4 (15%)	4 (31%)	0
HAL sum score (max 100 points)	97	96 (81-100)	96 (85-100)	92 (79-97)	86 (70-100)
Utility (EQ5D, max 1.0)	0.8	0.9 (0.7-1.0)	1 (0.8-1.0)	0.8 (0.7-1.0)	0.8 (0.6-0.8)

Values are medians (IQR) or numbers (%)

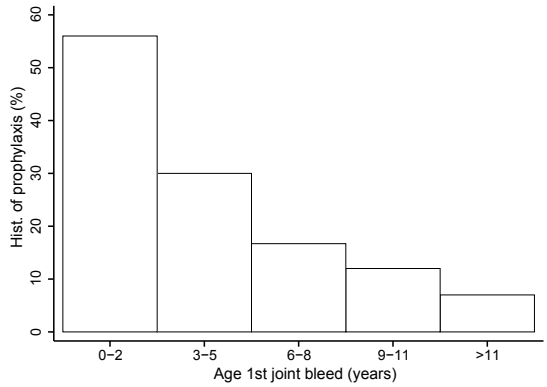
Median annual joint bleeding was 0 joint bleeds/year ranging from 0 to 8.8 joint bleeds/year. Thirty-three patients (44%) did not suffer any joint bleed during the last 5 years.

Patients with low baseline factor activities had higher bleeding frequencies than those with higher baseline factor activities. Patients with 1-2 IU/dl residual factor activity suffered median 2.9 bleeds/year (IQR 1.4-7.2 bleeds/year), including median 0.5 joint bleeds/year (IQR 0.5-2.5 joint bleeds/year), significantly more than patients with 3-5 IU/dl with median 1.4 bleeds/year (IQR 0.5-3.4 bleeds/year), including 0 joint bleeds/year (IQR 0-1.0 joint bleeds/year) (p=0.02). Prophylaxis was more frequently prescribed in patients with less than 3 IU/dl factor activity (39% vs. 13%). The more severe phenotype in patients receiving prophylaxis was obvious: patients requiring prophylaxis experienced median 6.1 bleeds/year (IQR 3.6-10.4 bleeds/year), including median 2.1 joint bleeds (IQR 1.4-4.5 joint bleeds/year), while those treated on demand suffered median 1.6 bleeds/year (IQR 0.8-3.9 bleeds/year), including median 0.5 joint bleeds (IQR 0.15-1.1 joint bleeds/year) (p<0.001). Although prophylaxis reduced bleeding, these patients continued to suffer joint bleeds: median 1.2 joint bleeds (IQR 0.8-3.4 joint bleeds/year) (p<0.01)

Median annual factor consumption was 148 IU/kg, ranging from 0-2903 IU/kg. Six patients (11%) had not used any replacement therapy in the last 5 years; one patient, with residual FVIII activity of 4 IU/dl, had never used factor replacement therapy. Similar to the bleeding frequencies and prophylactic use, factor consumption appeared higher in patients with 1-2 IU/dl (p<0.001). Patients treated with prophylaxis used significantly more factor concentrate than patients treated on demand, median 939 IU/kg (IQR 224-1964) versus 112 IU/kg (IQR 16-248 IU/kg) (p<0.001).

Orthopaedic outcome in patients with moderate haemophilia was good. Most patients (50/60; 82%) had minimal loss of function, less than 10 points on the HJHS scale (Table2). Equal to the bleeding and treatment parameters, orthopaedic outcome was similar across factor activity levels. Only 13 patients (17%), median age 53 years (range 22-78 years, 23% born before 1965), had a history of orthopaedic surgery (Table 2). Five patients had only minor orthopaedic surgery, like synovectomy or excision of cysts, while 3 patients had at least one ankle arthrodesis, and 6 patients had additional joint replacements. History of orthopaedic surgery appeared to be independent of treatment intensity, only 3 patients receiving orthopaedic surgery were ever treated with prophylaxis.

Figure 2. Percentage of patients with a history of prophylaxis according to age at first joint bleed



Outcome according to onset of joint bleeding

Residual factor activity levels were not clearly associated with the onset of joint bleeding. Age at first joint bleed was similar across residual factor activity levels (p=0.10), except for patients with 1 IU/dl who had all experienced their first joint bleed before the age of 3 years (Table 2). Age at first joint bleed was, however, associated with the need for prophylaxis. Patients who had their first joint bleed early, before the age of 5 years, more often required prophylaxis than patients suffering their first joint bleeds later or those without joint bleeding (Fig. 2).

Table 3 shows outcome according to age at first joint bleed. Patient with early onset of joint bleeding were younger than patients with late or no joint bleeding (p=0.03). Bleeding frequencies were higher in patients suffering their first joint bleed early. Patients with early onset of joint bleeding suffered more bleeds, median 2.9 bleeds/year, including 0.9 joint bleeds/year, compared to patients without joint bleeding or late onset, who suffered median 1.6 bleeds/year, including 0.5 joint bleeds/year. Patients with early onset of joint bleeding used more prophylaxis and showed subsequently a higher median factor consumption of 465 IU/kg/year (IQR 276-1203 IU/kg/year) than the other group, using median 113 IU/kg/year (IQR 54-253 IU/kg/year).

Although not significant, there was a trend towards slightly more loss of clinical function in patients with early onset of joint bleeding.

Table 3. Outcome according to onset of joint bleeding

	Early onset of joint bleeding (≤5 years)	Late onset (>5years) or no joint bleeding	p-value
n	33 (44%)	42 (56%)	
Age (years)	32 (20-43)	43 (24-53)	0.03
Haemophilia A	38 (90%)	29 (88%)	0.50
Bleeding and treatment			
Median annual no. bleeds	2.9 (1.0-5.9)	1.6 (0.5-2.8)	0.05
Median annual no. joint bleeds	0.9 (0-1.5)	0.5 (0-1.0)	0.25
Without joint bleeds last 5 years	0	6 (14%)	0.05
History of prophylaxis	17 (52%)	5 (12%)	<0.01
Annual factor consumption (IU/kg/year)	465 (276-1203)	113 (54-253)	<0.01
Orthopaedic outcome			
HJHS (max 128 points)	5 (0-10)	2 (0-6)	0.13
HAL sum score (max.100 points)	96 (82-100)	93 (85-100)	0.96
Quality of life (EQ5D, max. 1)	0.80 (0.73-1)	0.84 (0.73-1)	0.75

Values are medians (IQR) or numbers (%)

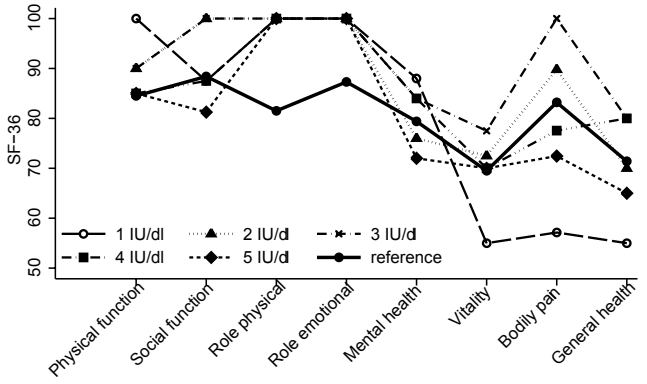
Activities and quality of life

Moderate haemophilia patients showed hardly any physical limitations as measured by the HAL. Median HAL score was 96 out of 100 (IQR 83-100). The HAL score was also similar across residual factor activities (p=0.98) and onset of joint bleeding (p=0.96) (Tables 2 and 3).

Quality of life, as measured by SF-36, was similar to the general population across all domains (0.31<p<0.97). The SF-36 domain scores were also similar across factor activity levels

(0.18<p<0.67) (Figure 3), and onset of joint bleeding (0.12<p<0.96). Utility confirmed the results of the SF-36. Median utility was 0.83 (IQR 0.7-1.0) and was similar across residual factor activity levels and onset of joint bleeding (p=0.33 and p=0.75, respectively).

Figure 3. Health related quality of life, as measured by SF-36, according to baseline factor activity



Solid black line represents the general male population in the Netherlands

Discussion

Moderate haemophilia is rare and to our knowledge this is the largest study on outcome in moderate haemophilia so far. Bleeding frequency was low, annual bleeding frequency was 2 bleeds/year, with hardly any joint bleeds. However, a small subgroup had high bleeding frequencies requiring prophylaxis. These patients mostly suffered their first joint bleed early, before the age of 5 years. Orthopaedic outcome was good. A few older patients, without access to replacement therapy in their childhood, required orthopaedic surgery. Quality of life and utility were comparable to the scores of the general population across all severities, as well as according to onset of joint bleeding.

In this study, patients were classified according to baseline factor activity level, determined by the lowest value measured. Moderate haemophilia patients have the ability to increase their endogenous factor activity under stress[11]. The assumption was made that this occurs only infrequently, therefore the lowest activity levels were used for classification. Very few patients had factor activity levels of 1 IU/dl, due to technical reasons. Because of difficulty with laboratory testing of patients in lower ranges, patients with baseline-factor levels of 0.5-0.99 IU/dl were classified as severe (<1 IU/dl)[12] and were therefore not included.

Although joint scores, orthopaedic surgery and quality of life usually deteriorate with age, no adjustment for age was performed, because the age distribution was similar across subgroups. Haemophilia B has a milder clinical phenotype than haemophilia A[13]. Adjustment was unnecessary, because haemophilia B was equally distributed over all subgroups.

As all studies on rare diseases, this study was hampered by low numbers, but the variation in clinical phenotype is corroborated by other reports. Already in 1965, Ahlberg et al[2] reported that 15-20% of patients with moderate haemophilia had unfavourable orthopaedic outcomes.

Surprisingly, this number does not seem to have changed a lot, since in the current study 13% had a history of orthopaedic surgery and 27% of patients had clinical loss of function. A major nationwide study on self-reported outcome in all haemophilia patients in the Netherlands in 2001, also showed a large variation in bleeding frequencies within moderate haemophilia patients[3,4]. Although 50% of patients from this study originated from the Van Creveldkliniek, the reported proportion of patients who required prophylaxis was comparable at 26%.

In 2000, the lack of association between factor activity level and joint bleeding was also reported in patients from our clinic, 39 of their patients are included in this study as well[14].

Generally, patients with moderate haemophilia seem well treated. Nevertheless, some patients had high bleeding frequencies, despite prophylactic treatment. These patients all started prophylaxis late, at median age of 14 years, while our severe haemophilia patients started at median age of 2.2 years (IQR 1.2-3.0)[15]. From studies in severe haemophilia, it is well known that secondary prophylaxis is less effective in preventing arthropathy[15,16]. Factor activity level and age first joint bleed were associated with the need for prophylaxis. These two parameters are also the earliest signs of bleeding phenotype available to the physician.

Although the majority of patients with moderate haemophilia had very few complications of haemophilia, some patients needed extra treatment. Physicians should be aware that 29% had high bleeding frequencies, with early onset of joint bleeding. Accordingly, these patients need close monitoring of their (joint) bleeding and in some cases early prophylaxis to avoid future joint damage.

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Chapter 8

Turning severe into moderate haemophilia by prophylaxis: Are we reaching our goal?

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Abstract

Since the introduction of prophylaxis, physicians have tried to convert the clinical phenotype of severe haemophilia into moderate haemophilia, but outcome of severe patients has never been compared to that of moderate patients.

Outcome of 80 patients with severe haemophilia on long term intermediate dose prophylaxis was compared to outcome in 40 patients with moderate haemophilia in a single centre study. Data on treatment history, activities (IPAQ, HAL), quality of life (SF-36, EQ5D), and 5-year bleeding and clotting factor consumption were collected of patients born 1970-1995.

Median age was 24 years (IQR 18-30 years). All severe patients received long-term prophylaxis, started at a median age of 4.8 years (IQR 3.2-6.2 years). Ten moderate haemophilia patients (25%) received prophylaxis, starting at median age 10.8 years (IQR 3.8-13.8 years). Annual number of bleeds, including joint bleeds was significantly higher in severe patients (median 2.0 joint bleeds/year; IQR0.8-3.7 joint bleeds/year) than moderate patients (median 0.8 joint bleeds/year; IQR 0-1.2 joint bleeds/year). Due to more use of prophylaxis, annual clotting factor consumption of severe patients (median 2120 IU/kg; IQR 1514-2768 IU/kg), was higher than in moderate patients (median 133 IU/kg; IQR 49-468 IU/kg). Patients with severe haemophilia showed slightly but significantly more loss of clinical function (HJHS): median 8 points (IQR 3-15) vs. median 2 points, (IQR 0-6). Quality of life as measured by SF-36 and EQ5D, and physical activity were similar between severities, as well as compared to the general population.

When comparing unselected cohorts, the bleeding pattern of severe haemophilia appears not fully converted into the milder bleeding pattern of moderate haemophilia by long term intermediate dose prophylaxis, although activities and quality of life were similar.

Introduction

Haemophilia is classified according to a patient's residual factor activity level. Severe haemophilia patients have <1 IU/dl and can bleed spontaneously into joints, muscles and other soft tissues. Moderate haemophilia patients have factor activity levels between 1-5 IU/dl. These patients bleed mostly after trauma and surgeries only. Mild haemophilia patients have factor activity levels of 6-40 IU/dl and hardly bleed at all, apart from after surgery. Haemophilia can be treated with replacement clotting factor, which has been available since 1965[1]. In the 1970s, prophylactic treatment was introduced. Prophylaxis was aimed to convert severe bleeding into the bleeding pattern of moderate haemophilia[2]. The goal of prophylaxis is to prevent bleeding, especially into joints, thus preventing arthropathy and consequent loss of quality of life[3].

Prophylaxis is a regular infusion of clotting factor concentrate to replace the lacking clotting factor in the plasma of haemophilia patients. This treatment is only available through intravenous administration. Due to a short half-life of the replacement factor, patients have to inject at least three times a week on a prophylactic regimen[4], which poses a considerable burden, especially in young children.

Traditionally, target trough levels of prophylaxis[5] have been set at 1-3 IU/dl[6,7], which are the residual factor activity levels of moderate patients. In theory, outcome in severe haemophilia patients on long term prophylaxis should therefore be comparable to that of moderate haemophilia patients. This study compares bleeding rates, orthopaedic outcome and quality of life of severe haemophilia patients on long-term prophylaxis with moderate haemophilia patients in a single centre cohort study.

Patients and methods

All patients with severe haemophilia (<1 IU/dl FVIII/FIX) on long-term intermediate dose prophylaxis and with moderate haemophilia (1-5 IU/dl FVIII/IX activity), born between 1970 and 1995, treated at the Van Creveldkliniek, UMC Utrecht, the Netherlands, were included in the study. Patients who ever had inhibitors >0.6 BU (19 patients), or those who did not have access to haemophilia care in their childhood (6 patients) were excluded. Two patients did not want to participate in the study.

Questionnaires on quality of life (SF 36, EQ5D), daily activities as measured by the Haemophilia Activities List (HAL), and sports activities as measured by the International Physical Activities Questionnaire (IPAQ), and a self designed sportslist specifying type of sports participation during the last year were administered to all patients aged >17 years. Prophylaxis was defined as at least one regular infusion per week for at least 45 weeks per year. Long term prophylaxis for severe haemophilia was defined as treatment with prophylaxis at least 30% of the time between diagnosis and last evaluation, with a minimum of 7 years. Joint bleeds were defined as complaints in ankles, knees, elbows, hips, wrists, or shoulders requiring treatment with concentrate at least once.

Weekly physical activity was measured by the IPAQ questionnaire, which allows for the computation of metabolic equivalence estimates (METs)[8]. To compare activity with the general population (controls), patients received an extra questionnaire to be filled in by a friend or colleague within

a 5 year age range of the patient. This questionnaire contained the IPAQ and the sportslist. All sports activities were categorized into medium risk activities, like swimming and cycling, and high risk activities, such as soccer, skiing and hockey[9]. Outcome in severe haemophilia was compared with outcome in moderate haemophilia. SF-36 and EQ5D results were compared to the male population in the Netherlands[10,11].

Statistical testing was performed using Kruskal-Wallis test, a non-parametric test for continuous variables and Fischer's exact test for categorical variables. Statistical analyses were performed with Stata SE11 (Statacorp, TX, USA).

Results

Eventually, history of treatment and bleeding of 80 patients with severe haemophilia and 40 patients with moderate haemophilia was collected. Table 1 shows the patient characteristics according to haemophilia severity. Median age at last evaluation was 24 years, ranging from 12 to 37 years in severe, and from 13 to 38 years in moderate haemophilia patients. Haemophilia A was diagnosed in 105 of the 120 patients (88%). Most patients were diagnosed before the age of 1 year (median 0.6 years, IQR 0.3-1.2 years). Evidently, patients with severe haemophilia had factor activity of <1 IU/dl, while patients with moderate haemophilia had median factor activity of 2 IU/dl, ranging from 1-5 IU/dl.

Bleeding and treatment

Bleeding and treatment characteristics are also provided in Table 1. Severe haemophilia patients suffered their first joint bleed at median age 2.0 years (IQR 1.1-3.0 years), much earlier than moderate patients, who had onset of joint bleeding at median age 4.8 years (IQR 3.0-8.5 years). Bleeding frequencies of severe patients on long-term prophylaxis were higher than of patients with moderate haemophilia ($p<0.001$). Patients with severe haemophilia had median 3.9 bleeds/year, including 2.0 joint bleeds (IQR 0.8-3.7 joint bleeds/year), while those with moderate haemophilia had median 2.2 bleeds, including 0.8 joint bleeds (IQR 0-1.2 joint bleeds/year). In the last 5 years, 7 patients with severe (9%) and 10 patients with moderate haemophilia (25%) did not suffer any joint bleeds.

Patients with severe haemophilia had used long term prophylaxis for a median of 18 years (IQR 7.8-36.7 years). Patients with severe haemophilia started prophylaxis at a median age of 4.8 years (IQR 3.2-6.2 years). Weekly dose at last evaluation was median 47 IU/kg (IQR 35-55 IU/kg). Twelve patients with severe haemophilia (15%) stopped prophylaxis completely during the study period, at a median age of 21 years (IQR 16-22 years). Bleeding frequencies in these patients remained low with median 1.0 joint bleed/year (IQR 0.3-4.0 joint bleed/year), comparable to patients with moderate haemophilia ($p=0.4$). Seven patients with severe haemophilia tried to stop, but restarted prophylaxis within a year, because of high bleeding frequencies (median 3.6 joint bleeds/year, IQR 0.6-6.3 joint bleeds/year).

Ten patients with moderate haemophilia (25%) also used prophylaxis during the study period. These patients used a median weekly dose of 29 IU/kg (IQR 17-54 IU/kg). Patients with moderate haemophilia started prophylaxis later ($p<0.05$), at median 10.4 years (IQR 2.8-13.8 years),

Table 1. Patient characteristics, bleeding and treatment of severe compared to moderate haemophilia

	Severe haemophilia	Moderate haemophilia	p-value
n	80	40	
Age in 2007 (years)	24 (18-30)	24 (19-32)	0.56
Haemophilia A	72 (90%)	33 (83%)	0.24
Age diagnosis (years)	0.7 (0.18-1.0)	0.5 (0-2.0)	0.90
Factor activity (IU/dl)	<1	2 (2-3)	<0.001
Bleeding			
Age 1st joint bleed (years)	2.0 (1.1-3.0)	4.8 (3.0-8.5)	<0.001
Annual no. bleeds	3.9 (1.9-6.1)	2.2 (0.8-3.1)	<0.001
Annual no. joint bleeds	2.0 (0.8-3.7)	0.8 (0-1.2)	<0.001
No joint bleed in 5 yrs	7 (9%)	10 (25%)	0.02
Treatment			
Age start prophylaxis (years)	4.8 (3.2-6.2)	10.4 (2.8-13.8)	0.05
History of long-term prophylaxis	80 (100%)	10 (25%)	<0.001
Weekly dose of prophylaxis (IU/kg)	47 (35-55)	29 (17-54)	0.16
Home treatment	80 (100%)	21 (53%)	<0.001
No treatment in 5 years	0	1 (3%)	0.33
Annual factor consumption in 5 years (IU/kg/yr)	2120(1514-2768)	133 (49-468)	<0.001

Values are median (IQR) or numbers (%)

compared to those with severe haemophilia. All patients on prophylaxis used home treatment, an additional 11 patients with moderate haemophilia also used home treatment.

Patients treated on demand had significantly lower bleeding rates ($p<0.01$) and better joint outcome ($p<0.01$) than patients with either severe or moderate haemophilia treated with prophylaxis. Patient treated on demand suffered median 0.5 joint bleeds/year (IQR 0-1 joint bleeds/year), while moderate patients treated with prophylaxis suffered median 1.1 joint bleeds/year (IQR 0.4-2.9 joint bleeds/year) and severe haemophilia patients median 2.0 joint bleeds/year (IQR 0.8-3.7 joint bleeds/year).

Due to more use of prophylaxis, factor consumption was much higher ($p<0.001$) in patients with severe haemophilia than in those with moderate haemophilia. Sixteen patients with severe haemophilia (12%) needed a central venous access device (CVAD) at one point in their lives, whereas none of the patients with moderate haemophilia ever did.

Joint outcome and quality of life

Joint outcome and quality of life according to severity, compared to the general population is shown in Table 2. Although clinical loss of function, as measured by the HJHS, varied widely for both severities, scores were slightly, but significantly, higher in severe patients. Overall, joint damage appeared only mild: in severe haemophilia 44 patients (55%) and in moderate haemophilia 29 patients (73%) had HJHS scores <10 points. The proportion of patients with a history of orthopaedic surgery was similar in both groups: 12 severe (15%) and 3 moderate (8%) patients ($p=0.38$).

Table 2. Joint outcome and quality of life according to severity, compared to the general population

	Severe haemophilia	Moderate haemophilia	Unaffected Controls	p-value
n	60	34	105	
Age (years)	27 (21-31)	25 (20-33)	24 (20-31)	0.56
Joint outcome				
Loss of function (HJHS, max 128 points)	8 (3-15)	2 (0-6)	-	<0.001
History of orthopaedic surgery	12 (15%)	3 (8%)	-	0.38
Quality of life				
Utility (EQ5D)	0.80 (0.72-1)	0.92 (0.72-1)	0.87 (0.84-1.0)	0.66
Physical activity (METs)	4294 (1554-10480)	2240 (880-5118)	3023 (1493-6936)	0.26
Participation in sports	47 (59%)	28 (70%)	92 (88%)	<0.01
Participation in high risk sports	27 (34%)	20 (50%)	64 (61%)	<0.01

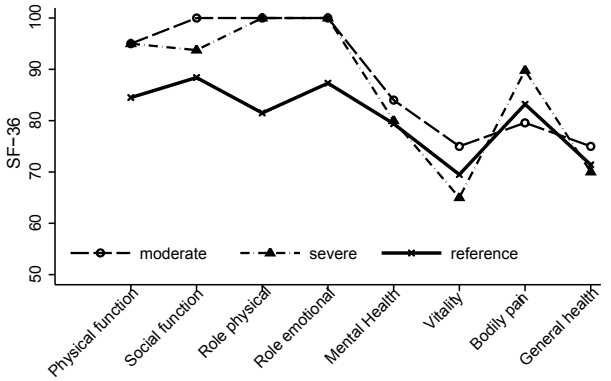
Values are median (IQR) or numbers (%)

Most of these patients, 9 severe and 2 moderate, underwent at least 1 major orthopaedic procedure, others had only had minor surgeries.

Of 120 patients, 94 (78%) returned the questionnaires. In total, 105 questionnaires of unaffected controls were received. Quality of life was similar across all groups. Patients with haemophilia, either severe or moderate, reported equal quality of life as measured by SF-36 (Fig. 1) or EQ5D (Table 2). Patients were also just as active as their peers. Median activity was 3276 METs (IQR 960-8640) in patients and 3023 METs (IQR 1493-6936) in peers. Severe patients reported the highest activity level, median 4294 METs (IQR 1554-10480 METs), while patients with moderate haemophilia reported least activity, median 2484 METs (IQR 942-5660 METs).

More than half of all haemophilia patients were involved in at least 1 sports activity. Most persons in the control group (61%) participated in at least 1 high risk sport. Haemophilia patients were more cautious: 50% of patients with moderate and 34% of those with severe haemophilia participated in high risk sports, which was significantly lower than in controls (Table 2).

Figure 1. SF-36 scores of patients with severe or moderate haemophilia compared to the male general population



Discussion

The goal of prophylaxis is to convert the bleeding pattern of patients with severe haemophilia into that of patients with moderate haemophilia. This study showed that, despite early prophylaxis, bleeding frequencies and joint function scores were slightly higher in severe patients treated with long-term intermediate dose prophylaxis than in patients with moderate haemophilia. For both severe and moderate haemophilia, a significant variation in bleeding phenotype was observed. The effects of 15% of severe patients who discontinued prophylaxis and 25% of moderate patients who required long-term prophylaxis, included in the study, could not be quantified separately.

It may seem odd that patients with moderate haemophilia treated with prophylaxis were included in the analyses. Excluding these moderate patients on prophylaxis would have introduced confounding by indication: leaving out patients with moderate haemophilia with high bleeding frequencies. Similarly, severe patients who discontinued because of low bleeding frequencies, but used long-term prophylaxis in their childhood were also included in the comparison. Moderate haemophilia may be considered a 'natural experiment' for trough levels in prophylaxis of severe haemophilia. Patients with moderate haemophilia treated with prophylaxis reported higher bleeding frequencies than those treated on demand. Without medical intervention, these patients with moderate haemophilia would not have received prophylaxis and their outcome would probably have been similar to that of severe haemophilia.

Quality of life and physical activity were similar in all groups as well as compared to the general population, which implies that the goal of prophylaxis to let patients be as active as their peers without haemophilia was reached. Evidently, one or two extra joint bleeds per year, if treated quickly and sufficiently, will not tremendously influence clinical function and quality of life. However, quality of life may have been overestimated due to coping effects. It has been shown that patients with chronic diseases rate their quality of life higher than the general population[11], and haemophilia is certainly a chronic condition. Patients suffered their first joint bleeds young, at median 2.0 years in severe and 4.8 years in moderate haemophilia. They learned to cope with their condition and the invasive treatment in their childhood, which may no longer be perceived as intrusive, but as a habit.

Patients on long-term prophylaxis spend a large part of their lives with more than 5 IU/dl factor activity[12]. In effect, they may be expected to have better outcome than patients with moderate haemophilia, who spend most of their lives <5 IU/dl. This study showed that bleeding and outcome were, however, slightly worse than in moderate haemophilia. Collins et al.[13] modelled time <1IU/dl and the association with joint bleeding. They predicted an increased number of joint bleeds when a patient spends more time per week <1 IU/dl, which may account for the worse outcome in severe haemophilia. At the Van Creveldkliniek, prophylaxis is targeted at bleeding pattern, rather than trough levels, hence relatively low dosages were used: median weekly dose was 47 IU/kg over three infusions. Even though a dose of 1000 IU every other day will keep an average adult male above 1% all the time, this will not be the case for all patients[13]. In order for the prophylactic regimen to work, patients need to adhere to the prescribed treatment. In a recent European study adherence to therapy was 80-87% in haemophilia patients[14]. Lack of compliance to prophylaxis can lead to increased time <1 IU/dl and increased bleeding.

Severe haemophilia could perhaps be converted to moderate haemophilia by using a higher dose of prophylaxis. Fischer et al.[15] compared outcome of Dutch intermediate dose prophylaxis compared to Swedish high dose prophylaxis. Compared to the Swedish patients, Dutch patients had higher bleeding rates and showed a trend towards more abnormalities on radiography.

The future of prophylactic treatment for severe haemophilia should be focussed on retaining at least the current clinical outcome and quality of life. It is clear that residual clotting factor activity is associated with better outcome, but it is not the only determinant of bleeding: moderate patients also showed considerable variation in bleeding, independent of factor activity.

Compared to patients with moderate haemophilia, increased bleeding in young adults with severe haemophilia was associated with a slightly increased loss of joint function and less participation in (high risk) sports, but normal physical activity levels and only minimal changes in quality of life. Future studies should investigate the benefits of more stringent bleeding control. Apparently, the goal as formulated by Inga-Marie Nillsson in the 1950s[16] of turning severe haemophilia into moderate haemophilia is not yet reached entirely.

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Chapter 9

Magnetic resonance imaging in teenagers and young adults with limited haemophilic arthropathy: baseline results from a prospective study

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Abstract

The clinical relevance of subtle changes on MRI for evaluating haemophilia treatment is unknown. To determine the association of findings on MRI with joint function and bleeding frequency in young adults and teenagers with apparently very mild arthropathy, a prospective study was performed.

Knees and ankles of 26 patients, aged 13-26 years, were scanned using a 60 min protocol on a 3 Tesla MRI. 15/16 patients with severe haemophilia, and 1/10 patients with moderate haemophilia used prophylactic treatment.

Two blinded radiologists scored the MRI (IPSG consensus score, max. 20 points/joint) and the radiographs (Pettersson score (PS), max. 13 points/joint), while clinical function (HJHS; max. 22 points/joint) was scored by one physiotherapist. Life-time number of bleeds was collected from patient files. Spearman's correlations were calculated at joint level.

Of 104 joints scanned, 3 were excluded because of previous arthrodesis or trauma. The remaining 101 MRI scores correlated weakly with clinical function ($r=0.21$; $p=0.03$) and less with lifetime number of bleeds ($r=0.14$; $p=0.1$). MRI scores were 0 in 58 joints, including 27 with recorded major bleeds. In 3 joints (2 knees, 1 ankle) of patients playing intensive sports, MRI showed minor changes (MRI score=1) in the absence of bleeds. Agreement was reasonable between PS and MRI scores ($r=0.41$; $p<0.01$). However, in 30% of the joints, MRI detected abnormalities in soft tissue and cartilage, while PS was 0 points.

Changes detected on MRI showed only weak correlation with clinical function, but none with number of joint bleeds. No evidence of occult haemorrhages was found. Instead, we found no abnormalities on MRI in 43 joints with a history of repeated joint bleeding, suggesting some soft tissue changes may be reversible. In teenagers, PS is a reliable instrument to detect bony changes. Abnormalities detected by MRI, but not by PS, were minor and their clinical implications are not yet clear.

Introduction

Haemophilia is an inherited, X-chromosomal bleeding disorder. The hallmark of haemophilia is recurrent joint bleeding. Repeated joint bleeds may lead to synovial hypertrophy and consequent cartilage and bone damage[1]. Recurrent bleeding may lead to disability or orthopaedic surgery, both compromising quality of life.

Since the 1980s, a haemophilia specific tool has been available to evaluate joints by radiography, i.e. the Pettersson score (PS)[2]. It has been adopted by the World Federation of Haemophilia (WFH) and has been widely used1. Magnetic Resonance Imaging (MRI) currently is the most sensitive imaging modality for early detection of joint lesions. As result of the efforts of the International Prophylaxis Study Group (IPSG), a scoring system for structural assessment using MRI[3] was developed to assess more subtle joint damage[4].

Except for a recent randomized controlled trial comparing prophylactic to on demand treatment in young children with severe haemophilia[5], MRI has not been subject to studies evaluating long-term outcome of prophylactic treatment. Hence we do not know if our teenage patients with a history of few bleeds, very little or no arthropathy on radiography, and good clinical function, will have structural changes on MRI. Moreover, what is the meaning of these formerly undetected changes? Are these changes the early, possibly reversible, manifestations of joint disease, and will they predict future arthropathy and loss of function?

The aim of this study was to investigate the value of MRI by comparison with findings on radiography, joint function and bleeding history as a baseline assessment in a study evaluating the predictive value of MRI.

Patients and methods

Since the impact of MRI studies is expected to be most pronounced and clinically relevant in teenagers and young adults with very minor or no known joint damage, MRI was performed in patients aged 12-26 years. Eligible patients had severe or moderate haemophilia A with minor joint damage. No more than one knee or ankle with a previous Pettersson score above 3 points and at least two ankles or knees with a previous Pettersson score of 0 points. Both knees and ankles of all patients were scanned by MRI.

Data from routinely performed plain radiographies, within two years from MRI assessment, were used for the analysis. To assess clinical function, routinely measured haemophilia joint health scores (HJHS)[6] performed within 1 year from MRI assessment were analyzed. Lifetime cumulative number of joint bleeds at the time of MRI scanning was recorded from patient files; only bleeds registered and treated were counted as joint bleeds. Major bleeds, defined as bleeds with loss of clinical function, pain and/or swelling requiring more than one infusion of clotting factor, were registered separately at each visit from 2002 onwards.

The research protocol was approved by the Institutional Review Board of UMC Utrecht, the Netherlands. All participants signed informed consent.

Imaging

All joints were imaged separately using MR equipment (Philips, type Achieva 3T, Koninklijke

Philips Electronics NV, The Netherlands) with field strength of 3 Tesla. Use of this high field strength results in a better signal-to-noise ratio, a higher spatial resolution and eventually in improved imaging of the cartilage (volume, thickness, surface area), cartilage lesions and subchondral bone lesions[7,8], especially in the ankles[9]. Moreover, use of higher magnetic fields allowed for the acquisition of more and thinner slices when compared with imaging at 1.5T. The strong susceptibility of 3T, which is one of the disadvantages of imaging on higher magnetic fields, will improve the detection of haemosiderin deposits and synovial hypertrophy.
Our protocol consisted of 3D WATSf (GE, time to repeat (TR) = 20 ms, time to echo (TE) = 4.5 ms, thickness 2 mm) sagittal for knees and coronal for ankles, with reconstruction in 3 planes for detection of synovial hypertrophy and haemosiderin deposits. PDW SPAIR (SE, TR = 3265.9 ms, TE = 30 ms), sagittal and transversal with section thickness 3.5 mm for knees and coronal with section thickness 2.5 mm for ankles for detection of cartilage loss and subchondral cysts. T1- weighted (SE, TR = 642.1 ms, TE = 20 ms) coronal with section thickness 3.5 mm for knees and sagittal with section thickness 2.5 mm for ankles were added because they provide the best anatomical overview of both joints.
The combination of parallel scanning and high-field MRI shortened the scanning time to less than 60 minutes for 4 joints: 14 minutes for each knee and 12 minutes for each ankle[9].

Scoring
MRI images and radiographs were scored by two blinded and independent radiologists, with long-term experience in musculoskeletal radiology. In case of disagreement, images were reviewed for consensus scores. All radiographs were assessed using the Pettersson score[2] (max. 13 points/joint). The MRI score followed the consensus score of the IPSG[3] (max. 20 points/joint). Clinical function was assessed by a physiotherapist, using the HJHS[6] (max. 22 points/joint).

Analysis
For each joint, the MRI score was compared to the HJHS and to available bleeding history to assess agreement between the available scores. Agreement was defined when all scores were positive or when all scores were negative. Another comparison was made between MRI scores and recent PS.
Correlations were calculated on joint level, using true values of all scores, to analyze the association between the scores in a more numeric manner. Spearman's correlation was used, because the association of bleeding and clinical function with MRI might not be linear. All analyses were also performed separately for moderate and severe patients.

Results

In total 26 patients with haemophilia A (104 joints, 52 knees and 52 ankles) were scanned by MRI. Median age was 20 years (range 13-26), 16 (62%) patients had severe (FVIII <1 IU/dl), and 10 (38%) had moderate haemophilia (median FVIII activity 4 IU/dl; range 2-5 IU/dl). All patients with severe haemophilia were on home treatment and used prophylaxis from a median age of 4 years (range 0.9-5.5 years) onwards. One discontinued prophylaxis at the age of 17 years while the

other 15 continued with a mean dose of 1000 IU FVIII 3 times per week. The majority of patients with moderate haemophilia (7 patients, 70%) were on home treatment. Only one patient used prophylaxis (started at age 12 years, current dose 3 times weekly 1000 IU), while others used on demand treatment.

Table1. Patient and joint characteristics

	n=101 joints
Age (years)	20 (17-23)
Severe haemophilia	16 (62%)
Prophylaxis	16 (62%)
Age start prophylaxis (years)	4.2 (1.7-5.0)
Current dose of prophylaxis (IU/week)	3000 (3000-3563)
Home treatment	23 (89%)
Lifetime cum. joint bleeds	2 (0-5)
Joints without joint bleeds	18 incl. 6 ankles (33%)
Major joint bleeds (from 2002 onwards)	53 incl. 26 ankles (49%)
Clinical Function (HJHS)	0 (0-1)
Joints with perfect clinical function	76 incl. 35 ankles (46%)
MRI score	0 (0-1)
Joints without MRI changes	59 incl. 23 ankles (39%)

Values are medians (IQR) or numbers (%)

Data on the cumulative number of joint bleeds at joint level were available for all joints, median follow-up 14 years (IQR 5-19 years). Median cumulative number of joint bleeds was 2 per joint, ranging from 0-19 joint bleeds until time of evaluation. Only 18 joints (18%, including 6 (33%) ankles) had not yet suffered any joint bleeds. HJHS scores were available for all patients, within two years of MRI scanning (median 0.81 years; IQR 0.61-1.53 years). Clinical function was very good in most patients: 76 joints (75%) showed no signs of loss of function as measured by the HJHS (Table 1).

Imaging

Of 104 joints scanned, images of 3 ankle joints were excluded (1 because of a previous arthrodesis, and 2 because of previous trauma, unrelated to haemophilia), leaving 101 joints available for analysis. Median MRI score was 0 points, ranging from 0 to18 points, and 96 joints (95%) scored less than 5 points out of the maximum of 20 points on the IPSG scale. Knees were less frequently affected: MRI score was zero in 59 joints (57%, including 23 (39%) ankles). The remaining 42 joints (including 26 (62%) ankles) showed mostly minimal changes. Components that most often scored were: any loss of cartilage height (28 joints, of which 16 (57%) ankles), haemosiderin (18 joints, of which 11 (61%) ankles), synovial hypertrophy (10 joints, of which 9 (90%) ankles), any surface erosion (15 joints, of which 10 (67%) ankles), and one subchondral cyst (12 joints, of which 10 (83%) ankles).

Agreement of scores

Table 2 shows the agreement between the scores. MRI score did not agree well with either bleed-

ing history or clinical function. Correlation of MRI scores with clinical function was low, r=0.27 (p=0.01), and correlation with bleeding was negligible, r=0.16 (p=0.14). The correlations were similar across severities (r<0.22), and for knees (r<0.24). In ankles, MRI scores did not correlate with clinical function, but MRI scores showed a very weak correlation with bleeding (r=0.29, p=0.04). Unfortunately, correlations did not improve when only major bleeds were taken into account (r=0.11, p=0.29).

Table 2. Agreement of evaluation scores in 52 knee- and 48 ankle joints of 16 severe and 10 moderate haemophilia patients

Joint bleeds	MRI score	HJHS	n
+	+	+	13
+	+	-	26
+	-	-	39
+	-	+	5
-	-	-	10
-	-	+	5
-	+	+	2
-	+	-	1

+ present or positive score; - absent or negative score

Only 13 joints (13%) scored positive on all scores, these joints were exposed to median 2.5 joint bleeds (IQR 1-11 bleeds; range 1-19 bleeds), had MRI scores of median 2.5 points (IQR 1-11 points; range 1-18 points) and showed little loss of clinical function (HJHS: median 2.5 points; IQR 1-4 points). Twenty-six joints (including 16 (62%) ankles) had positive MRI score (median 1; IQR 1-3) and a history of joint bleeding (median 2 bleeds; IQR 1-6 bleeds), but reported no loss of clinical function. However, more than a third of the joints (39%, including 27 (69%) ankles) with a history of joint bleeds (median 3 bleeds; IQR 2-5 bleeds; range 1-19 bleeds) showed neither changes on MRI, nor in clinical function. In this group, 27 (69%) joints had at least one registered major joint bleed. Finally the remaining 5 joints (5%, including 3 (60%) ankles) that had a history of joint bleeding (median 4.5 bleeds, IQR 2-6 bleeds), showed no changes on MRI, but did have a little loss of clinical function (HJHS median 1 point, IQR 1-3 points).

Table 3. Agreement between MRI score and Pettersson score, n=93

MRI Score	Pettersson score	n
+	+	10 (11%)
+	-	28 (30%)
-	+	2 (2%)
-	-	53 (57%)

+ present or positive score; - absent or negative score

Out of 18 joints without any recorded joint bleeds, 10 showed no changes on MRI, or loss of clinical function. Five joints without joint bleeding were normal on MRI, but showed loss of clinical function (HJHS: median 1 point; IQR 1-6 points). Surprisingly, 3 joints, 2 knees and 1 ankle

without history of bleeds, showed minor changes on MRI, only 1 point: any loss of cartilage height in all cases. Both knees also showed loss of flexion (HJHS, 3 points in both joints). These 3 joints belonged to 3 different patients aged 14, 15 and 19 years. All three patients were very active in sports, playing soccer 2-3 times per week and participating in sports activities like basketball, volleyball and baseball 1-3 times per week.

Table 4 Discordant items between radiography (PS) and MRI, n=29 joints

MRI score	Pettersson score	Enlargement epiphysis	Small synovial hypertrophy	Haemosiderin	Small surface erosion	One subchondral cyst	Small loss of cartilage height	Other
-	+	2						
+	-		9	12	9	6	18	6

+ positive score; - negative score

MRI scores were mostly similar to Pettersson scores (68%), and correlated reasonably (r=0.41, p<0.01). In 28 cases (30%), MRI detected more than Pettersson score did. In all cases these changes were minor changes to soft-tissue, synovium or cartilage. In 2 cases, Pettersson scores were positive, while MRI scores were negative. In these patients, the epiphysis of the knee was enlarged, which was confirmed on MRI, but was not part of the MRI score. In all discordant items in which MRI detected abnormalities, while radiography did not, small changes in synovium or cartilage were detected by MRI. Small loss of cartilage height was detected in 18 joints by MRI, while PS were 0 points. Haemosiderin, which is only detectable by MRI, not with radiography, was present in 12 joints without abnormalities on plain radiography. Small synovial hypertrophy and small surface erosion were detected by MRI in 9 joints each, which were negative on radiography.

Haemosiderin

Joints negative for haemosiderin had significantly more time (p<0.001) between their last joint bleed and MRI than joint positive for haemosiderin. Joints with a positive bleeding history and no haemosiderin suffered their last joint bleed median 4.5 years (IQR 2.8-7.6 years) prior to MRI. Joints with a positive bleeding history and haemosiderin detected by MRI, suffered their last joint bleed median 1.8 years (IQR 1.0-3.0 years) prior to MRI.

Discussion

In this group of active young adults and teenagers with haemophilia A and only minor haemophilic arthropathy previously detected by radiography, MRI hardly correlated with clinical function or bleeding history. All three scores were concordant in only 22%, while disagreeing in various combinations in 78% of joints. Almost half of bleeding scores were discordant with MRI scores, as were 40% of clinical function scores. Patients with a history of joint bleeds, even major bleeds, showed hardly any or no changes at all on MRI. Three patients had no history of joint bleeds,

but did show minor changes on MRI. These changes may be related to intensive physical activity, rather than joint bleeding. MRI scores compared well to PS. In only a third of patients, MRI detected more changes in soft-tissue and cartilage than radiography.

As expected, MRI scores did correlate with PS, only MRI detected more soft-tissue changes than PS. MRI scores did not correlate with joint bleeding, and the correlation with clinical function was very weak. Apparently, the minor changes detected by MRI do not hamper patients in their range of motion. The weak correlation with bleeding could be explained by a time delay needed for the changes to appear on MRI after a joint bleed. Perhaps the effects of joint bleeding are less pronounced if the joint has been free of bleeds for a long time. Recovery after joint bleeding seems to be possible, because 44 joints showed no abnormalities on MRI, despite registered joint bleeds, in 28 cases even major joint bleeds. Additionally, haemosiderin was only detected in joints with a recent bleed. Most joints that had suffered their last bleeds more than 3 years prior to MRI did not show any haemosiderin, despite having a history of major joint bleeds.

Joint bleeds were defined as registered and treated bleeds. Despite keeping detailed logs of patient's bleeds and treatment, misclassification could have occurred, as all events were self-reported. However, analysis based on more objective major bleeds, classified by swelling, pain and treated with a minimum of two infusions of FVIII, did not improve score correlations.

Although this study is small, it is the first to address the issue of whether MRI is appropriate in evaluating long-term therapy in haemophilia. If MRI could pick up any changes leading to further deterioration in joints, treatment could still be intensified to benefit the life of these young patients. However, long term follow-up is of the essence in these kinds of studies, and repeated imaging after 5 years is planned in the present study. As expected, this cross-sectional study could not address the predictive value of MRI.

Unlike in the recent trial by Manco-Johnson et al[5], no evidence for occult haemorrhaging was found. The current study has actually found the opposite: the presence of joints with a history of numerous major joint bleeds but normal MR images. Similar to the Manco-Johnson study[5,] as well as others[10,11], the current study found a weak correlation between MRI and clinical function. This is corroborated by the findings of Pergantou et al.[11] who reported 50% discordant scores between orthopaedic joint scores and MRI scores. This is in contrast with the study of Lundin et al[12], who reported a correlation of 0.32-0.39 between joint bleeding, clinical function and MRI scores, while validating the MRI score. However, this validation study was performed on patients with relatively high bleeding frequencies. They reported median 11 joint bleeds (range 0-80 bleeds), which is much higher than the median 2 joint bleeds (range 0-19 bleeds) reported in the current study.

Patients with recorded joint bleeds, even major joint bleeds, showed unaffected healthy joints on MRI. This is probably due to timely and sufficient, prophylactic treatment available, in combination with home treatment for all patients. Or can it be that the changes found on MRI are reversible? In vitro tests have shown that cartilage has the ability to heal from small injuries and haemosiderin can be removed by the body[13]. In this study joints with recent joint bleeds had evidence of haemosiderin, while joints that had been bleed-free for a longer period had no visible traces of haemosiderin.

MRI detected changes in cartilage in joints without a history of bleeding, but with a positive history of sports. Are we treating our patients so well, that they participate in normal daily life like

any other teenager? And does this mean that they will not only show changes because of their disease, but also “normal” changes caused by sports injuries, just like their peers?

All studies comparing traditional radiography to MRI report that MRI detects more abnormalities[14-16]. This study showed that in only a third of patients MRI detected more abnormalities. What the clinical meaning of these abnormalities will be, was not yet clear. Therefore, diagnostic tools should not only be evaluated on their added information, but should also be evaluated for their use in clinical management[17].

MRI is very sensitive in detecting soft tissue changes as well as early cartilage changes[15,18]. Before implementing MRI as a standard therapy evaluation tool, a careful study should be carried out whether changes on MRI images provide more valuable therapeutic information than the readily available and cheaper[18] plain radiography and clinical function scores[19]. Unfortunately, the present study provides only the first step in the evaluation of the predictive value of MRI in haemophilic arthropathy. The changes found by MRI were inconclusive, indicating that it is not yet clear whether these changes are reversible or whether they are meaningful in the deterioration of joints. The patients included in this study will be scanned by MRI and radiography in five years’ time and clinical function will be measured every year, which will prove or disprove the added value of MRI for preventing and diagnosing haemophilic arthropathy. Until then, these patients will be as active as they are now and be treated according to their protocol as appropriate by current standards.

In young adults and teenagers with moderate and severe haemophilia, MRI of knees and ankles correlated only weakly with clinical function, but not with cumulative number of joint bleeds. No evidence of occult haemorrhages was found. Further research will have to determine which abnormalities detected on MRI are permanent and which are reversible. Until then, MRI should be used with caution while evaluating therapy in clinically unaffected joints.

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Chapter 10

Protected by nature? Effects of strenuous exercise on FVIII activity in moderate and mild haemophilia A patients; a pilot study

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Abstract

Increase of factor VIII (FVIII) activity after physical exercise has been reported in healthy subjects and patients with von Willebrand disease. The mechanism of FVIII activity increase through exercise is thought to be similar to that of desmopressin. We aimed to study whether moderate and mild haemophilia A patients are able to increase their endogenous FVIII activity levels by strenuous physical activity.

We studied changes in FVIII activity levels after high intensity exercise of 15 haemophilia A patients, aged 20-39 years. Eight patients suffered from moderate haemophilia and 7 patients had mild haemophilia. Patients cycled until volitional exhaustion. Blood samples were drawn before and 10 minutes after the exercise test. Plasma was analyzed for FVIII, Von Willebrand factor (VWF) and Von Willebrand propeptide (VWFpp). In mild haemophilia patients, changes in FVIII activity were compared to DDAVP tolerance tests.

FVIII activity increased 2.5 times (IQR 1.2-4.0 times), similar across severities. Absolute increases were markedly different: median 9 IU/dl (IQR 8-11 IU/dl) in patients with moderate, compared to median 21 IU/dl (IQR 19-44 IU/dl) in mild haemophilia patients. VWF and VWFpp increased independently of severity: median 50% (IQR 30-79%) and median 165% (IQR 130-244%), respectively, reflecting acute release of VWF. Relative increase after DDAVP administration was median 3.5 times (IQR 2.5-4.5 times), similar to increase in FVIII activity after exercise.

These observations may be used to promote high intensity warm up activities before participating in sports and other strenuous activities for moderate and mild haemophilia A patients with modest endogenous FVIII activity levels, who might be considered at high bleeding risk. Further studies are warranted to fully appreciate the clinical significance of exercise on different levels of intensity in patients with mild or moderate haemophilia A.

Introduction

Exercise is an essential part of a healthy lifestyle, as it reduces the risk of coronary disease[1] and diabetes[2], and promotes well-being[3,4]. Yet sports participation is lower in patients with haemophilia than in healthy peers[5,6]. Haemophilia A patients lack clotting factor VIII (FVIII), resulting in high risk of joint bleeding. Severe haemophilia patients (FVIII <1IU/dl, or <1% of normal) suffer from spontaneous joint bleeds, which, if untreated, will lead to crippling haemophilic arthropathy[7]. Patients with moderate (FVIII 1-5 IU/dl) or mild (FVIII 6-40 IU/dl) haemophilia generally only bleed after trauma or overexertion. The bleeding risk has hampered participation in sports in many haemophilia patients[5]. Historically, these patients were recommended low impact sports such as swimming[6]. Fortunately, treatment has been intensified and currently more than half of haemophilia patients in the Netherlands actually participate in a variety of sports, even so-called contact sports such as soccer[8].

An increase of FVIII activity within 10 minutes after physical exercise has been reported in healthy subjects[9,10] and patients with von Willebrand disease[11]. We performed a pilot study to assess whether moderate and mild haemophilia A patients are also able to increase their endogenous FVIII activity levels after exercise.

Patients and methods

In this pilot study, fifteen non-severe haemophilia A patients with FVIII activity levels of 1-15 IU/dl volunteered consecutively during routine control visits. After signing informed consent, coagulation parameters were measured before and after a standardised incremental exercise test. Patients were considered able to complete the test if they did not have any disability preventing them from cycling or strenuous physical activity.

Patients performed a graded exercise test on an electronically braked cycle ergometer (Ergoline 9000, Germany). Subjects started with one minute of unloaded cycling, after which the load was increased with 25W every minute. Pedalling frequency was 60-80 revolutions/min. This protocol was continued until the patient stopped because of volitional exhaustion, despite strong verbal encouragement of the investigator. During the test heart rate (HR), oxygen consumption (VO2) and carbon dioxide production (VCO2) were measured with a calibrated portable breath-by-breath system (metamax 3B, Cortex biophysik, Germany). Respiratory Exchange Ratio (RER) was calculated as VCO2/VO2. A test was rated as maximal when at least 2 out of 3 of the following criteria were met: 1) plateau in VO2, 2) peak RER >1.0, and 3) maximal HR within 10 beats of age predicted maximum for age (220 minus age).

Laboratory assays

The optimum sampling time after exercise was determined based on the literature[12,13] and confirmed by repeated blood sampling after exercise of a healthy volunteer. Plasma samples were frozen and analyzed in one batch to avoid inter-assay differences. Plasma levels of FVIII activity, Von Willebrand factor (VWF) and Von Willebrand propeptide (VWFpp) were assessed in all samples. FVIII activity was assessed using the one-stage assay on a STA-Rack evolution[14]. VWF antigen was measured by enzyme-linked immunoabsorbent assay (ELISA), using a polyclonal

antibody against human VWF for capture and detection[15,16]. The concentrations of VWFpp in patient plasma were determined with a standard ELISA on a Tecan Freedom EVO pipetting robot[17].

Statistical analysis

For each patient, the increase in FVIII activity was calculated. The relative increase was calculated by dividing the absolute increase in FVIII by the patient's baseline FVIII activity level.

Changes in FVIII activity, VWF and VWFpp after exercise and across severities were compared using Mann-Whitney U tests. In patients with mild haemophilia the changes in FVIII activity after exercise was compared to previously determined DDAVP response (increase in FVIII activity 1 hour after intravenous administration of 0.3 µg/kg DDAVP), using a Wilcoxon matched-pairs rank test.

Results

Repeated blood sampling from a healthy volunteer showed peak levels of FVIII activity, followed by a plateau before decreasing. Ten minutes after exercise, FVIII activity was 1.5 times baseline and remained high up to 60 minutes after exercise, before slowly decreasing. At 120 minutes, FVIII activity was still elevated 1.3 times baseline.

Table 1. Characteristics of patients (n=15) before, during and after the incremental exercise test

	n=15
Age (years)	27 (20-39)
Moderate haemophilia (n, %)	8 (53%)
BMI	23.8 (20.3-33.5)
Time to exhaustion (minutes)	11 (9-15)
Peak Workload (Watt)	304 (242-375)
Peak Heart Rate (beats/min)	188 (164-199)
Peak Respiratory Exchange Ratio (VCO2/VO2; maximum exercise: RER>1)	1.25 (1.02-1.54)

Values are median (range) or n(%)

Patient characteristics and test results of the exercise test are shown in Table 1. A total of 15 patients with a median age of 27 years (range 20-39 years) volunteered. Eight patients had moderate (FVIII 1-5 IU/dl) and 7 mild haemophilia (FVIII 6-15 IU/dl). All patients completed the exercise test without complications and all patients met at least 2 out of 3 criteria of a maximal exercise test. Median peak workload was 304 Watt (range 242-375 Watt). Median peak heart rate was 185 beats/min (range 164-199 beats/min).

Median baseline FVIII activity was 5 IU/dl (range 2-15 IU/dl). After the incremental exercise test, FVIII activity had increased in all patients, to median 11 IU/dl (range 7-77 IU/dl), although the absolute change in FVIII activity varied widely (Fig.1). Median relative increase was 2.5 times (IQR 1.2-4.0 times) and was similar (p=0.8) for moderate (2.4 times; IQR 1.3-4.0 times) and mild haemophilia patients (2.5 times; IQR 1.2-4.0 times). The absolute increase was higher in patients with mild haemophilia (p=0.01), resulting in higher FVIII activity levels after exercise for mild (median 21 IU/dl; IQR 19-44 IU/dl) than for moderate haemophilia patients (9 IU/dl; IQR 8-11 IU/dl).

Table 2. Increase in coagulation parameters after exercise in moderate (n=8) and mild (n=7) haemophilia patients

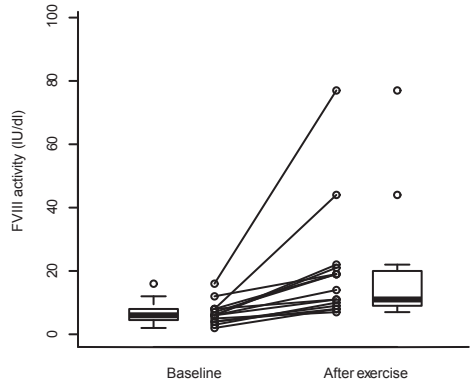
	Moderate	Mild	p-value
Absolute increase			
FVIII (IU/dl)	7 (3-9)	15 (6-62)	0.01
VWF (%)	50 (8-117)	57 (23-123)	0.42
VWFpp (%)	164 (48-346)	186 (90-350)	0.67
Relative increase			
FVIII	2.4 (1.8-7.0)	2.5 (1.9-4.5)	0.82
VWF	1.5 (1.1-2.7)	1.8 (1.2-2.9)	0.30
VWFpp	2.6 (1.5-4.1)	2.7 (2.0-4.9)	0.64

Values are medians (range)

VWF and VWFpp also increased independent of severity (p=0.3-0.6) in all patients, median 50%; IQR 30-79% and median 165%; IQR 130-244% respectively. The proportional increase in VWF was lower than in VWFpp. (Table 2)

DDAVP tolerance tests were available only for patients with mild haemophilia. Relative increase after DDAVP administration was median 3.5 times (IQR 2.5-4.5 times), similar to increase in FVIII activity after exercise (p=0.14).

Figure 1. Baseline FVIII activity levels before and after an incremental exercise test in patients (n=15) with moderate and mild haemophilia



Discussion

The results of this study showed that patients with moderate or mild haemophilia increase their endogenous FVIII activity levels during high intensity exercise. The relative increase of FVIII activity after exercise was independent from baseline FVIII activity levels. All patients showed at least doubling of FVIII activity after exercise.

The reason for choosing patients with <15 IU/dl FVIII activity was that baseline bleeding risk is higher in these patients than in patients with >15 IU/dl[18]. These patients, who are treated on demand, have most to gain by an increase of their FVIII activity levels.

The increase in FVIII activity levels coincides with an increase of VWF and VWFpp levels. The

proportional increase was higher in VWFpp, indicating acute release of VWF from endothelial cells[12]. During exercise, as after DDAVP administration[19], muscular perfusion increases, VWF is washed out of the endothelial of the muscles and subsequently binds more FVIII.

In this pilot study, it appeared that the increase after exercise was lower than after DDAVP administration. In moderate haemophilia, the response to DDAVP is generally very low[20]. DDAVP testing was not included in the study protocol, and only 7 patients with mild haemophilia were tested. At our clinic, response for DDAVP is only tested in mild haemophilia patients, as DDAVP treatment is only considered for these patients. By including patients with low baseline FVIII activities in the exercise, the overall increase in FVIII could have been negatively influenced.

In addition to baseline level, other factors could have impacted the increase in FVIII activity. Training level influences the increase in FVIII activity in healthy people[21,22]. Persons performing sports on a semi-professional level have lower increases of FVIII activity, but increased baseline levels.[22]. The patients in this pilot study were not practising high intensity sports regularly, as was established by a short interview before participation in which participants were asked to describe their physical activities. None of the patients participated in high-level endurance sports, such as cycling or running, at least three times a week, but all participated in sports at least once a week. Although numbers were low, the increase in FVIII and VWF seem to be consistent throughout this study. There is additional evidence that exercise lower than maximum intensity sports also results in increase in FVIII activity, albeit less pronounced. Koch et al.[23] reported grouped data on the increase of FVIII activity after an incremental exercise test in seven severe and three mild haemophilia A patients aged 8-15 years, with FVIII activity levels ranging from 14.5 to 17.3 IU/dl. As can be expected, FVIII activity did not increase in patients with severe haemophilia. Three patients with mild haemophilia showed, however, a 1.15 times increase of FVIII activity levels after submaximal exercise. The exertion in the study of Koch et al. was clearly submaximal as reflected by low mean peak workload (45W; range 37-60W), as well as the mean peak heart rate (177 bpm), which is considered quite low for children. Roya et al.[13] tested 10 patients with mild haemophilia, with mean FVIII activity levels of 12 IU/dl (sd 3.8 IU/dl). They reported a consistent increase of FVIII activity of 1.3 times after an incremental exercise test, which was similar to that of Koch et al. Mean exercise time in the study of Roya et al. was 37.4 minutes (range 23-46), corresponding to approximately 212W (range142-267), which was almost 100W lower than in our study (mean peak workload of 304W). Unfortunately Roya et al. did not report values for peak RER or peak HR which would have defined exercise intensity more clearly, but they also reported a slight increase in VWF post exercise. The age distribution from the study of Roya et al[13] (24.5 years (17-38 years)) matched that of our study (26.5 (20-39)), and therefore age-related effects on VWF and FVIII[24] do not affect the comparison of the studies. In contrast with both Koch et al[23] and Roya et al[13] who performed a submaximal exercise tests, we used a maximum incremental exercise test, which could explain the larger increase[24]. Apparently, the intensity of the physical activity influences the increase in FVIII activity. The target level of the intensity of physical activity to reduce bleeding risk remains to be studied. The increase (median 2.5 times) found in the currents study is comparable to the increase found in von Willebrand patients (mean increase 1.6-2.3 times)[11] and healthy subjects[10].

The mechanism of increase in coagulation components during exercise or DDAVP is not yet fully

understood. There have also been reports that endothelial cells could release FVIII[25,26] or even produce FVIII[25,27]. Future studies should investigate whether both DDAVP and exercise are two parts of the same mechanism or target different systems of release of extra endogenous FVIII.
In this study, patients exercised up to a maximum workload, with heart rates of median 188 beats per minute, resulting in doubling of the endogenous FVIII activity level. If patients would perform a high intensity warmup activities, before participating in sports and other strenuous activities, they could profit from a maximal increase in FVIII activity. Although normal levels are not achieved, the increase of FVIII activity may help to reduce bleeding risk during physical activity, especially in patients with moderate haemophilia. This information may be taken into consideration when counselling patients or tailoring treatment. Sports and other strenuous activities should not be discouraged in moderate and mild haemophilia A patients, because of increased bleeding risks. Rather, these patients may benefit from their ability to raise FVIII levels during sports activities as a natural mechanism to reduce the risk of bleeding.

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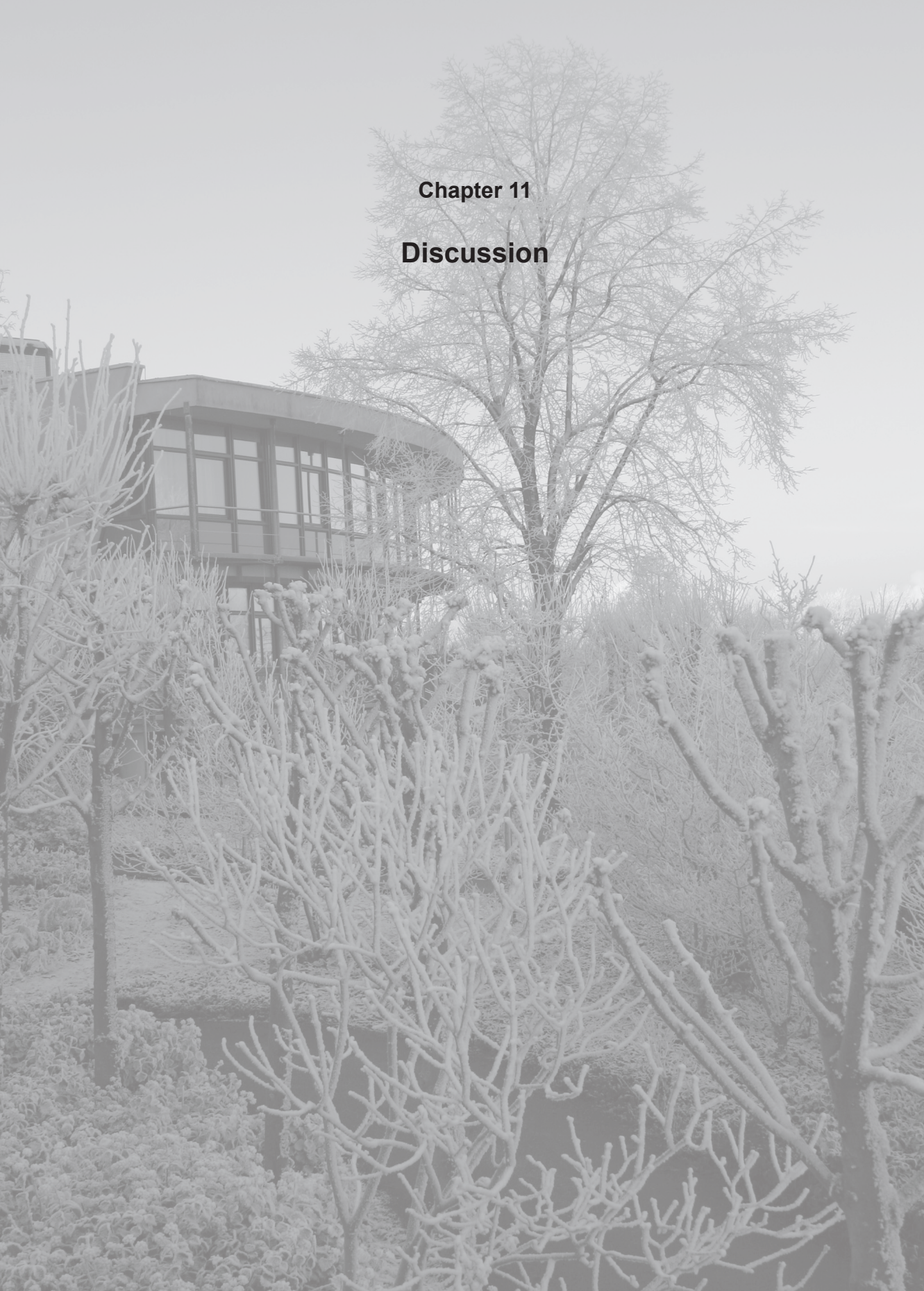


Chapter 11

Discussion

Patients

We studied bleeding and outcome in two populations of haemophilia patients. The first population consisted of all Dutch haemophilia patients, who completed a nationwide questionnaire on treatment and burden of disease, 'Hemofilie in Nederland (HIN)', in 2001. Most patients (68%) returned the questionnaire[1], providing a large cohort comprising patients of all severities and both haemophilia types. The second population consisted of about 50% of all Dutch haemophilia patients, treated at the Van Creveldkliniek in Utrecht. This centre was the first haemophilia treatment centre in the Netherlands, founded by professor Van Creveld in 1964[2]. From 1970 onwards, outcome and treatment of all patients has been collected according to a standardised format, providing a well-documented cohort[3].

Because the van Creveldkliniek is a multidisciplinary and specialised referral centre, it attracted more patients with complications due to haemophilia. Patients with hardly any bleeding problems were mostly treated in hospitals closer to their residence, which was reflected by the under representation of mild haemophilia. Mild haemophilia comprises 44% of the Dutch haemophilia population, while at the van Creveldkliniek only 32% of patients have mild haemophilia. Those with mild haemophilia treated at the van Creveldkliniek showed more bleeding complications, which was reflected by the large proportion of patients with mild haemophilia that had suffered joint bleeding. In contrast, half of the patients at the van Creveldkliniek had severe haemophilia (48%), while in the general haemophilia population severe haemophilia was diagnosed in 39% of patients.

Duration of follow-up varied. For some patients, lifelong bleeding and treatment data were available, while others came to the clinic at a later age. When appropriate, we corrected for this difference via statistical multilevel methods.

Measuring outcome: self-reported or objective

We studied joint bleeding according to factor activity using self-reported data from questionnaires and using more objective data from patient files. With both methods, we generated similar results. On the one hand this was to be expected, because the populations consist of the same patients. On the other, hand self-reported outcome is generally more biased than outcome assessed using standardized measurements. This bias seemed to be minimised in this well-informed and experienced population of patients[4].

Soft-tissue bleeds can easily be misclassified. Some patients report an extensive bruise or a nosebleed, while others will only classify muscle bleeds as 'real' bleeds. To avoid misclassification we mostly used joint bleeding as outcome, instead of total number of bleeds. Joint bleeds were classified as bleeds into joints that were treated at least once, in moderate and mild haemophilia A patients[5].

Patients with inhibitors against FVIII/FIX were excluded. Patients with inhibitors did not receive prophylaxis and bleeds were more difficult to treat, because bypassing agents are less effective than FVIII/FIX, which affects long-term outcome. Additionally, the inhibitor interferes with baseline factor activity levels. In our studies, most patients with moderate or mild haemophilia showed decreased baseline levels in the presence of inhibitors, and half of them had no detectable FVIII, leading to an increased risk of bleeding and adverse outcome.

Determinants of bleeding frequency independent of factor activity

Variation in bleeding pattern, also called phenotype, occurs independent of factor activity levels, and is seen in moderate as well as severe haemophilia. Patients with severe bleeding phenotypes required prophylaxis and often experienced their first joint bleed early, before the age of five years. The role of other coagulation components is being studied, including work on platelets[6], thrombin generation[7] or endothelial function[8,9]. So far, no explanation has been found in the coagulation proteins[10]. Instead of one factor causing the heterogeneity, interactions of these factors with baseline clotting factor activity and external influences may explain the additional variation in bleeding patterns.

Physical activity is known to have an impact on bleeding[11]. Not only trauma and injuries, associated with high risk sports, are detrimental to joints. Underdeveloped muscles, due to lack of physical activity, can also lead to increased joint bleeding[12]. Unfortunately, data on physical activity was only available for a proportion of patients. Activity levels were similar across severe and moderate haemophilia patients and to activity levels in unaffected peers. Therefore, we expect that physical activity have not influenced our results.

Age is another determinant of joint bleeding frequency as well as of physical activities[13,14]. Younger persons generally report more vigorous activities than older patients[15]. Therefore, in this thesis bleeding frequencies were always adjusted for age.

Variations in laboratory assays

Another source of variation is difference in laboratory assays. Determination of factor activity is difficult especially in the lower ranges (<2 IU/dl)[16]. The inter-assay difference of our lab was small (max. 4%, Albert Huisman, personal communication) and the laboratory is certified and regularly audited[17]. Nevertheless, misclassification in the lower ranges, around 1-2 IU/dl especially, cannot be excluded. In order to minimize this effect, the lowest factor activity ever measured was considered the baseline level of endogenous clotting factor activity.

Another source of laboratory misclassification is the type of assay used. Some mutations in mild haemophilia will have higher FVIII activity levels when measured with one-stage assay than with other methods like two-stage or chromogenic assays[18].

Scores

To quantify joint deterioration, we used scores specifically designed for haemophilia. The Haemophilia Joint Health Score (HJHS) measures loss of clinical function[19], the Pettersson Score[20] determines joint damage on radiography (x-ray) and the MRI Score assess the joint as imaged by Magnetic Resonance Imaging (MRI)[21]. By using these scores, our observations were standardised, which made our studies comparable to other studies. However, all these scores are limited to the included items. Anything observed outside of the items of the score could not be quantified. In the HJHS score the physiotherapists noticed that patient with hip problems had difficulty in performing some parts of the score. Since the hip is not included in the score, consensus was reached to include it in the gait score. In a second example, we observed enlarged epiphyses in the knees of 2 patients. This item is part of the Pettersson score for radiography, but not of the MRI score, thus creating a discrepancy between the two scores, even though it was observed

with both modalities. We chose to describe all discrepant items, rather than solely compare quantitative score, to assess agreement between scores and the added value of MRI.

Classification of haemophilia right or wrong?

Biggs and MacFarlane[22] designed a very robust classification of haemophilia in 1958, based on simple clinical observations, without fancy laboratory work or statistical tools (Table 1). This classification has remained unchallenged for 60 years. We studied whether this was justified. In this thesis, bleeding pattern were compared according to this classification, which was adopted by the ISTH in 2001[23].

Table 1. Classification of haemophilia [23]

Severity	Factor activity
Severe haemophilia	<1 IU/dl
Moderate haemophilia	1-5 IU/dl
Mild haemophilia	6-40 IU/dl

The current classification was tested in both study populations, and generated the same results. Although dating back 60 years, the classification still stands. However, a wide variation in bleeding pattern was observed, especially in patients with moderate haemophilia.

Generally, severe haemophilia had the highest bleeding frequency and most severe orthopaedic outcome, and bleeding phenotypes were progressively milder in patients with moderate and mild haemophilia. Modelling joint bleeding according to factor activity level confirmed the current classification. The association was asymptotic: joint bleeding frequency was high in patients with factor activity levels of 1-2 IU/dl, decreased steeply in patients with 3-5 IU/dl and levelled off to zero in patients with >15 IU/dl. On average with every IU/dl increase in factor activity, joint bleeding decreased by approximately 17%.

The group of moderate haemophilia patients with the highest bleeding frequencies had baseline factor activity levels of 1-2 IU/dl, suggesting a division in moderate haemophilia not reflected in the current classification. In 2001, the UK Haemophilia Alliance suggested to classify severe haemophilia by <2 IU/dl, moderate 2-10 IU/dl, and mild haemophilia by >10 IU/dl[16]. Our studies agree with a cut-off at 2 IU/dl, but additionally, patients with >5 IU/dl were significantly better than those with 3-5 IU/dl, which is not reflected by this proposed classification.

The classification of haemophilia was not only confirmed with bleeding data. The milestones of haemophilia (diagnosis, onset of treatment and bleeding) were observed at a later age with increasing factor activity level. However, age at diagnosis was only a good indicator in patients with a negative family history of haemophilia; due to genetic counselling, patients with a family history of haemophilia were diagnosed early, irrespective of bleeding signs[24].

Although the severities are well classified, clinical impression was that haemophilia B had a milder phenotype than haemophilia A. In our study we confirmed this opinion. Haemophilia B patients showed 18% less bleeding than haemophilia A patients with the same factor activity level. The asymptotic relation of decreased bleeding with increasing factor activity was observed in both haemophilia types. Future studies on outcome in haemophilia should take into account that pa-

tients with haemophilia B have lower bleeding rates than those with haemophilia A. As a result a population with more haemophilia B patients will show lower bleeding rates and probably better outcome than populations consisting mainly of haemophilia A patients. Populations should have similar distributions of haemophilia types or have to be analysed separately, even though it may lead to low numbers in haemophilia B.

Are patients with moderate haemophilia undertreated?

Moderate haemophilia is the rarest and least studied form of haemophilia. Of all 176 patients with moderate haemophilia who returned the HIN questionnaire, 11% of patients aged 19-40 years and 33% of patients aged 41-64 years reported severe hindrance in their daily activities.

Generally, patients with moderate haemophilia had low bleeding frequencies and hardly any joint bleeds. However,a subgroup showed a more severe bleeding phenotype. Patients with factor activity levels <3 IU/dl and those with early onset of joint bleeding, before the age of 5 years, had significantly higher bleeding rates than the majority of moderate haemophilia patients. These patients were more often treated with prophylaxis than patients with higher factor activity levels or late onset of joint bleeding.

This thesis provides the first step towards answering the question whether moderate haemophilia patients are undertreated. The majority of patients with moderate haemophilia had a mild phenotype. About a quarter of patients with moderate haemophilia required treatment by prophylaxis. Despite prophylaxis, these patients continued having high bleeding rates. This may be caused by inadequate dosing, long intervals between infusions or the late start of prophylaxis.

From studies in severe haemophilia, it is well known that postponing prophylaxis is less effective in preventing arthropathy[25,26]. It is therefore paramount to monitor bleeding phenotypes closely and identify patients with severe bleeding phenotypes early. Most of those patients had low factor activities and early onset of joint bleeding.

Future studies need to focus on whether an increased prophylactic dose or earlier start of prophylaxis can decrease bleeding and joint dysfunction in patients with moderate haemophilia and a severe bleeding phenotype

Is the bleeding pattern of severe haemophilia converted to moderate haemophilia with long-term prophylaxis?

Since the introduction of prophylaxis at the end of the 1960s, physicians have been trying to convert the bleeding pattern of severe haemophilia into the milder bleeding pattern of moderate haemophilia[27]. We compared outcome in moderate haemophilia to outcome in severe haemophilia patients on long-term prophylaxis. Even during intermediate dose prophylaxis, bleeding occurred more frequently and joint function was more reduced in patients with severe haemophilia on long-term prophylaxis, as compared to moderate haemophilia. Early changes of joint deterioration, as detected by MRI, were similar for both teenage and young adolescent moderate and severe haemophilia patients. Both patients with severe and moderate haemophilia reported similar quality of life and physical activity levels as the general male population in the Netherlands.

The equality in quality of life and physical activity suggest that patients with severe haemophilia experience haemophilia not as a burdensome disease. The explanation for this is that they can

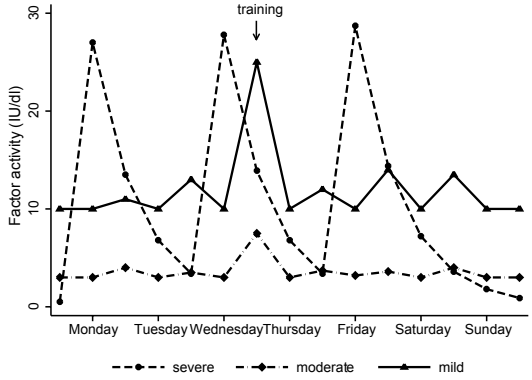
live nearly normal lives, because of long-term prophylaxis, but coping mechanisms also played a role.

The goal of Inga-Marie Nilsson of turning severe haemophilia into moderate haemophilia[27] was not yet reached entirely.

Physical activity a blessing or a curse?

Baseline factor activity levels are only part of the variation in clinical outcome. Severe haemophilia patients treated with prophylaxis rarely have plasma factor activity levels <1 IU/dl. Quite the opposite, they spend most of their life above the factor level of moderate haemophilia, due to prophylaxis. Figure 1 depicts a week in the lives of three imaginary 20-year old haemophilia patients in the absence of inhibitors and bleeds. The first patient has severe haemophilia and is treated with prophylaxis: thrice weekly with a weekly dose of 40 IU/kg[28], assuming an in vivo recovery of 2 (IU/dl)/(IU/kg)[29]. The second patient has moderate haemophilia with a baseline factor activity level of 3 IU/dl. The third patient has mild haemophilia with a baseline factor activity level of 10 IU/dl. All patients have soccer training on Wednesday evenings. Who has the highest bleeding risk?

Figure 1. Factor activity levels during the week of three patients with haemophilia; 1 severe treated with prophylaxis, 1 moderate and 1 mild patient



The patient with severe haemophilia has received prophylaxis on Monday, Wednesday and Friday morning. During soccer practice, he had a factor activity of around 15 IU/dl. After sprinting exercises during warming up, both patients with moderate and mild haemophilia increased their factor activity levels. The patient with mild haemophilia increased his factor activity to almost 25 IU/dl, reducing his risk of bleeding. The patient with moderate haemophilia also had an elevated factor level, but unfortunately it did not increase above 10 IU/dl. Eventually, the patient with moderate haemophilia had the lowest plasma factor activity level and the highest bleeding risk during training.

Should severe patients on prophylaxis therefore not have less bleeding and better joint outcome than patients with moderate haemophilia? The graph depicted is for an average patient. Individual patients might have shorter half-lives[30] or less compliance[31], resulting in increased bleeding. Ad-

ditionally, patients with severe haemophilia on this three times per week regimen drop below 1 IU/dl during weekends, which is associated with increased risk of bleeding[32].

The mechanism of increase in FVIII after intense physical activity is thought to be similar to that of DDAVP[5]. DDAVP increases FVIII in all probability by activation of the β-adrenergic system; the complete mechanism has not yet been determined. This β-adrenergic system is also activated when people are experiencing fear. If mental stress could also increase FVIII levels of haemophilia A patients, this would confirm the mechanism, although of course it could not be used for treatment.

Treatment options

Recurrent joint bleeding causes irreversible joint damage, leading to severe arthropathy[33]. Although, factor activity levels provide some indication of bleeding phenotype, they do not tell the whole story. Some patients report high bleeding frequencies in severe and moderate haemophilia, while patients with similar factor activity levels experience hardly any bleeding. Moderate haemophilia is rare, patients with a severe bleeding phenotype are even rarer. These patients will therefore often be classified as outliers. This thesis shows that these patients are not outliers, but a considerable subgroup. In our studies, about a quarter to a third required prophylaxis because of high bleeding frequencies.

Patients with moderate haemophilia received secondary prophylaxis, starting at a median age of 14 years, while they suffered their first joint bleed before the age of 5 years. Early onset of joint bleeding was associated with the need for prophylaxis later in life. These patients could benefit from close monitoring of bleeding phenotype, similar to severe haemophilia, in order to start primary prophylaxis rather than secondary prophylaxis.

Maintaining minimum trough levels of 1 IU/dl does not convert severe haemophilia into moderate haemophilia. This thesis suggested that patients with moderate haemophilia and baseline factor activity levels <3 IU/dl had worse bleeding and joint function than patients with baseline factor levels of 3 IU/dl or higher. Ideally trough levels should therefore be aimed at >3 IU/dl. Higher doses of prophylaxis raise trough levels and prevent bleeding more effectively, resulting in better long-term outcome[28]. Another possibility would be to increase the infusion frequency from three times a week to every other day[29].Longer acting factor concentrates may also be very effective at raising trough levels, but these are not yet available[34].

Conclusions

- The classification of haemophilia still stands after 60 years
- Generally, moderate haemophilia patients show only infrequent bleeding, but a considerable subgroup shows a more severe phenotype requiring prophylactic treatment
- Prophylaxis improves outcome in patients with high bleeding frequencies. The goal of converting severe haemophilia into the milder phenotype of moderate haemophilia is, however, not yet attained by intermediate dose prophylaxis

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Summary

Haemophilia is an inherited, X-linked bleeding disorder. Patients with haemophilia lack clotting factor VIII (FVIII) or IX (FIX) causing spontaneous or prolonged bleeding. The hallmark of severe haemophilia is recurrent joint bleeding, eventually leading to severe crippling arthropathy. Functional limitations and the need for lifelong treatment have a large impact on a patient's daily life.
Two populations of haemophilia patients were studied in this thesis. The first population consisted of 1066 Dutch haemophilia patients, who completed a questionnaire on treatment and burden of disease, 'Hemofilie in Nederland', in 2001. The second population consisted of about 50% of all Dutch haemophilia patients; those treated at the Van Creveldkliniek in Utrecht. This centre was the first haemophilia treatment centre in the Netherlands, founded by professor van Creveld, in 1964.

Classification of haemophilia
Haemophilia is classified into two types and according to quantity of clotting factor deficiency. Haemophilia A patients lack FVIII, while haemophilia B patients lack FIX. In 1958 haemophilia was classified in three severities based on clinical observations; <1 IU/dl FVIII/FIX (<1% of normal activity levels) was classified as severe haemophilia, 1-5 IU/dl FVIII/FIX (1-5% of normal activity levels) as moderate haemophilia and 6-40 IU/dl FVIII/FIX (6-40 % of normal activity levels) as mild haemophilia. In 2001 this classification has been adopted by the International Society of Thrombosis and Haemostasis as standard. However since 1958 this classification has remained unchallenged. Is this justified?
This thesis showed that, without fancy laboratory work or statistical tools, Biggs and MacFarlane designed a very robust classification of haemophilia.

In *Chapter 2* haemophilia was categorized according to the current classification in Dutch haemophilia patients. Data on bleeding, treatment and disability of 420 patients with severe, 176 with moderate and 470 with mild haemophilia were analysed. The results confirmed different clinical outcomes between severe, moderate and mild haemophilia, as described by Biggs and MacFarlane. Severe haemophilia had the highest bleeding frequency and the worst orthopaedic outcome, followed by patients with moderate haemophilia. Patients with mild haemophilia had the lowest bleeding frequencies and few orthopaedic issues. Despite the differences in clinical outcome, quality of life was similar across all severities and showed no clear association with factor activity levels. Patients with moderate haemophilia showed a wide variety in outcome. A quarter had to be treated by prophylaxis at some point, because of high bleeding frequencies.

Joint bleeding was modelled according to factor activity level in *Chapter 3.* The association between annual number of joint bleeds and factor activity level also confirmed the current classification in 119 patients with moderate and 314 patients with mild haemophilia. This association was asymptotic: joint bleeding was high in patients with <2 IU/dl and decreased steeply in patients with 3-5 IU/dl, while it levelled off to zero in patients with 6-15 IU/dl. On average with every IU/dl increase in factor activity, joint bleeding decreased by 18%.

The classification of haemophilia was not only confirmed with bleeding data. In *Chapter 4*, the

milestones of haemophilia (age at diagnosis, age at first treatment and age at onset of bleeding) were modelled according to factor activity levels. In 377 patients, born after 1970 and treated at the Van Creveldkliniek, all milestones were observed at a later age with increasing factor activity level. However, probably due to more diligent genetic counselling, age at diagnosis only increased with factor activity levels in patients with a negative family history. When modelled according to the current classification the distinction between severe and non-severe haemophilia was very clear. The distinction between moderate and mild haemophilia, however, was less pronounced.

When comparing the two types of haemophilia (A and B), as performed in *Chapter 5*, this asymptotic relation was observed in both haemophilia types. Clinical opinion was that the phenotype of haemophilia B was less severe than that of haemophilia A. Bleeding and treatment data on almost 8000 years of follow-up of 507 patients with haemophilia A and 800 years of follow-up of 51 patients with haemophilia B were available from medical records at the Van Creveldkliniek. In the crude comparison both haemophilia types seemed similar. After adjustment for treatment, factor activity level and age, however, patients with haemophilia B showed slightly lower bleeding rates than those with haemophilia A. Patients with haemophilia B had an average reduction of bleeding risk of 17% .

In *Chapter 6* inhibitor development was assessed in 231 patients with mild and 79 with moderate haemophilia, treated at least once with FVIII at the Van Creveldkliniek. Five patients with moderate haemophilia (6.3%) and 14 with mild haemophilia (6.1%) developed inhibitors. In the presence of an inhibitor most inhibitor patients showed decreased baseline factor activity levels. Half of them had no detectable FVIII, leading to an increased risk of bleeding and possibly worse outcomes. Compared to severe haemophilia, inhibitors were less common in mild and moderate haemophilia. Peak treatments, the Arg593Cys mutation, and older age were risk factors for inhibitor development in mild haemophilia.

Outcome in moderate haemophilia

Moderate haemophilia is the rarest and least studied form of haemophilia. Only 20% of haemophilia patients have 1-5 IU/dl FVIII/FIX. Since the introduction of prophylaxis at the end of the 1960s, physicians have been trying to convert the bleeding pattern of severe haemophilia into the milder bleeding pattern of moderate haemophilia. Some studies have addressed moderate haemophilia patients, but usually combined with mild haemophilia. Although moderate haemophilia is the target for prophylaxis in severe haemophilia, little is known about long term outcome in moderate haemophilia.

In *Chapter 7*, moderate haemophilia was studied more closely in 75 patients treated at the Van Creveldkliniek. Most patients had low bleeding frequencies and hardly any joint dysfunction. Nevertheless, 25% of patients with moderate haemophilia showed complications comparable to those in severe haemophilia, even warranting prophylaxis. Joint outcome was unfavourable in this group. Most patients using prophylaxis had baseline factor activity levels of 1-2 IU/dl. It

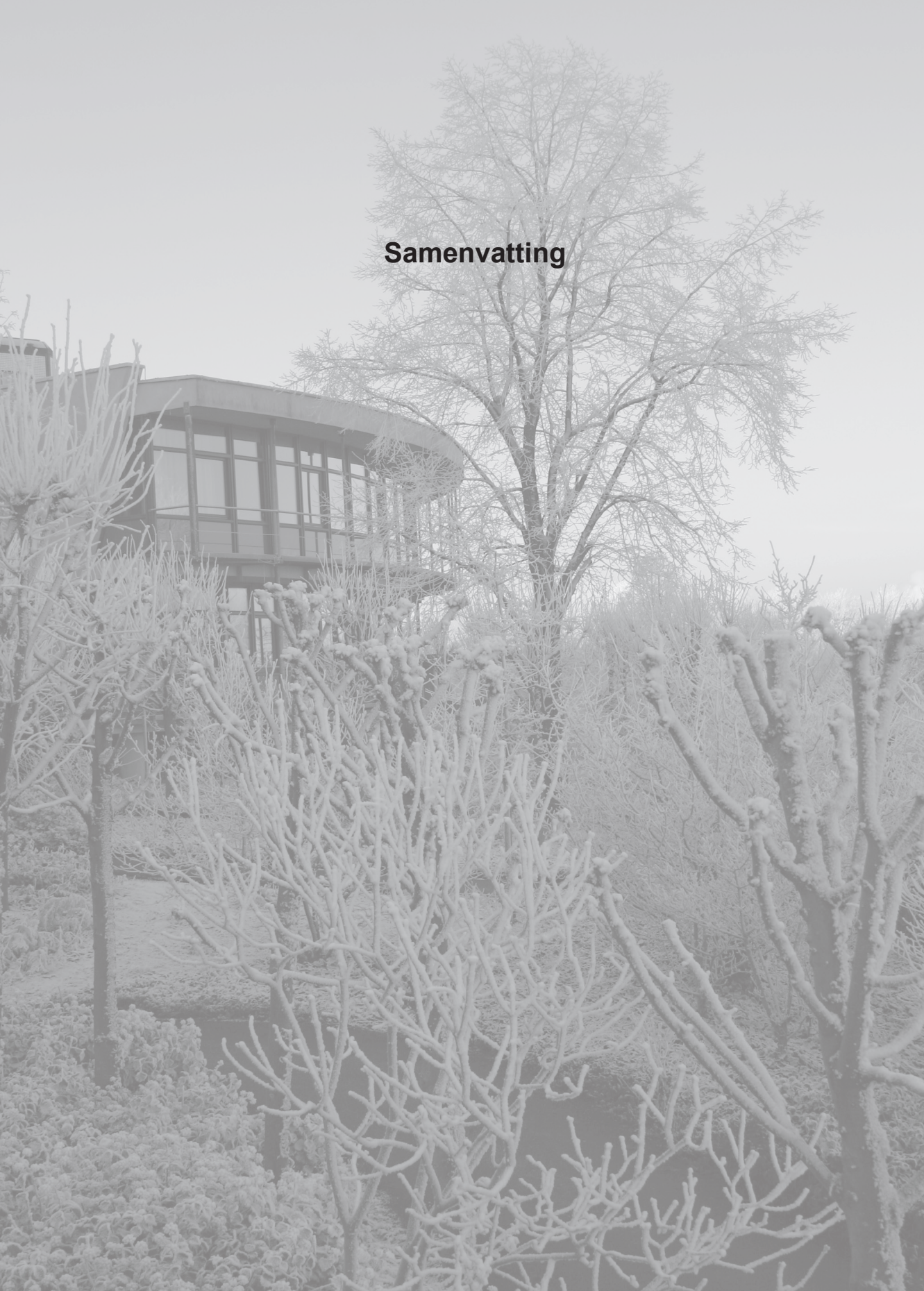
appeared that patients with baseline factor activity levels of ≥3 IU/dl had less complications of haemophilia than patients with baseline levels <3 IU/dl.

Severe haemophilia is not yet completely turned into moderate haemophilia by prophylaxis. In *Chapter 8*, bleeding and treatment histories were compared of 80 patients with severe and 40 with moderate haemophilia, born between 1970 and 1995 and treated at the Van Creveldkliniek. Bleeding occurred more frequently and joint function was more reduced in patients with severe haemophilia on long-term prophylaxis than in patients with moderate haemophilia. Patients of both severities reported similar quality of life and physical activity levels compared to the general population. When comparing severe haemophilia patients on prophylaxis to patients with moderate haemophilia treated by prophylaxis, bleeding rates and orthopaedic outcome was similar in both groups.

In *Chapter 9* the value of Magnetic Resonance Imaging (MRI) for detection of early signs of joint deterioration was studied in adolescents and teenagers (12-26 years) with severe (16 patients) or moderate (10 patients) haemophilia. They had little joint damage on plain radiography and hardly any loss of clinical function in the measured joints, knees and ankles. In these patients clinical function and bleeding history did not correlate with MRI scores. Plain radiography did correlate with MRI, but obviously detected less soft-tissue changes. In some joints abnormalities were detected by MRI, without prior joint bleeding. These abnormalities may have been caused by sports-injuries rather than be haemophilia related. Haemosiderin on MRI was associated with the time between assessment and last bleed. Joints that had suffered a bleed long before MRI had less haemosiderin than those with a bleed shortly prior to imaging, suggesting regeneration of the joint after a bleed, provided sufficient time to recuperate in the absence of bleeding.

Professor van Creveld used to let healthy blood donors run up and down the stairs to increase FVIII in donorplasma. Therefore, in *Chapter 10*, we studied whether FVIII would also increase after strenuous physical activity in patients with moderate or mild haemophilia. Fifteen patients performed an incremental exercise tests on a bicycle. FVIII increased on average 2,5 times, 10 minutes after exercise. The absolute increase was much higher in patients with mild haemophilia than in those with moderate haemophilia. Nevertheless, after an intensive warm-up prior to exercise, patients with moderate or mild haemophilia may profit from reduced bleeding risks.

Samenvatting



Hemofilie, ook wel bloederziekte genoemd, is een erfelijke stollingsziekte. Hemofilie wordt doorgegeven via het X-chromosoom en komt daarom vooral bij mannen voor. Hemofilie is een zeldzame ziekte, in Nederland hebben ongeveer 1600 mensen hemofilie. Patiënten met hemofilie missen een stollingsfactor, namelijk factor VIII (FVIII) of factor IX (FIX). Het gebrek aan FVIII of FIX veroorzaakt bloedingen in weefsels en gewrichten. Gewrichtschade, veroorzaakt door herhaalde gewrichtsbloedingen, is de grootste complicatie van hemofilie en wordt hemofilie-arthropathie genoemd. Patiënten met hemofilie-arthropathie ondervinden veel hinder in hun dagelijks leven door verminderde mobiliteit en pijn.
Hemofilie kan behandeld worden door het intraveneus toedienen van de ontbrekende stollingsfactor. Om zoveel mogelijk gewrichtsbloedingen te voorkomen kunnen stollingsfactoren profylactisch gebruikt worden, voor dat gewrichtsbloedingen ontstaan. Door de hoge kosten kan de stollingsfactor niet volledig worden vervangen, waardoor hemofiliepatiënten nog steeds gewrichtsbloedingen hebben.
De functionele beperkingen en de levenslange intraveneuze behandeling hebben veel impact op het leven van een hemofiliepatiënt.
Voor de studies in dit proefschrift zijn twee groepen patiënten met hemofilie bestudeerd. Allereerst een groep van 1066 hemofiliepatiënten uit heel Nederland, die hebben deelgenomen aan het onderzoek ‘Hemofilie in Nederland’. Zij hebben in 2001 een vragenlijst ingevuld over hun dagelijks leven, bloedingen en behandelingen. De tweede groep patiënten bestond uit patiënten die werden behandeld bij de Van Creveldkliniek, UMC Utrecht. De Van Creveldkliniek is in 1964 opgericht door professor Van Creveld en was het allereerste hemofilie behandelcentrum in Nederland. Het team van de Van Creveldkliniek behandelt ongeveer de helft van alle hemofiliepatiënten in Nederland.

Classificatie van hemofilie
Hemofilie kan op 2 manieren worden geclassificeerd, kwalitatief en kwantitatief. Er worden twee typen hemofilie onderscheiden: hemofilie A, waarbij FVIII ontbreekt, en hemofilie B, waarbij FIX ontbreekt. In 1958 hebben Biggs and MacFarlane hemofilie geclassificeerd op basis van de hoeveelheid FVIII/FIX activiteit die nog door het eigen lichaam wordt gemaakt en de klinische verschijnselen. Deze classificatie bestaat uit 3 vormen: patiënten met ernstige hemofilie hebben < 1 IU/dl (<1% van normale FVIII/FIX activiteit), patiënten met matige hemofilie hebben 1-5 IU/dl (1-5% van normale FVIII/FIX activiteit) en patiënten met milde hemofilie hebben 6-40 IU/dl (6-40% van normale FVIII/FIX activiteit). Sinds 2001 is deze 60-jaar oude classificatie de internationale standaard, maar is dat wel terecht?
De resultaten van dit proefschrift laten zien dat Biggs en MacFarlane, zonder geavanceerde laboratoriumtechnieken of statistische methodes, een zeer goede en robuuste classificatie hebben ontworpen.

In *Hoofdstuk 2* zijn de Nederlandse hemofiliepatiënten geclassificeerd volgens de internationale classificatie. Van 420 patiënten met ernstige, 176 met matige en 470 met milde hemofilie zijn bloedingshistorie, behandelingen en beperkingen vergeleken. De resultaten bevestigden het verschil in klinische verschijnselen tussen ernstige, matige en milde hemofilie, net zoals

beschreven in 1958 door Biggs en MacFarlane. Patiënten met ernstige hemofilie hadden de meeste bloedingen en beperkingen door hemofilie-arthopathie, gevolgd door patiënten met matige hemofilie. Patiënten met milde hemofilie hadden nauwelijks bloedingen en beperkingen. Ondanks deze klinische verschillen was de kwaliteit van leven gelijk, onafhankelijk van de ernst van de hemofilie. Het klinische beeld van patiënten met matige hemofilie varieerde sterk. Een kwart van deze patiënten had op enig moment in zijn leven profylaxe nodig vanwege een hoge bloedingsfrequentie.

In *Hoofdstuk 3* is de associatie tussen gewrichtsbloedingen en factor activiteit gemodelleerd. De huidige classificatie werd bevestigd in 119 patiënten met matige hemofilie en 314 met mild hemofilie. De relatie tussen het gemiddelde aantal gewrichtsbloedingen per jaar en factor activiteit was asymptotisch. Het jaarlijkse aantal gewrichtsbloedingen was zeer hoog in patiënten met < 2 IU/dl, daalde sterk in patiënten met 3-5 IU/dl en daalde verder naar nul in patiënten met 6-15 IU/dl. Gemiddeld nam het risico op gewrichtsbloedingen af met 18% per toename van 1 IU/dl factor activiteit.

De hemofilie classificatie werd niet alleen bevestigd door middel van gewrichtsbloedingen. In *Hoofdstuk 4* worden de mijlpalen van hemofilie (leeftijd van diagnose, eerste behandeling en de eerste gewrichtsbloedingen) vergeleken met de huidige classificatie en gemodelleerd volgens factor activiteit. In 377 patiënten van de Van Creveldkliniek, geboren na 1970, kwamen alle mijlpalen op latere leeftijd naarmate de factor activiteit toenam. Volgens de huidige classificatie was er een groot verschil tussen ernstige en niet-ernstige hemofilie, maar het verschil tussen matige en milde hemofilie was minder duidelijk.

Bij de vergelijking tussen de 2 hemofilietypes (hemofilie A en hemofilie B), zoals uitgevoerd in *Hoofdstuk 5*, werd deze asymptotische relatie ook gevonden. De indruk van hemofilie behandelaren was dat de klinische verschijnselen in hemofilie B minder erg waren dan in hemofilie A. Bloedingshistorie en behandelingen van bijna 8000 evaluatiejaren, verdeeld over 507 patiënten met hemofilie A en 800 evaluatiejaren, verdeeld over 51 patiënten met hemofilie B, geregistreerd in patiëntendossiers van de Van Creveldkliniek zijn geanalyseerd. In de eerste, ongecorrigeerde vergelijking, leek er geen verschil tussen beide hemofilietypes. Echter, na correctie voor behandeling, factor activiteit en leeftijd, bleek hemofilie B een lager bloedingsrisico te hebben dan hemofilie A: een gemiddelde verlaging van het risico met 17%.

Het lichaam van hemofiliepatiënten kan antilichamen maken tegen de lichaamsvreemde stollingsfactoren waarmee ze worden behandeld. Deze antistoffen worden ‘remmers’ genoemd. Door de aanwezigheid van remmers werkt het toedienen van stollingsfactoren minder goed. In *Hoofdstuk 6* is gekeken naar de ontwikkeling van remmers bij 119 patiënten met matige en 231 patiënten met milde hemofilie. Deze patiënten waren minstens eenmaal behandeld met FVIII bij de van Creveldkliniek. In totaal 5 patiënten met matige (6.3%) en 14 patiënten met milde (6.1%) hemofilie ontwikkelden een remmer. In de aanwezigheid van een remmer hadden de meeste patiënten ook een lagere eigen factor activiteit. De helft had zelfs helemaal geen

meetbare factor activiteit meer, wat leidde tot meer bloedingen en mogelijk meer hemofilie-arthropathie. Ongeveer een kwart van de patiënten met ernstige hemofilie ontwikkelt een remmer, dit was veel lager (iets meer dan 6%) in patiënten met matige en milde hemofilie. Risicofactoren voor het ontwikkelen van een remmer bij milde hemofilie waren, langdurig achter elkaar behandelen, zoals rondom operaties, oudere leeftijd en de genmutatie Arg593Cys.

Matige hemofilie
Matige hemofilie is de meest zeldzame vorm van hemofilie, het komt maar bij 20% van de hemofiliepatiënten voor. Sinds de introductie van profylaxe, aan het eind van de 60er jaren, hebben artsen geprobeerd om het bloedingspatroon van ernstige hemofilie te veranderen in het wat mildere bloedingspatroon van matige hemofilie door middel van profylaxe. Er is een klein aantal studies gedaan naar matige hemofilie, maar meestal werd matige hemofilie dan gecombineerd met milde hemofilie vanwege het kleine aantal patiënten. Ondanks dat geprobeerd wordt om ernstige hemofilie te veranderen in matige hemofilie, weten we maar heel weinig van de klinische verschijnselen en orthopedische beperkingen als gevolg van matige hemofilie.

In *Hoofdstuk 7* wordt matige hemofilie uitvoeriger bestudeerd. In totaal zijn 75 patiënten met 1-5 IU/dl, die behandeld werden bij de Van Creveldkliniek gevolgd. De meeste patiënten hadden weinig bloedingen en nauwelijks functieverlies van gewrichten. Niettemin had 25% van de patiënten met matige hemofilie zoveel bloedingen, dat zelfs profylaxe nodig was. Hierdoor had deze groep patiënten ook meer gewrichtsschade. De meeste patiënten met matige hemofilie die profylaxe nodig hadden, hadden een eigen factor activiteit van 1-2 IU/dl. Het lijkt er dus op dat patiënten met ≥3IU/dl factor activiteit minder complicaties van hemofilie ondervinden dan patiënten met <3 IU/dl.

Ernstige hemofilie wordt nog niet compleet veranderd in matige hemofilie met behulp van profylaxe. In *Hoofdstuk 8* zijn bloedingen en behandelingen van 80 patiënten met ernstige hemofilie, die lange tijd profylaxe hebben gebruikt, vergeleken met die van 40 patiënten met matige hemofilie. Alle patiënten waren geboren tussen 1970 en 1995 en behandeld bij de Van Creveldkliniek. Ondanks langdurige profylaxe, hadden patiënten met ernstige hemofilie meer bloedingen en gewrichtsfunctieverlies dan patiënten met matige hemofilie. Beide groepen patiënten, zowel de ernstige als de matige hemofilie patiënten, rapporteerden een kwaliteit van leven en activiteiten patroon vergelijkbaar met de Nederlandse populatie. Patiënten met matige hemofilie die ook profylaxe gebruikten, hadden wel een verglijkbaar klinisch beeld met de ernstige patiënten op profylaxe.

In *Hoofdstuk 9* is de waarde van Magnetic Resonance Imaging (MRI) voor het opsporen van vroege aanwijzingen voor gewrichtsschade onderzocht in jongeren (12-26 jaar) met ernstige (16 patiënten) en matige (10 patiënten) hemofilie. Vooraf hadden deze patiënten weinig schade op de röntgenfoto en nauwelijks functieverlies van de gemeten gewrichten, knieën en enkels. Gewrichtsfunctie en bloedingshistorie correleerden nauwelijks met de MRI scores. Röntgenscores correleerden wel met MRI, hoewel met MRI meer kapsel- en weefselafwijkingen werden

opgespoord dan met röntgen. In sommige gewrichten werden kleine afwijkingen gevonden, zonder dat er bloedingen geweest waren. Deze afwijkingen kunnen veroorzaakt zijn door sportblessures in plaats van door bloedingen. Hemosiderine (ijzerafzetting na bloedingen in het gewricht) bleek geassocieerd met de tijd tussen de MRI en de laatste bloeding. Hoe langer de tijd tussen de MRI en de laatste (voorafgaande) bloeding, des te minder vaak werd er hemosiderine aangetroffen in een gewricht. Dit wijst erop dat een gewricht zich kan herstellen wanneer er genoeg tijd is, zonder dat er opnieuw een bloeding plaatsvindt.

Vroeger liet professor van Creveld gezonde bloeddonoren de trap op en neer rennen om het FVIII niveau in donorplasma te verhogen. Daarom is in *Hoofdstuk 10* onderzocht of de verhoging van de factor activiteit na inspanning ook mogelijk was in patiënten met matige en milde hemofilie. Vijftien patiënten hebben een maximale inspanningstest op een hometrainer uitgevoerd. De gemiddelde stijging van FVIII bedroeg 2,5 maal, 10 minuten na de inspanningstest. De absolute stijging was veel hoger in patiënten met milde dan in patiënten met matige hemofilie. Toch zouden patiënten met matige en milde hemofilie, na een intensieve warming-up voor inspanning, kunnen profiteren van een verminderd bloedingsrisico.

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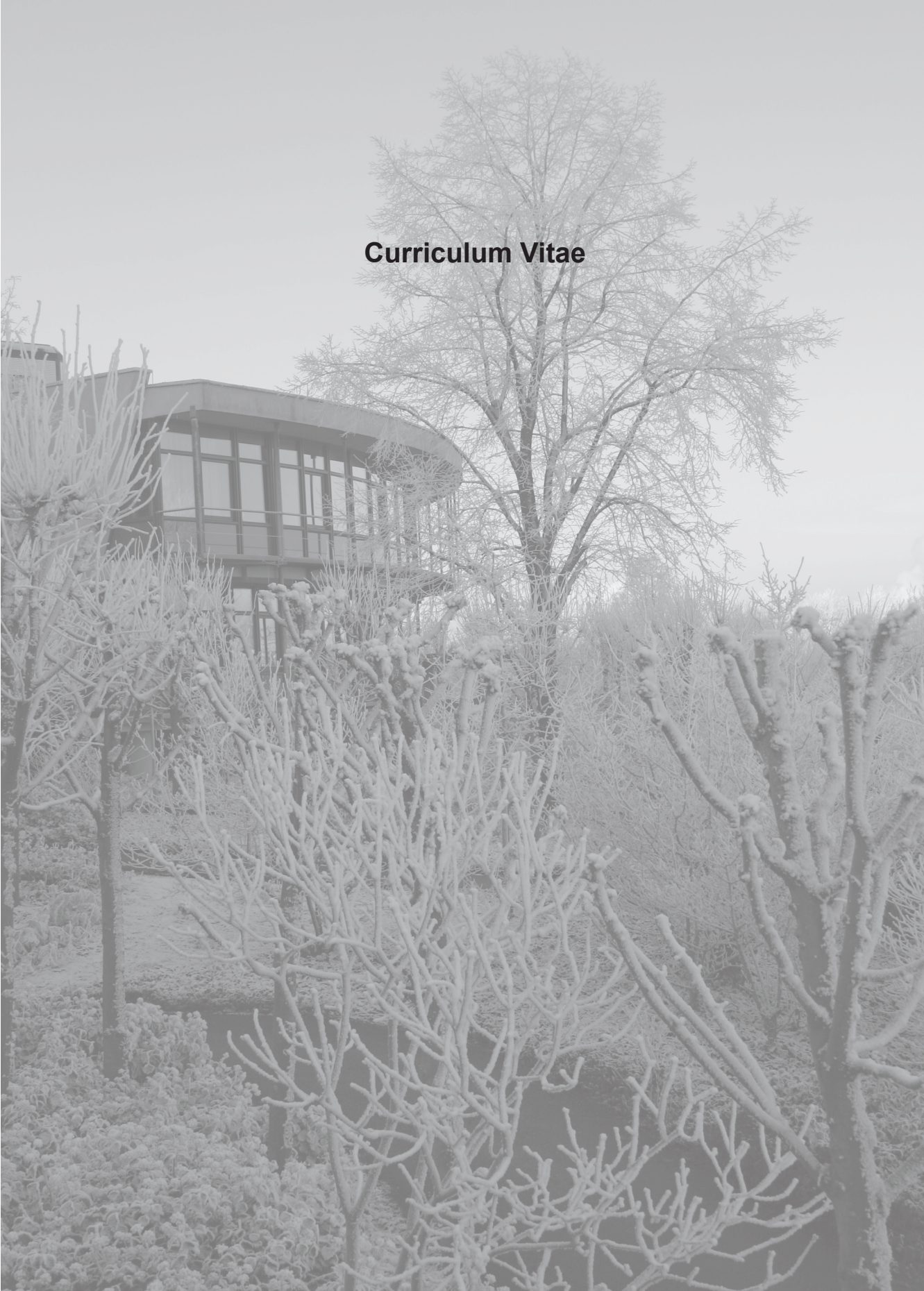
Curriculum Vitae



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For this research, she received three awards: In 2009, at the ISTH in Boston, she received a 'Young investigator's award' for Chapter 3. Also at the ISTH 2009, 'Top third of posters by score' for the development of the protocol used in Chapter 9. Finally at WFH 2010 in Buenos Aires, she received the 'H.R. Roberts award' for Chapter 9.
From 2008 she has combined this research with teaching statistical courses at the department of biostatistics from the Julius Center.

Currently, she has returned to her first interest, veterinary sciences, and combines it with epidemiology as a veterinary epidemiologist at the Animal Health Service (GD) in Deventer, the Netherlands.