VILNIUS UNIVERSITY

SONATA ŠAULYTĖ TRAKYMIENĖ

ASSESSMENT OF THE DEVELOPMENT OF BLOOD-INDUCED JOINT DAMAGE IN CHILDREN WITH HEMOPHILIA IN RELATION TO DIFFERENT TREATMENT STRATEGIES

Summary of doctoral dissertation
Biomedical sciences, medicine (06 B)

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List of abbreviations

APTT – activated partial thromboplastin time

CFCs – clotting factor concentrates

CI – confidence interval

DK – Denmark

FVIII – coagulation factor VIII

FIX – coagulation factor IX

HJHS – Haemophilia Joint Health Score

HTCs – hemophilia treatment centers

IQR – interquartile range

IUs – international units

LT – Lithuania

LHA – Lithuanian Hemophilia Association

MRI – magnetic resonance imaging

NHR – National Hemophilia Registry

PedNet – European Paediatric Network

PWH – people with hemophilia

QoL – quality of life

RCT – randomized controlled trial

SD – standard deviation

WFH – World Federation of Hemophilia

WHO – World Health Organization

1. Introduction

1.1 Hemophilia care in Lithuania

Hemophilia, a rare hereditary bleeding disorder caused by an X-linked recessive deficiency of coagulation factor VIII or IX, may lead to chronic disease and life-long disabilities if not managed properly (1). Over the past 50 years, the great advances in the treatment of hemophilia have produced far-reaching changes in the prognosis of people with hemophilia (PWH). The natural history of severe hemophilia, which was formerly described as recurrent bleeding into joints (hemarthrosis), leading to a progressive blood-induced joint damage and subsequent development of hemophilic arthropathy in 90% of patients (2), has been radically transformed thanks to prophylactic administration of clotting factor concentrates (CFCs). The primary objective of hemophilia treatment has shifted from the resolution of hemorrhage to the prevention of joint bleeds. A person with hemophilia can now lead an almost normal life without fear of frequent bleeding into joints, and experience an essentially normal mean life expectancy (3-5). The benefits of prophylaxis have been demonstrated in numerous long-term retrospective observational studies, case reports and comparative studies, and definitely proven through prospective randomized controlled multicenter trials (6-22). It is now well established, that the introduction of optimal treatment, i.e. early prophylactic replacement therapy with CFCs, results in life-changing improvements of outcome regardless of whether the outcome focus is on number of joint or life-threatening bleeds (23), on arthropathy, evaluated by physical examination, radiography or magnetic resonance imaging (MRI) (24), or on quality of life for PWH (25). Primary prophylaxis is considered the treatment of choice in severe hemophilia and has been recommended by the World Health Organisation (WHO) and the World Federation of Hemophilia (WFH) since 1994 (26). However, hemophilia care in Lithuania, especially for children, was largely inadequate in 2007 compared to modern standards which were clearly outlined in other European countries (27, 28). Thus, in response to the rapid and enormous advances in the treatment of hemophilia, initiatives were taken to focus on the hemophilia care of patients under 18 years of age in Lithuania in order to improve their treatment and follow-up.

The first steps towards the improvement of hemophilia care in Lithuania were taken more than a decade ago. In 1994, the collaboration between Malmö and Klaipėda (Sweden-Lithuania) twinning centres resulted in a comparative study between matched cohorts of patients. Outcomes of the study gave way to the conclusion that more intensive replacement therapy with CFCs would be required for Lithuanian PWH, which should be initiated at a younger age. (29). An attempt to set up a registry of the hemophilia patients in the Klaipėda area was unfortunately not completed. Several years later, in 1998, a study that assessed phenotypic and genotypic data comprising about 80% of Lithuanian PWH, provided data that was expected to improve hemophilia care in Lithuania and result in an establishment of a National Hemophilia Registry (NHR) (30). Despite the efforts and recognition of the need of a NHR, there was a failure to establish a registry at that time, however major genetic findings obtained over this period for diagnostic and therapeutic purposes have dramatically facilitated the evolution of hemophilia care in Lithuania. In 2007, a set of "Baltic guidelines for the care and

treatment of hemophiliacs" were published. Subsequently, continuous prophylaxis has been recommended as the treatment of choice for children with severe hemophilia A and B in Lithuania. In order to start implementing these guidelines into clinical practice, detailed information concerning demography, baseline factor level, clotting factor consumption, and objective data of clinical outcomes were fundamental. Therefore, an accurate and comprehensive database on all children up to the age of 18 years in Lithuania suffering from hemophilia, including those who had not received treatment, can be established in order to register current status and be ready to initiate delivery of prophylactic regimen.

1.2 Assessment of the development of joint damage

Hemophilia is characterized by frequent bleeds that occur either spontaneously or following trauma at any time during the entire life of the patient. Around 90% of bleeding episodes in PWH occur within musculoskeletal system; and of these 80% of bleeds occur into joints (31). With time, recurrent joint bleeds lead to irreversible blood-induced joint damage and eventual development of hemophilic arthropathy. Prevention of irreversible arthropathy is possible by regular replacement of the deficient clotting factor using a preventive regimen known as prophylaxis (32). Where prophylaxis is not available, an on-demand (episodic) treatment strategy is adopted as an alternative treatment modality, where deficient clotting factor is administered in response to a bleeding episode using doses that are considered adequate for arresting the bleeding. Episodic treatment fails to control recurrent joint bleeds and prevent progression of irreversible joint damage, and the outcome will evidently be the development of hemophilic arthropathy. As a result of recurrent hemarthroses, musculoskeletal outcome remains an important hallmark of treatment efficacy and outcome in hemophilia. Therefore, measurement of the musculoskeletal condition applying standardized physical joint-assessment tools is critically important in individuals with hemophilia, especially children. Data are important in documenting the signs of chronic changes related to recurrent joint hemorrhages over time and may further be useful in comparing the efficacy of various treatment principles (33, 34). In the past, several different systems existed for use as musculoskeletal assessment instruments for children and adults (35-38). However, with the introduction of prophylaxis and heightened interest in preserving joints and preventing complications related to recurrent hemarthroses, older systems were found to lack sensitivity in detecting the earliest signs of joint disease and inadequate in the evaluation of joints in children (34, 37, 39). Progress has been achieved recently through the work delivered by multinational expert groups to develop a new international consensus musculoskeletal assessment tool, the Haemophilia Joint Health Score (HJHS) (40, 41). It was the first standardized, highly sensitive, reliable and valid physical joint-assessment tool for children with hemophilia (42-44), however its utility was assessed in children receiving prophylaxis and its power was not known in children treated by on-demand principles.

Replacement treatment with clotting factors VIII and IX at bleeding episodes only was previously the standard practice in most children with hemophilia in Lithuania. Joint damage remains an inevitable complication in patients treated for bleeding episodes

only. However, it is unclear at which age arthropathy will manifest itself. The long-term impact of repeated hemarthroses and associated risk of joint damage increases proportionally with the number of bleeding episodes (45). In contrast, there is evidence that progression of joint disease may occur early in life following relatively few joint bleeds (6, 46). Furthermore, based on MRI findings, subclinical joint hemorrhages may exist that cause chronic deterioration (13, 47-49). The clinical implications of such early and subtle changes are not clear today, even if their presence is suspected of causing harm only in a subgroup of patients with clinically unaffected joints (49-51). Based on the clinical experience and observation that joint abnormalities may be minimal on physical examination in very young children – even those receiving on-demand treatment, it has been hypothesized that although development of hemophilic arthropathy is a continuous process during childhood and adolescence (45), it seems likely that the most aggravating development occurs once the child grows rapidly, i.e. from the age of 10 and onwards.

While it has been convincingly established that prophylactic treatment improves joint outcome (52), comparative studies of the long-term musculoskeletal outcomes using different treatment strategies were scant (53). Furthermore, HJHS as a new joint-assessment tool for PWH needed further evaluation in different patient populations. Comparative study of clinical outcomes using the HJHS in patients treated with prophylaxis as compared to those managed with on-demand treatment might provide insight into early and late manifestations of joint impairment based on the HJHS in relation to different treatment regimens, and thereby extend our understanding of the interpretation of the HJHS scores. Based on that knowledge it was decided to evaluate physical joint status based on the HJHS during childhood and puberty in relation to different treatment regimens.

2. Study purpose and objectives

Assess demographic data, analyze treatment and study the development of musculoskeletal damage in patients with hemophilia in order to improve diagnosis, treatment and follow-up for children with hemophilia in Lithuania.

Objectives of the study

1. Create credible database of Lithuanian patients less than 18 years of age suffering from hemophilia: investigate and describe demographic, disease-specific baseline laboratory data (total number of patients with hemophilia, age distribution, distribution of type and severity, factor VIII/IX mutation profile, inhibitor frequency in 2008-2011) and incidence in 1992-2011.
2. Analyze annual consumption of CFCs in patients with severe hemophilia for the period 2003-2008.
3. Present changes in the treatment regimen occurring since 2007.
4. Assess the development of changes in musculoskeletal status (joint scores) in patients with severe haemophilia:

a) utilize a new joint evaluation method (Haemophilia Joint Health Score, HJHS) to investigate its applicability and utility in children treated by on-demand principles;
b) investigate the progression of hemophilic arthropathy during childhood and puberty focusing on the age of remarkable changes in musculoskeletal health based on the HJHS;
c) investigate and characterise musculoskeletal damage in boys with severe hemophilia treated by on-demand treatment strategy, and compare their physical joint health with age-matched Danish patients who received prophylaxis from an early age.

Relevance

Hemophilia is a rare genetic and life-long bleeding disorder associated with significant morbidity. It is complex to manage and requires development of specialist care at special treatment centers (54). Owing to the low prevalence and phenotypic heterogeneity of hemophilia, central patient registry database, providing exact and credible data, is of paramount importance. It could provide not only the epidemiological status of hemophilia in Lithuania, but also be a very valuable resource for following the natural history of hemophilia, its treatment and complications. Generation of accurate and comprehensive baseline data of children with hemophilia could serve as a useful tool for medical experts and healthcare providers for treatment, statistical, economic and research purposes as well as be a platform for the future establishment of the NHR.

Treatment of hemophilia requires very expensive CFCs replacement and in parallel adequate follow-up to ensure treatment efficacy, therefore it must be carefully monitored by physicians and health authorities. Models of care have been developed in Western countries based on careful documentation of outcome over many years. Such data were lacking from our country. Carefully documented data on the main outcome –

joint health of PWH in Lithuania were not available. Neither was treatment of hemophilia in terms of its efficacy ever regularly monitored in our country. Studies from other disciplines suggested that the use of standardized outcome measures in daily practice led to improvement in the quality of care (55). Introduction of a new standardized method for the assessment of joints in PWH was very important to evaluate the efficacy of the existing treatment principles and be ready for follow-up of children initiating a new treatment strategy (based on the guidelines for the care and treatment of patients with hemophilia in the Baltic countries). Furthermore, analysis of consumption of CFCs by the physicians can help to optimize factor use and anticipate future demand. In addition, because of the rareness of hemophilia, both baseline and clinical outcome data were very important not only for the start of the new treatment period in Lithuania, but also in the perspective of international collaboration and research. The ability for a single centre and often even single country to provide robust outcome research in hemophilia, is limited (56, 57).

Scientific novelty

No accurate and credible national data were available about the size and distribution of hemophilia population less than 18 years of age in Lithuania and even less was known about the rate of complications from the disease or its treatment. This is the first comprehensive national survey of PWH below 18 years of age on demography, disease-specific data, treatment modalities and musculoskeletal status in hemophilia.

The HJHS is a new, recently developed and validated international scoring tool for assessing joint impairment in boys with hemophilia from 4 to 18 years of age (42, 44). However, as a new joint-assessment tool, it needed further evaluation in different patient populations and in centres that were not involved in its design in order to assess its applicability and usefulness in clinical practice and research. Our study was the first that used this standardized clinical outcome measure to investigate its utility on musculoskeletal assessment in patients receiving exclusively on-demand treatment. This is also the first study that used this standardized clinical outcome measure to compare joint outcome in different treatment groups in order to provide new knowledge for the interpretation of the HJHS scores in nearly normal versus damaged joints. Finally, available evidence comparing the impact of prophylactic versus on-demand therapy has largely been limited to clinical trials. Real-world long-term evidence was scanty.

4. Materials and methods

The study was conducted in the period year 2008 to 2011 at two centres: Children's Hospital, Affiliate of Vilnius University Hospital Santariskiu Klinikos, Lithuania (LT), and Aarhus University Hospital Skejby, Denmark (DK). The study was approved by the Lithuanian Ethics Committee and the Ethics Committee of Region Midtjylland. Signed informed consent was obtained from all patients and/or their parents.

4.1 Development of hemophilia patient database in Lithuania

Study subjects

Hemophilia care in Lithuania was not well coordinated in 2007. There was no joint registry of PWH. Data were collated from three distinct sources: from the local patient lists of Centre for Pediatric Hematology/Oncology, Children's Hospital, Affiliate of Vilnius University Hospital Santariskiu Klinikos, Klaipeda Seamen's Hospital and Lithuanian Hemophilia Association (LHA). Pooling of information from the three sources, patients aged 0-17 years with the diagnosis of hemophilia were selected to create one compile list. Database of the National Health Insurance Fund served as a double check for treated PWH. Selected patients were contacted by phone and received an invitation to come to the hospital (Centre for Pediatric Hematology/Oncology, Children's Hospital, Affiliate of Vilnius University Hospital Santariskiu Klinikos) for the study.

Information about demographics, family history, including pedigree, contacts and treatment was collected by interviewing the patients. Data on the type and severity of hemophilia and FVIII or FIX genotype (where available) were collected from medical records or from the database of the previous genotype study in Lithuanian PWH (30).

The demographic data on Lithuanian population were taken from the Statistical Yearbook of Lithuania 2011 and Lithuania in Figures 2012 (www.stat.gov.lt, last access 11 December, 2012).

The age-specific prevalence of hemophilia for the five-year period (2008-2011) was the number of identified hemophilia cases residing in Lithuania in the period 2008-2011 divided by the estimated Lithuanian male population for corresponding age in 2008-2011 and multiplied by 100 000 (cases per 100 000 males). The incidence was estimated by the number of new cases of hemophilia during a one year period of time divided by the number of boys born and multiplied by 10 000 (cases per 10 000 boys born).

Laboratory tests

Coagulation tests were performed specifically. All patients who suffered from mild or moderate hemophilia or whose previous test had yielded borderline values were re-investigated. FVIII and FIX activity (FVIII:C and FIX:C) assays were performed in the biochemical laboratory of Children's Hospital, Affiliate of Vilnius University Hospital Santariskiu Klinikos. FVIII:C and FIX:C was assessed in platelet-poor plasma using a one-stage activated partial thromboplastin time (APTT-based assay). Established normal reference value for FVIII:C and FIX:C 60-150%. Three degrees of hemophilia severity were distinguished: severe, moderate and mild, according to plasma clotting factor activity levels <1%, 1-5% and >5-40%, respectively (58).

All patients were screened for inhibitors against FVIII. Blood samples were collected, processed in the biochemical laboratory of Children's Hospital, Affiliate of Vilnius University Hospital Santariskiu Klinikos and stored at minus 70^0C. Frozen samples were sent for the inhibitor determination (Bethesda assay) to the laboratory of

Klaipeda Seamen's hospital. Established normal reference value <0.6 BU/mL. There were no laboratory facilities to screen patients against inhibitors for FIX.

In cases where FVIII or FIX genotype was unknown, blood samples were taken and sent to the laboratory of Hemophilia Center, Institute of Experimental Haematology and Transfusion Medicine, University Clinic Bonn, Germany.

Treatment data

To exclude factors associated with variations in annual CFCs use (most of the CFCs are used by patients with severe hemophilia A), we have chosen to analyze consumption of CFCs in severe hemophilia A cases (FVIII activity <1%). CFCs consumption in severe hemophilia A cases was obtained from the database of the National Health Insurance Fund. Electronic database was available since 2003. Data were obtained for the period 2003-2008. Year 2009 and onwards were not included because treatment and monitoring of hemophilia had been subject to several changes after 2009 in Lithuania. FVIII use was analyzed in terms of reported international units (IUs) per person with severe hemophilia A each year. Patients with inhibitors were excluded.

In order to analyze the changes in treatment pattern occurring since 2007 onwards, the accurate and comprehensive documentation related to the type of treatment received (i.e. prophylaxis or on-demand) and product used, including patients's diary, has been started, providing the opportunity to study changes in the treatment pattern over the last five years. All patients visited Children's Hospital, Affiliate of Vilnius University Hospital Santariskiu Klinikos, at least once a year. Prophylaxis was defined as infusion of FVIII for at least twice a weak and FIX at least once a week for a minimum of 45 weeks per year. On-demand treatment was defined as treatment per bleed only, or lees than 3 weeks of prophylaxis per year.

4.2 Joint assessment

Study subjects

Boys, aged 4-17 years were enrolled, meeting the following inclusion criteria: males with severe hemophilia A or B as defined by factor VIII or IX level <1%, with no past or present history of inhibitors (<0.6 BU/mL, Bethesda method) and without an acute bleed within two weeks prior to testing. According to study hypothesis, patients were stratified into two age groups: patients 4-9 years of age (group I) and patients 10-17 years of age (group II).

Joint evaluation

Evaluation of the six index joints (elbows, knees and ankles) and gait was performed by the same assessor in both countries using the Haemophilia Joint Health

Score (HJHS), version 2.0. A manual and supporting Instructional video were used to master the HJHS and use it reliably. Each of the six index joints were assessed individually on eight items (swelling, duration of swelling, muscle atrophy, crepitus on motion, flexion loss, extension loss, joint pain and strength) and numerically scored in categories of severity, with a total single joint score ranging from zero, representing the best possible joint health, to 20. A global gait score was assessed separately from zero indicating that all skills (walking, stairs, and running, hopping on one leg) were within normal limits, to four indicating that no skills were within normal limits. The total joint score (the sum of the six joint scores) and the global gait score, when combined, provided an overall total score range from zero to 124 with a score of zero representing no identifiable joint impairment. Raw data worksheets for each patient during the examination of six index joints and gait were completed. Following the examination, the HJHS scoring sheet was completed.

Treatment and follow-up strategy

Based on the treatment strategy, patients were stratified in to two groups: on-demand group (Lithuania, LT) and prophylaxis group (Denmark, DK).

Treatment in LT (on-demand group) was primarily given in response to a bleed. During their entire lifetime, LT patients had received treatment only to resolve acute bleeding episodes, but had never been subjected to prophylaxis. Patients received treatment at home or in a clinic. Home treatment was defined as access to replacement therapy with CFCs by venous access in the home setting. Clinic treatment was defined as patient's or parent's inability to self-infusion of CFCs. According to the Lithuanian National Health Service system, CFCs spending was limited to three single doses for one patient at one visit and prescribed by a general practitioner or hematologist. The follow-up on this group of patients was irregular, and patients visited general practitioner in case of demand of CFCs or were seen at a hospital in case of major bleeds.

Prophylaxis was the primary treatment strategy in DK (prophylaxis group), defined as a long-term therapy with regular injections of CFCs following the principles devised by Nilsson *et al*. (21) and consisted of at least three infusions per week for FVIII deficient patients or two to three infusions per week for FIX deficient patients. Patients were on early home treatment and had free access to therapy. Joint bleeds were treated with one or more infusions of 25-40 IU kg, according to severity, until bleeding episode stopped. Patients were monitored regularly to evaluate the effects of treatment. Follow-up visits took place every six months and included medical examination, physical joint evaluation, inhibitor testing and dosing evaluation.

Data collection

For the prophylaxis group, data were obtained from consecutive evaluation of the patients regularly attending hemophilia center from January 2010 to June 2011. All patients were randomly selected depending on their date of birth. In this group, age at start of prophylaxis and annual frequency of bleeding episodes were collected from treatment records at hemophilia center. The annual amount of CFCs (period January

2010 – December 2011) for each patient was retrieved from hospital pharmacy dispensing database.

For the on-demand group, patients were enrolled into the study from November 2008 to April 2009. Amongst LT patients, 16 were found to be age-matched with 16 DK patients according to our pre-set matching criterion of identical year of birth (+/- one year). Demographic characteristics such as year of birth, height and body weight, type and severity of hemophilia, treatment schedule (e.g. factor VIII or IX concentrate, doses, regimen, and home/clinic treatment), and inhibitor history were collected. Information was gathered on individual treatment doses and total annual amounts of CFCs consumed during January 2007 to December 2008. Observations were made concerning possible development of target joints, here defined as ≥3 bleeds in the same joint during any six months' period. Since systematic collection of bleeding episodes in the form of diaries had not been established in LT patients at the time of the study, no data on annual frequency of joint bleeds were available. The age at start of replacement therapy and home treatment was not collected because of unavailability of precise recordings.

4.3 Statistical analysis

Statistical analyses were performed using STATA version 10 (Stata Corp., College Station, TX, USA). Data are presented as means with standard deviation (SD) or medians with Interquartile range (IQR) or ranges, and percentages.

Satterthwaite's two-sample t-test with unequal variances was used for statistical comparison of the mean values of HJHS between age groups. The differences in mean values of the HJHS in elbows, knees and ankles were tested using one-way analysis of variance. Multiple comparisons were performed using Bonferroni test. The significance level was taken as 0.05.

Student's *t* test or the non-parametric Mann-Whitney *U* test was used for unpaired data. Pairs of matched patient scores were analyzed using a t-test for paired differences or the non-parametric Wilcoxon signed-rank test between treatment groups by two-sided approach at $\alpha=0.05$.

Other study data were analyzed using descriptive statistics.

5. Results

5.1 Demographic data and analysis of replacement therapy

On December 31, 2011, the overall number of patients aged 0-17 years diagnosed with hemophilia A and B in Lithuania was 48 (population of 3.2 million as in January, 2011) (59). The ratio hemophilia A/B was 41/7. Severe hemophilia A and B constituted 60% (28/48) of all cases (Table 1). The mean age of all patients was 10.3 years (SD 5.2, range 8 months – 17.4 years). Around half (48%) of all hemophilia cases were older then 12 years of age.

Table 1. Occurrence of hemophilia in children <18 years of age in Lithuania

	Severe	**Moderate**	**Mild**	**Total**
Hemophilia A	22	5	14	41 (85%)
Hemophilia B	6	1	0	7 (15%)
Total	28	6	14	48 (100%)
	60%	10%	30%	100%

At re-investigation of factor level in non-severe hemophilia A (n=20), five patients, all born between 1994-1999, displayed discrepancies between previous and current FVIII level. In four patients biochemical phenotype has shifted from moderate to mild hemophilia and in one patient from mild to moderate.

Inhibitor screening was performed in 31 out of 41 patients with hemophilia A. Three (13.6 %) out of 22 patients with severe hemophilia A have been registered with inhibitors against FVIII.

On December 31, 2011, in 87.5% of all hemophilia cases <18 years of age results of genotype were available. In hemophilia B, all individuals had missense mutations. In the case of severe hemophilia A, two inversion mutations (intron 22 and intron 1) were responsible for 50% and 4.5% of all cases, respectively. The remainder of mutations responsible for hemophilia A included a wide array of missense (71% of all mild hemophilia A cases), nonsense and insertion/deletion.

In Lithuania, the number of babies born and diagnosed with hemophilia each year from 1992 to 2011 was fluctuating from 0 to 8 with a mean of 3 (SD 1.9, 95% CI 2.1-3.9). The incidence rate between 1992-2011 was with a mean of 1.57 (SD 0.9) (CI 1.1-2.0, range 0-4) child with hemophilia born per year per 10 000 boys. It was fairly constant between this period except for the year 1996 (4/10 000) and 1999 (3.2/10 000) where the incidence was higher. The trend in incidence of hemophilia A and B in Lithuania is given in Fig. 1.

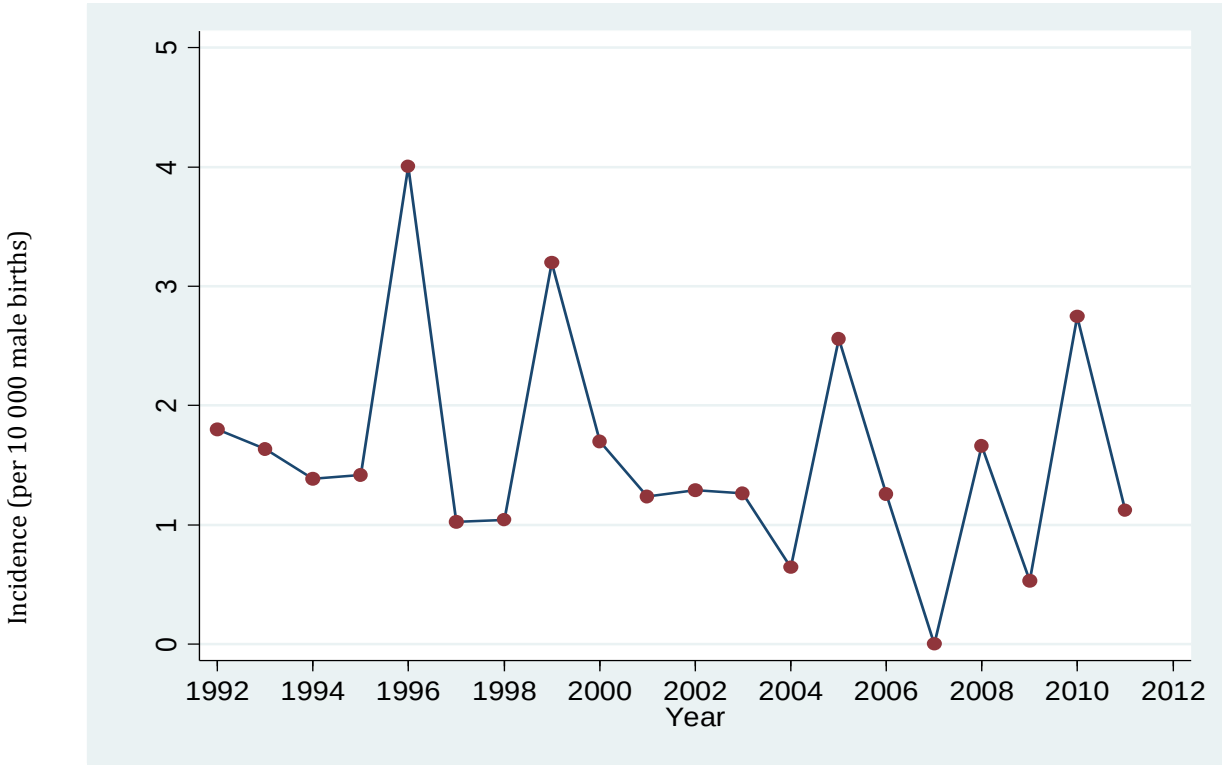

Figure 1. Trend in incidence of hemophilia A and B in Lithuania, 1992-2011

The prevalence of hemophilia for males 0-17 years of age in Lithuania in 2011 was 15.3/100 000. The prevalence in the age group 13-17 years was 7.34/100 000.

Treatment in all PWH <18 years of age was primarily given in response to a bleed (on-demand) until 2007. In 2007 the first patient with severe hemophilia A started primary prophylaxis. Since 2007 steady progress has been made in implementing prophylaxis for PWH <18 years of age. The number of severe hemophilia A patients receiving on-demand therapy each year from 2003 and 2008 ranged from 21 to 31. Median annual FVIII consumption among children 0-17 years with severe hemophilia A treated on-demand for the period 2003-2008 was 30 938 IUs (IQR 16 042-55 333). But the median annual FVIII use was different across the years with a tendency to increase every year since 2003 (Table 2). Over the six-year period from 2003 to 2008 median annual clotting factor consumption increased from 21 000 IU/year in 2003 to 50 250 IU/year in 2008: an increase by 140%.

Table 2. Reported median annual FVIII use (IUs per person with severe hemophilia A)

Year	Number of patients	Median	IQR	Min, max
2003	29	21 000	9 000–35 000	1 000–265 000
2004	30	23 125	11 500–42 500	750–165 500
2005	30	25 500	15 000–51 000	750–121 500
2006	31	29 000	12 000–60 500	1 500–157 500
2007	26	36 750	18 750–67 500	3 000–186 000
2008	21	50 250	30 000–75 500	11 250–187 500

IQR, interquartile range; min, minimum; max, maximum

The increase in CFCs consumption over this period was seen among all patients, however FVIII use was significantly different across the patients even of the same age. Distribution of total annual FVIII consumption per patient each year was strikingly different across the range of FVIII usage in the same type of patients, representing a few observations with a very high FVIII consumption each year (Fig 2).

An analysis by age group showed that starting from the age of 10 years and onwards, the difference in median annual FVIII consumption increased from approximately 1.5 to 2 times (Table 3). Persistent rising of factor consumption over the time in both age groups was distinct and from the 2005 onwards each year it was significantly higher in the group of ≥10 years of age ($p<0.05$). Median annual FVIII consumption for the studied period was comparable in the group of ≥10 years of age: 47 770 IU/year for boys of 10-12 years, 57 125 IU/year for boys of 13-15 years, 51 750 IU/year for boys of 16-17 years.

Table 3. Reported median annual FVIII use (IUs per person with severe hemophilia A) by age group

Year	Group <10 years Median (IQR)	Group ≥10 years Median (IQR)
2003	13 750 (10 750–21 000)	25 500 (7 500–39 000)
2004	21 000 (10 500–26 500)	29 000 (14 750–45 500)
2005	19 125 (1500–27 750)	40 500 (24 500–69 000)
2006	19 500 (6000–38 250)	34 500 (19 000–73 000)
2007	35 000 (9 375–49 250)	80 250 (27 000–109 500)
2008	43 500 (18 250–66 000)	89 000 (42 000–109 500)

IQR, interquartile range

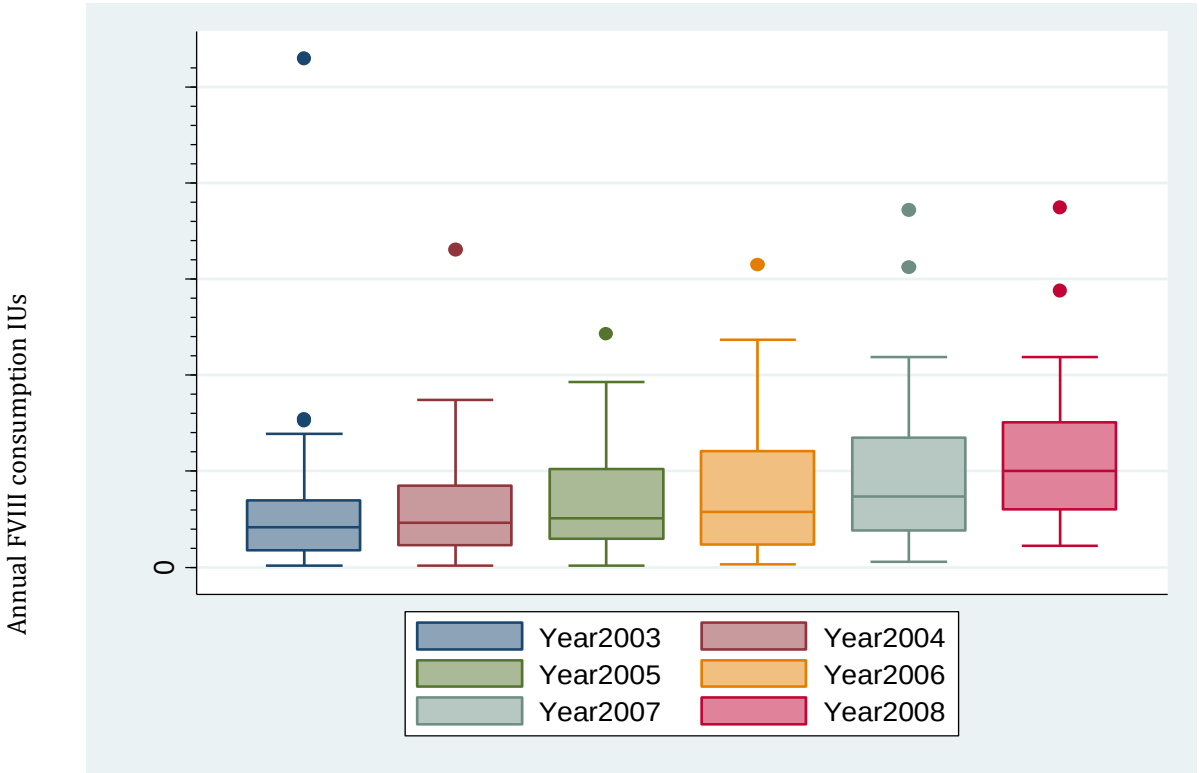

Figure 2. Annual FVIII consumption for the period 2003-2008. The boxes contain all values from the 25th to the 75th percentile (= interquartile range; IQR). The horizontal bar in the middle of the boxes is the median. The whiskers represent the 5th and 95th percentile and dots represent outliers. IUs, international units

Since the release of the region specific guidelines called "Baltic guidelines for the care and treatment of hemophiliacs" in the Baltic countries, when primary and secondary continuous prophylaxis has been recommended as the treatment of choice for boys with severe hemophilia A and B in Lithuania, start of important changes have occurred for hemophilia patients. From 2007 onwards, regular follow-up of the patients with careful documentation of the number of joint bleeds, and type of treatment received (i.e. prophylactic or on-demand), was introduced.

On December 31, 2011, the overall rate of use of some type of long-term prophylaxis treatment was 44% (21/48) for children <18 years of age with hemophilia A or B. When patients with severe hemophilia were considered, 71% of them were on the long-term prophylaxis: 40% (*n*=8) were on primary prophylaxis with a median age of 1.2 years (range 0.6-1.8 years) at the start and the rest 60% (*n*=12) received secondary prophylaxis, started at the median age of 6.2 years (range 2.3-15.9 years) (Fig. 3).

All severe hemophilia cases aged 0-12 years received long-term prophylaxis. Patients with severe hemophilia showed progressive increase in the use of long-term prophylaxis: from 3% in 2007 to 30% in 2009 and 71% in 2011 (Fig. 4 and 5).

Analysis of replacement therapy revealed that all previously untreated patients (PUPs) since 2009 were commenced on recombinant FVIII (rFVIII). Overall, recombinant CFCs were administered to 44% of patients, and plasma-derived products were used in 56% of cases. Rates of recombinant concentrate use were highest among the patients on prophylaxis (75%) *vs.* among those on on-demand (25%). Choice of clotting factor concentrates for patients on prophylaxis is presented in Fig. 6.

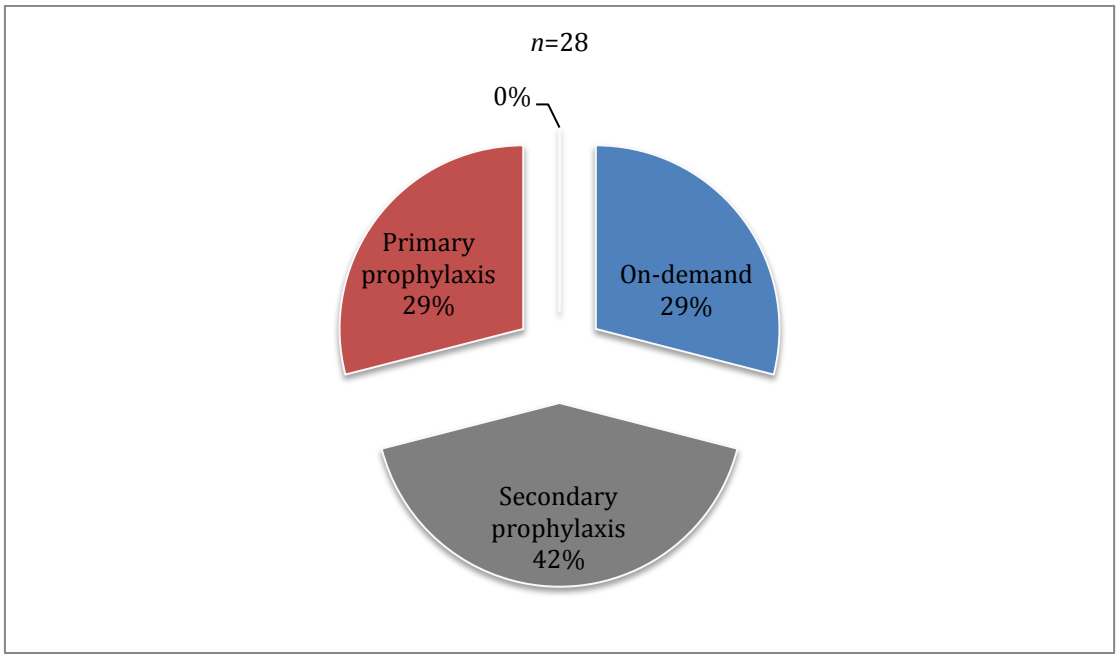

Figure 3. Treatment regimens in severe hemophilia A and B patients <18 years of age

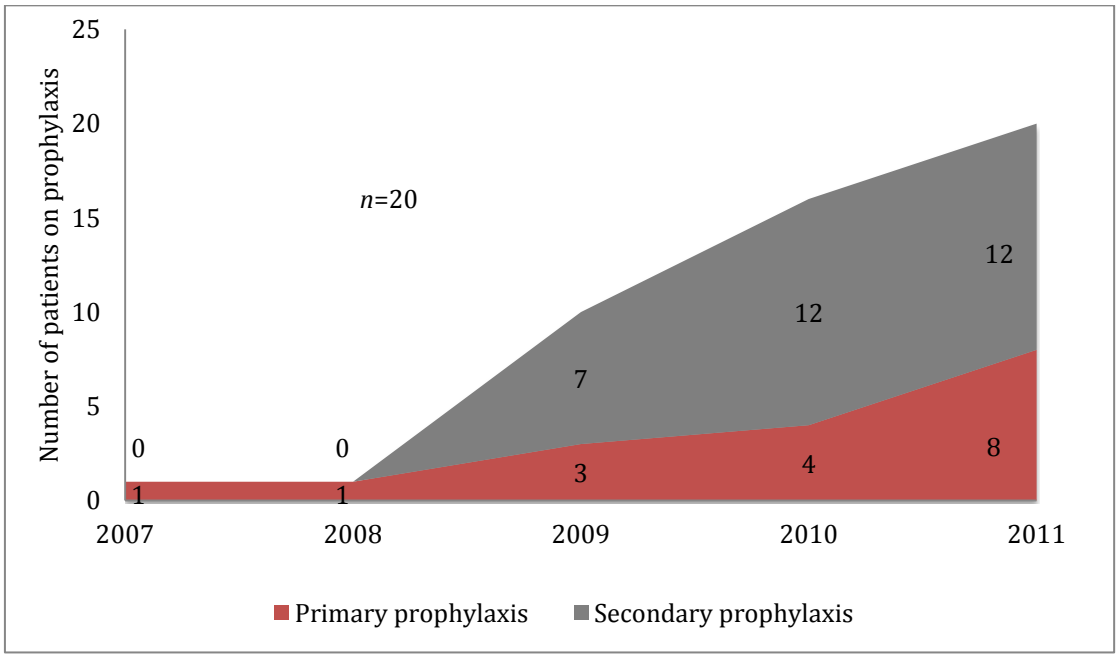

Figure 4. Expansion of long-term prophylaxis: adoption of long-term prophylaxis (primary or secondary) into clinical practice in children with severe hemophilia over the past five years

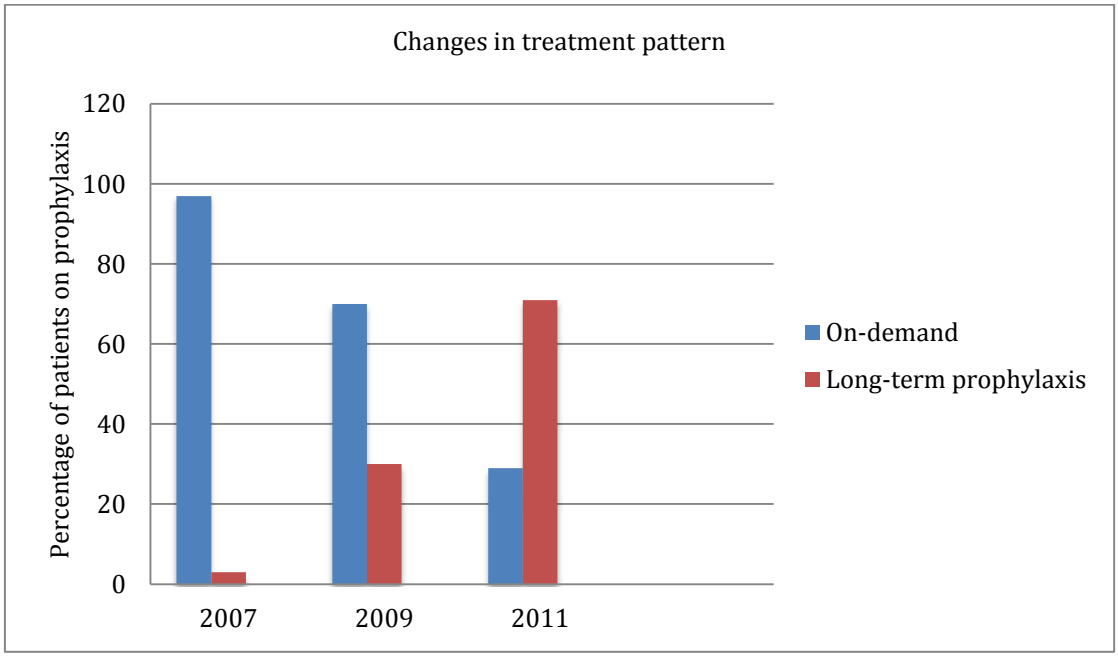

Figure 5. Treatment pattern in PWH <18 years of age in Lithuania over the past five years

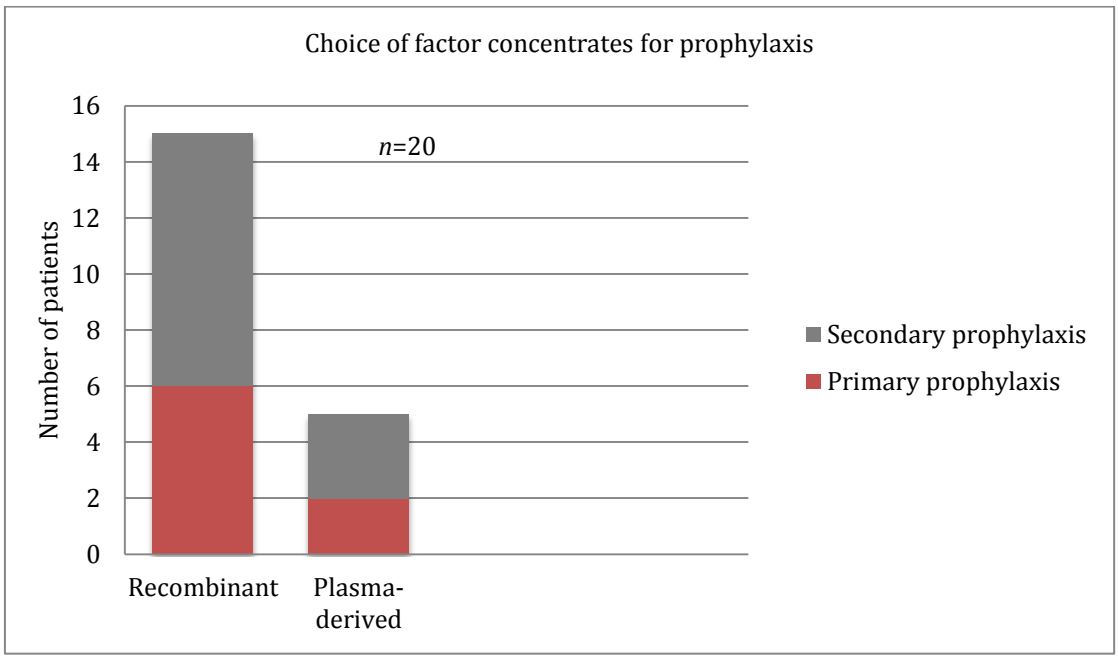

Figure 6. Type of clotting factor concentrate in PWH<18 years of age on prophylaxis

5.2 Utility of the HJHS

Twenty-one patients with congenital severe hemophilia A and B were enrolled in the joint assessment study. One patient was excluded from the analysis because of the detection of inhibitors to FVIII for the first time concurrent with the enrollment process. Hence, 20 patients constituted the study material. Of these, 18 were FVIII deficient (hemophilia A), and 2 were FIX deficient (hemophilia B). All individuals received replacement therapy on demand for their bleeding episodes from the time of diagnosis. Mean FVIII infusion dose was 25 (range 15-35) IU/kg and FIX 40 IU/kg body weight. The age of the study patients' ranged from 4 to 17.2 years, with a mean of 11.5 years (SD 4.3) and a median 12.5 years. There were 7 patients younger than ten years of age and 13 patients ten years of age or older. Accordingly, patients were subdivided into those of 4-9 years (group I) and those of 10-17 (group II) years. The baseline characteristics of the patients included in this analysis are presented in Table 4.

Table 4. Demographic characteristics of patients with hemophilia enrolled in the study

Characteristics	
Total patients	20
Mean age (years)	11.5
Range (years)	4-17.2
Patients <10 years of age	7 (35%)
Patients ≥10 years of age	13 (65%)
Severe hemophilia A	18 (90%)
Severe hemophilia B	2 (10%)

All six main joints and gait were evaluated separately using the HJHS. Mean total HJHS score for the six main joints and gait was 24.5 (SD 14.5, median 23.5) out of possible maximum of 124. It ranged from 5 to 50 and the score of 50 was identified in one patient. No patient had a total score of 0. Distribution of patients according to the HJHS obtained for all six joints and gait are presented in Fig. 7.

The most affected joints were ankles, followed by knees and elbows (Table 5). Elbows were the least affected joints and 25% of patients had a score of zero and the left elbow was found to unaffected in 45% of the study cohort having a score of zero for this joint on a scale varying from zero to 20. None of the patients had a zero score for both knees or both ankles. In the case of left knee and left ankle, none of the patients were scored at zero. The highest score of 16 per joint (maximum 20 per joint) was detected in the left knee in one patient. There was a significant variation between mean of the HJHS in elbows, knees and ankles ($p<0.001$). The mean scores for both elbows were significantly lower compared with both knees as well as both ankles (Table 5).

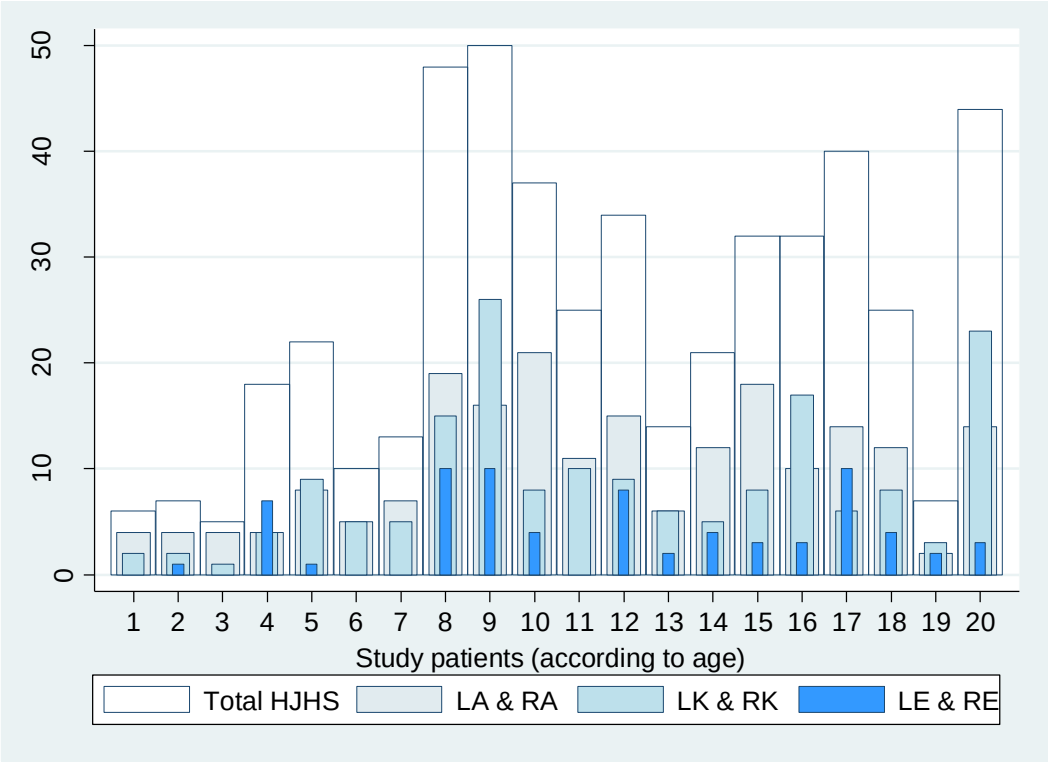

Figure 7. Distribution of the Haemophilia Joint Health Score (HJHS) scores according to age (patients nos from 1 to 7 are those 4-9 years of age, patients nos from 8 to 20 are those 10-17 years old). LA, left ankle; RA, right ankle; LK, left knee; RK, right knee; LE, left elbow; RE, right elbow

Table 5. Comparison of the Hemophilia Joint Health Score for ankles, knees and elbows

Mean (SD, range)			**Difference**	
Ankles	Knees	Elbows	(95% CI)	*p* value
10.3 (5.7, 2–21)	8.6 (6.7, 1–26)		1.7 (-1.0 to 4.4)	1
10.3 (5.7, 2–21)		3.6 (3.5, 0–10)	6.7 (4.5 to 8.9)	0.001
	8.6 (6.7, 1–26)	3.6 (3.5, 0–10)	5.0 (2.2 to 7.8)	0.02

SD, standard deviation; CI, confidence interval

Mean total HJHS score in group I (patients 4-9 years of age) was 11.6 (range 5-22) and in group II (patients 10-17 years of age) – with a mean of 31.5 (range 7-50) (Fig. 8). The mean total HJHS score was 19.9 points higher in group II compared to that of group I and the difference was statistically significant (Table 6).

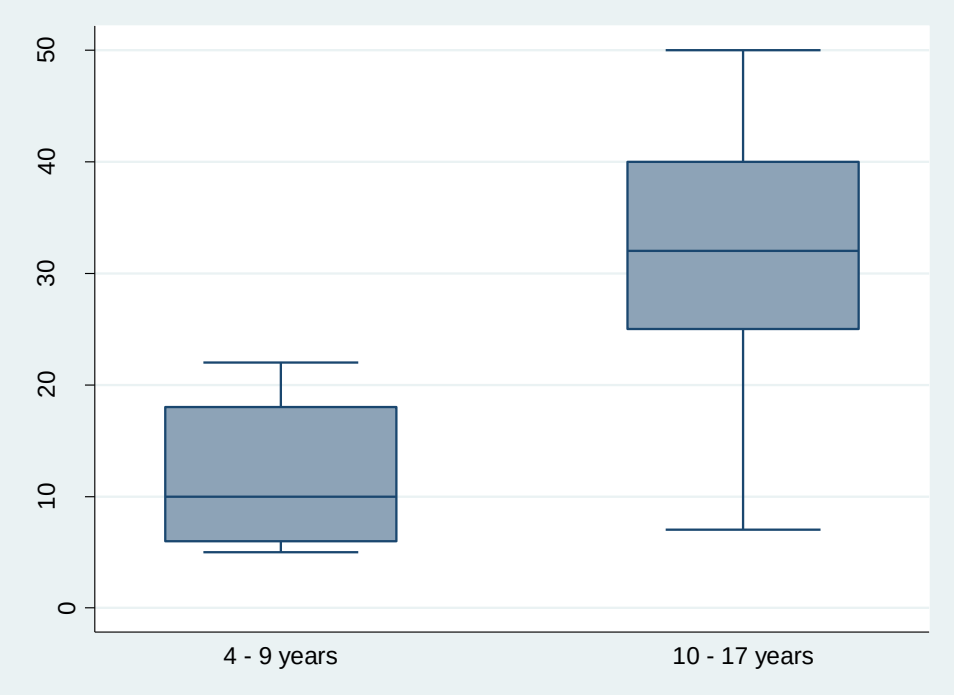

Figure 8. Distribution of the total Haemophilia Joint Health Score (HJHS) according to age

Table 6. Comparison of overall scores of the HJHS in two age groups

	Mean (SD)		**Difference**	
	Group I (*n*=7)	Group II (*n*=13)	(95% CI)	*p* value
Total (max=124)	11.6 (6.5)	31.5 (12.8)	19.9 (10.8 to 29)	0.0002
Elbows (max=40)	1.3 (2.5)	4.8 (3.4)	3.5 (0.7 to 6.4)	0.02
Knees (max=40)	4 (2.7)	11 (7)	7 (2.4 to 11.8)	0.005
Ankles (max=40)	5.1 (1.6)	13 (5.2)	8 (4.6 to 11.2)	0.0001

SD, standard deviation; max, maximum score; CI, confidence interval

An overview of the overall HJHS scores achieved for each age group for six major joints are also presented in Table 6. Both knees and both ankles were highly significantly worse in terms of the HJHS scores in older patients and the difference in elbow joints according to the age group was also significant. The lowest HJHS score in group II was 7 for a boy nearly 17 years of age (16 years 11 months), who presented with scores of 2 for left ankle and left knee and scores at 1 for each elbow, right ankle and right knee.

Home treatment was practiced by half of the study patients (*n*=10). The other half of the study patients or their family members had difficulties with venous access and could not infuse factor at home for their bleeds. These patients received in-clinic treatment, i.e. infusion of the factor at a health care providing institution. Musculoskeletal outcome in terms of the total HJHS scores comparing patients that had home treatment with those receiving in-clinic treatment did not result in detection of a significant difference.

5.3 Comparative study

Patients and treatment characteristics

In total, 32 (16 in each treatment group) patients were enrolled. Demographic characteristics of study subjects are provided in Table 7, showing a good comparability between the two treatment groups.

Table 7. Patient characteristics according to the primary treatment strategy

Patients	**On-demand (Lithuania)**	**Prophylaxis (Denmark)**
Patients (*n*)	16	16
Hemophilia type A/B	15/1	15/1
Age (years)	11.5 (4.2)	11.3 (3.9)
Weight (kg)	42.1 (18.5)	43.9 (20.0)

Values are means (SD) or numbers (*n*); SD, standard deviation

For the on-demand group, median clotting factor dose administered in response to a bleed was 20 IU kg (IQR 16-30). Half of the patients were on home therapy the remaining 50% visited a clinic to receive factor infusion. Median annual clotting factor consumption was 1179 IU kg year (IQR 609-1848).

In the group treated with prophylaxis, all patients received treatment from a median age of 1.4 years (IQR 0.9-2.2). The frequency of infusions varied from daily to three times per week at a median dose of 23.5 IU kg. Most patients had been administered factor concentrate doses ranging from 21.5 to 32.5 IU kg (IQR), however, individual doses ranged from 7 IU kg (minimum) given in daily infusions to 45 IU kg (maximum) three times per week. Median annual clotting factor consumption was 4500 IU kg year (IQR 3319-7157). Median number of joint bleeds per year was 0 (IQR 0-1.5). All patients were on home treatment.

Outcome – joint status

In total, 192 joints in 32 patients were evaluated. Joint outcome based on the HJHS score was analyzed in two different treatment groups and compared within the matched pairs.

On-demand group. Mean total (global 6-joint and gait) HJHS was 27.4 (SD 14.5). The HJHS for each joint pair was highest for the ankles at mean 11.4, followed by knees at 9.4 and elbows with a mean score of 4.2 (Fig. 9). None of the patients had a zero score for both knees and ankles. The proportion of joints with zero scores for both elbows was 18.7 %. The mean scores for both elbows were significantly lower compared to both knees and ankles (Table 8).

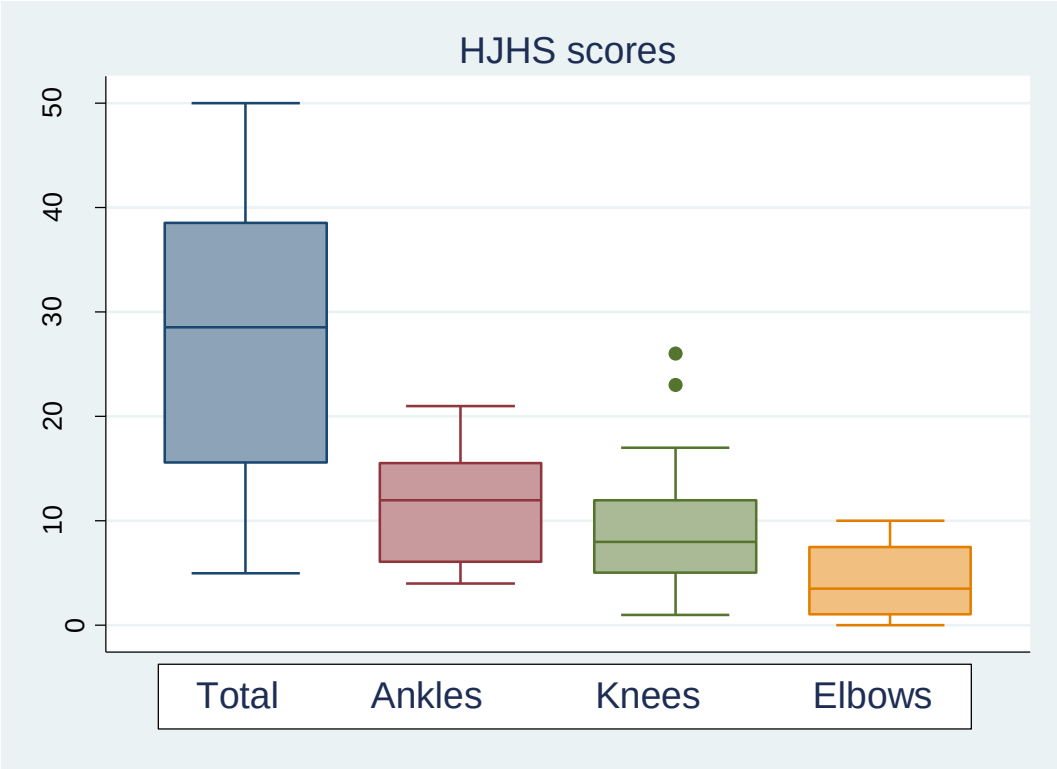

Figure 9. HJHS total score and for the ankles, knees and elbows. The boxes contain all values from the 25th to the 75th percentile (= interquartile range; IQR). The horizontal bar in the middle of the boxes is the median. The whiskers represent the 5th and 95th percentile and dots represent outliers. HJHS, Hemophilia Joint Health Score

Table 8. Comparison of the HJHS for the ankles, knees and elbows in the on-demand group

Mean (SD)			**Difference**	
Ankles	Knees	Elbows	(95% CI)	*p* value
11.4 (5.6)	9.4 (7.2)		2.0 (-1.5 to 5.5)	0.24
11.4 (5.6)		4.2 (3.7)	7.2 (4.7 to 9.7)	< 0.0001
	9.4 (7.2)	4.2 (3.7)	5.2 (1.7 to 8.7)	0.006

SD, standard deviation; CI, confidence interval

Patients younger than 10 years of age (4-9 years, group I) had lower HJHS scores than patients 10 years of age and older (10-17 years, group II). The mean total HJHS score was 23.8 points higher in the group II compared to that of group I. An overview of the overall HJHS scores achieved in each age group for six major joints are presented in Table 9. All pairs of joints were highly significantly worse in terms of the HJHS scores in the older subset of patients.

Table 9. Comparison of the overall HJHS scores for the on-demand group in two age groups

	Mean (SD)		Difference	
	Group I (*n*=6)	Group II (*n*=10)	(95% CI)	*p* value
Total (max=124)	12.5 (6.5)	36.3 (9.4)	23.8 (15.2 to 32.4)	<0.0001
Elbows (max=40)	1.5 (2.7)	5.9 (3.2)	4.4 (1.1 to 7.7)	0.01
Knees (max=40)	4.3 (2.8)	12.5 (7.4)	8.2 (2.5 to 13.8)	0.008
Ankles (max=40)	5.3 (1.7)	15.1 (3.4)	9.8 (7.0 to 12.6)	<0.0001

SD, standard deviation; max, maximum score; CI, confidence interval

Prophylaxis group. Mean total (global 6-joint and gait) HJHS was 3.3 (SD 2.3). The HJHS for each joint pair was comparable for the ankles at mean 1.25 (SD 1.9) and knees at 1.5 (SD 2.2). The lowest score was for the elbows at mean 0.4 (SD 0.8) (Fig. 10). The proportions of joints with zero scores for the ankles, knees and elbows were 56.2, 31.2 and 68.7%, respectively. Components that most often scored were: mild crepitation on motion (slightly audible/or palpable) in 24 joints, of which 17 joints (71%) were knees. In half of the patients (*n*=8, 15 joints) crepitus on motion was the only component resulting in total HJHS score above 0.

HJHS scores for patients less than 10 years of age (4-9 years, group I) did not differ significantly from those of patients 10 years of age and older (10-17 years, group II) with a mean of 2.2 and 3.9, respectively (Table 10).

Table 10. Comparison of the overall HJHS scores for the prophylaxis group in two age groups

	Mean (SD)		Difference	
	Group I (*n*=6)	Group II (*n*=10)	(95% CI)	*p* value
Total (max=124)	2.2 (1.5)	3.9 (2.5)	1.7 (-0.4 to 3.9)	NS
Elbows (max=40)	0.8 (0.9)	0.2 (0.6)	0.6 (-0.4 to 1.6)	NS
Knees (max=40)	0.5 (0.8)	2.1 (1.7)	-1.6 (-2.9 to 0.25)	0.02
Ankles (max=40)	0.8 (1.3)	1.5 (2.2)	-0.7 (-2.5 to 1.2)	NS

SD, standard deviation; max, maximum score; CI, confidence interval; NS, not significant

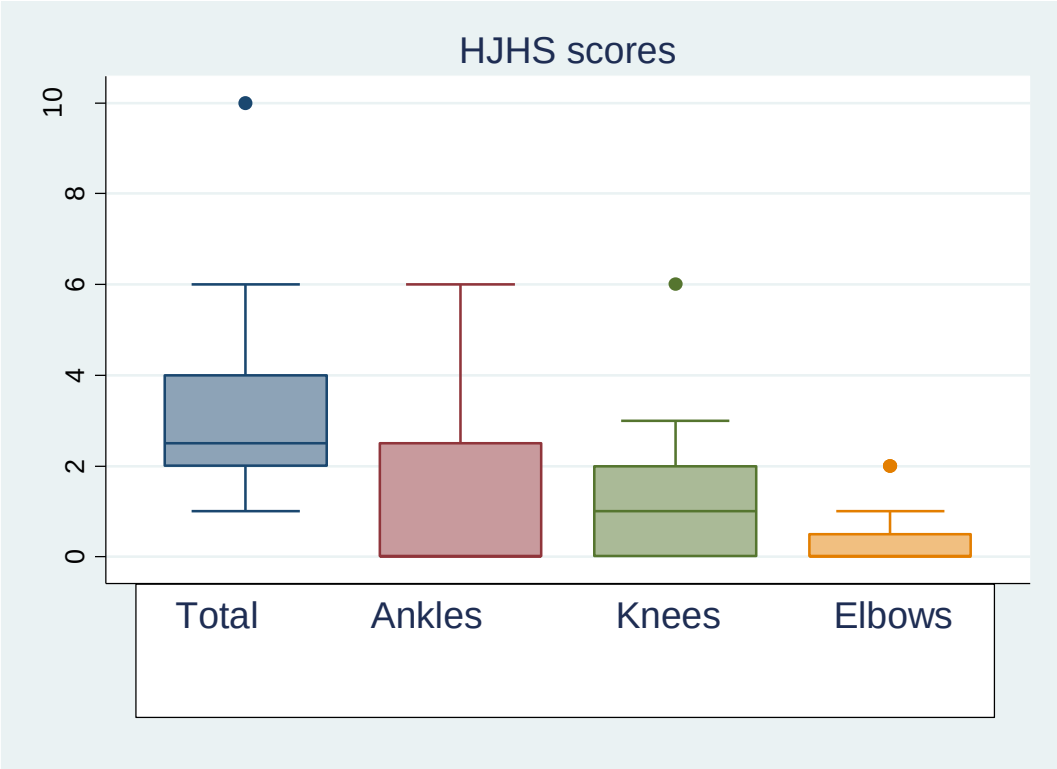

Figure 10. HJHS total score and for the elbows, knees and ankles. The boxes contain all values from the 25th to the 75th percentile (= interquartile range; IQR). The horizontal bar in the middle of the boxes is the median. The whiskers represent the 5th and 95th percentile and dots represent outliers. HJHS, Hemophilia Joint Health Score

Comparison between different treatment groups. Joint status according to the treatment strategy is presented in Table 11. Compared to the patients who were on prophylaxis, patients who were treated on-demand had significantly higher scores based on the mean total HJHS (Fig. 11). Difference between two treatment strategies was seen from early age: both patients in group I (4-9 years) and group II (10-17 years) had significantly better joint scores with the preventive treatment than those in on-demand group.

Table 11. Outcome according to the primary treatment strategy

	Mean (SD)		**Difference**	
	On-demand	Prophylaxis	(95% CI)	*p* value
HJHS (max=124)	27.4 (14.5)	3.3 (2.3)	24.1 (16.7–31.6)	<0.0001
HJHS *group I* (*n*=12)	12.5 (6.5)	2.2 (1.5)	10.2 (4.0–16.3)	0.0002
HJHS *group II* (*n*=20)	36.3 (9.4)	3.9 (2.5)	32.5 (25.4–40.0)	<0.0001

SD, standard deviation; max, maximum score; CI, confidence interval

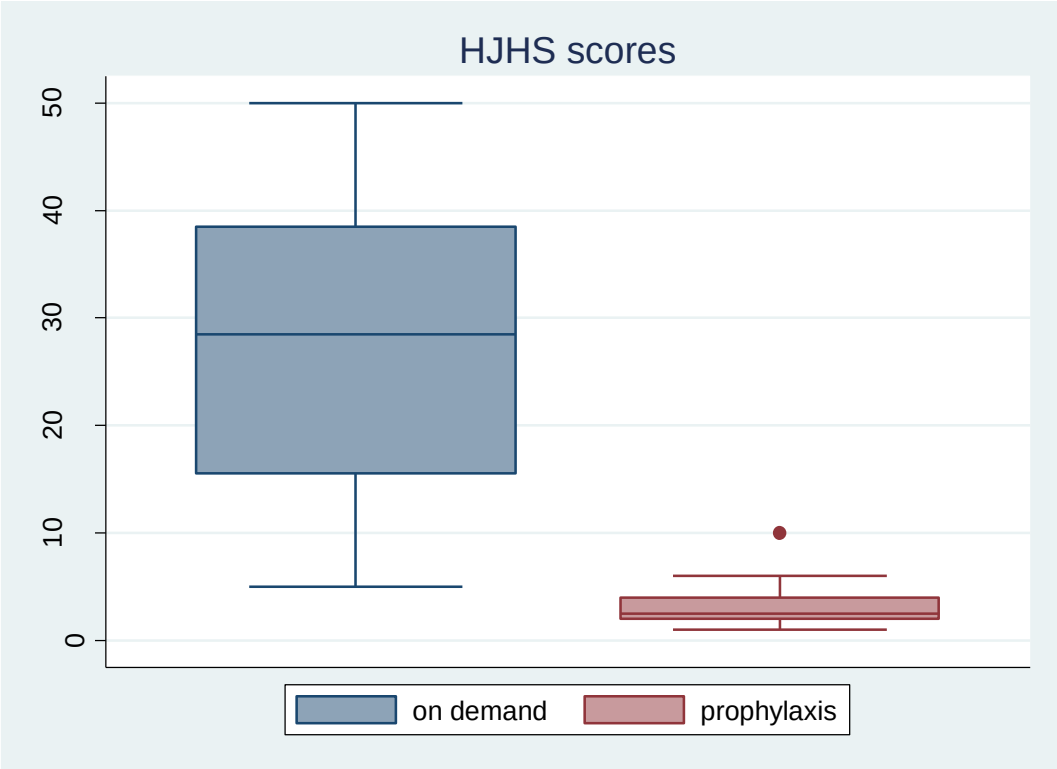

Figure 11. HJHS total score in different treatment groups. The boxes contain all values from the 25th to the 75th percentile (= interquartile range; IQR). The horizontal bar in the middle of the boxes is the median. The whiskers represent the 5th and 95th percentile and dots represent outliers. HJHS, Hemophilia Joint Health Score

The presence of a target joint was reported in five out of 16 Lithuanian matched patients, while none amongst 16 Danish patients except for one historical target joint.

6. Discussion

6.1 Demographic characteristics and treatment changes

The intention of this thesis was to estimate the past, realize the present and plan the future of hemophilia care in Lithuania. To plan and organize comprehensive hemophilia care requires a thorough knowledge of the basic demographic data concerning the hemophilic population. National hemophilia databases, including data on prevalence, treatment and outcome, have been established in many countries including UK, Italy, Germany, France, Spain, Australia, Canada, and the USA (60). Despite to the way hemophilia care was organized in Lithuania, it is likely that all cases were put on the list in separate healthcare institutions along with LHA, which helped us to make a joint final list of good quality and provide basic information regarding total numbers of PWH <18 years of age in Lithuania. The overall number of recognized cases of hemophilia <18 years of age in Lithuania was fairly steady from 2008 to 2011 ranging from 45 to 50. However, despite all efforts, there is a possibility, that patients who were not diagnosed until the end of 2011 or do not require treatment were not counted. Data from

the US showed that by 15 years of age, over 95% of PWH of all severities had been diagnosed and by age above 30 years, almost all were diagnosed (61).

The allocation of PWH into severities was based on laboratory determination of the plasma level of FVIII or FIX. The variation in the proportion of severe hemophilia found in the literature is great. Our series showed a proportion of severe cases of 60%, which is similar to that reported in the Polish (62), US (63), Italian (64) and most recently Japanese (65, 66) studies. These figures are significantly lower in other published series of patients from Canada (67, 68), Sweden (69), the Netherlands (70), Greece (71), Spain (72), UK (73) and Western Cape (74). A high proportion of severe hemophilia is sometimes explained by a low detection rate of mild cases of PWH, which is not excluded in our study. Despite the fact that the proportion of mild cases increased from 15% in 2008 to 30% in 2011, it is not a result of greater awareness of the disease by medical personnel but because of re-classification of non-severe hemophilia cases possibly resulting from the effect of newer and improved laboratory techniques today comparing to that of the past when the diagnosis of hemophilia was made.

The observed ratio of hemophilia A to B was 5.9:1, similar as to the reported in studies from Poland (6.2:1) (62), Greece (6.6:1) (71), Spain (6.5:1) (75), Portugal (6.6:1) (76), but higher than in Sweden and Scotland (4:1 and 3:1, respectively) (69, 77).

Frequencies of FVIII mutation types by mutation type and severity in Lithuania was comparable as it is reported in Germany (78), US hemophilia A patients (79) and other countries. In severe hemophilia A, the most prevalent mutation was intron 22 inversion (50% of the mutations) while in non-severe hemophilia A cases almost all gene defects were missense mutations (71% of all mutations). In hemophilia B, inversion mutations were not reported in our cohort. All individuals had missense mutations as it is reported in the literature that null mutations are much less common in hemophilia B than in hemophilia A (80).

In the current study, inhibitors towards FVIII were present in 13.6% (n=3) of severe hemophilia A cases, i.e. 7.3% of all registered hemophilia A patients up to 18 years of age. These results do not differ significantly from those reported in the UK, France and Holland (70, 73, 81). It is interesting that the same number – only three hemophilia A patients with an inhibitor have been registered in a study by Ivaskevicius *et al*. in 1998 in Lithuania (30), however it corresponded to a lower inhibitor prevalence of 6% (3 out of 50) in severe hemophilia A. The prevalence of inhibitors to FIX in severe hemophilia B Lithuanian patients remains unknown until the availability of laboratory facilities to detect them.

Such simple demographic variable as prevalence would be of value, however, without an accurate registry, it is very difficult to know the exact total number of PWH in Lithuania, therefore we could not describe actual (true) prevalence of hemophilia except the prevalence among people of 0-17 years age. According to the WFH report on the annual global survey 2010, the prevalence in our country was reported of 9.7 PWH per 100 000 males or 4.5. per 100 000 population (82). The hemophilia A and B prevalence separately per 100 000 males from 1998 to 2006 in Lithuania was provided by other recent studies evaluating variations of hemophilia prevalence around the world (Table 12) (83, 84), however, these data were also based on the WFH annual global surveys where the prevalence data provided could be of variable quality (discussed later).

Table 12. The reported hemophilia A and B prevalence (per 100 000 males) determined from the reported number of patients with hemophilia A and B in Lithuania from 1998-2006 divided by its male population in the relevant year (83, 84)

Hemophilia	1998	1999	2000	2001	2002	2003	2004	2005	2006	Mean	SD	CV
A	5.6	5.8	6.9	6.8	NA	6.9	NA	8.0	8.1	6.9	0.9	14%
B	1.02	0.97	1.10	1.11	NA	1.24	NA	1.25	1.32	1.14	0.13	11%

SD, standard deviation; CV, coefficient of variation; NA, not available

According to both of these studies results, the prevalence in the high-income countries was significantly greater than in the other economic classifications. The mean prevalence of hemophilia A (per 100 000 males) for high income countries was 12.8 (SD 6.0), whereas it was 6.6 (SD 4.8) for the rest of the world (83). The hemophilia B prevalence (per 100 000 males) for the highest income countries was 2.69 (SD 1.61), whereas the prevalence for the rest of the world was 1.20 (SD 1.33) (84). Reported prevalence rates, based primarily on cases identified through contact with specialized hemophilia treatment centers, in older studies by other countries, ranged from 8.2 to 20.5 cases per 100 000 males (64, 69, 71, 73, 85). Indeed, in reality prevalence of hemophilia varies widely from country to country for several reasons. One of them is a national registry, because the data from countries that use NHR provide higher quality prevalence data than the other data sources (83). As our country does not have a NHR, the reported data from Lithuania might include variations in the completeness of case finding. Therefore, compared to high-income countries, in Lithuania persons with non-severe hemophilia might be not diagnosed, falsely lowering the identified prevalence of hemophilia. Furthermore, as persons in normal population have lower life expectancy than in high-income countries, PWH in Lithuania might have even shorter life span (mean male life expectancy at birth at the beginning of 2011 was 68.5 years) (59), thereby further reducing hemophilia prevalence in our country. The estimated age-specific prevalence of hemophilia in Lithuania in 2011 in our study was 15.3 cases per 100 000 0-17 year-old males and might be declined with the age. This estimate is comparable to the recently reported both hemophilia A and B prevalence in high-income countries of around 15.5. per 100 000 males. Higher prevalence is associated with a smaller percent of PWH in the 0-17 years age groups as more PWH survive into adult life, which seems not the case in our country. However, at the time of the study, there were 149 records of the known PWH in Lithuania (Klaipeda Seamen's Hospital database), of which approximately one third were children <18 years of age (*n*=48), which suggested that we did not loose adults in a population because of early death. Hopefully, more coordinated structure of hemophilia care in our country, especially for adults, might increase the numbers of hemophilia patients living to adulthood.

However, despite not very well coordinated hemophilia care in Lithuania, like in other countries, there was a strong trend of increasing both hemophilia A and B prevalence over time (Table 12). Higher prevalence over time could reflect improved diagnosis, improved access of care for hemophilia, increased survival and improved data collection and reporting. Based on the reported prevalence of hemophilia A (83) which ranges from 6.6-12.8 per 100 000 males, the number of known patients in Lithuania as of December 31, 2011 was equal to 67% (*n*=129, Klaipeda Seamen's Hospital database) by applying the higher prevalence figure or more than 100% by applying the lower prevalence figure of the expected number for Lithuania. By using the same reported prevalence figures for hemophilia B (84) , i.e. 1.2-2.69 per 100 000 males, the number of known hemophilia B cases in Lithuania (*n*=20, Klaipeda Seamen's Hospital database), is approximately to 100% as the expected number of cases by applying the lower prevalence figure, however, only about 50% by applying the higher prevalence figure, suggesting that on a country-wide basis the identification of hemophilia B is poor. If the prevalence in Lithuania were equal to that of reported in high-income countries, rough estimates of the expected numbers of hemophilia would then be 193 hemophilia A patients and 40 hemophilia B patients, i.e. 233 in total. Calculation of overall hemophilia

prevalence in our country according to the WFH formula (86) gives a total number of 215 PWH in Lithuania.

The WFH annual global surveys, on which the only documented data on the prevalence of hemophilia in Lithuania are based, have some limitations, because the numbers in these reports are as reported by the members of the National Hemophilia Societies in the country and they are not independently verified by the WFH. However, despite its limitations, the WFH global database is the most robust, comprehensive and best available source of worldwide hemophilia data (87), which help address this lack of documented data and evidence in Lithuania. Prevalence data are extremely valuable information for the planning of national healthcare priorities and allocating resources for the treatment of hemophilia. Furthermore, as it was stated in 1997 meeting of the WHO and the WFH, the NHR is an essential step for improving care and lives of PWH (83). While there remain challenges in collecting reliable prevalence data in Lithuania, we believe that considerable progress has been made by collecting, recording and reporting both quantity and quality of the basic data of children <18 years of age in Lithuania.

Hemophilia incidence has been widely cited as 1 in 5000-10 000 male births (88) and considered to be the same for all populations and racial groups (54, 89). The yearly incidence of hemophilia in Lithuania appears to have been relatively variable for the last 20 years, with a mean of 1.57 per 10 000 or 1 in 6369 live-born males. Few data have been published regarding the incidence of hemophilia in other countries. In 1982, the reported incidence in the Swedish PWH has remained fairly constant since 1957 and was 1.67 per year per 10 000 males born (69). In contrast, the incidence of both hemophilia A and B was increasing in Italy from around 1 per 10 000 male births for the period 1952-1961 compared to 1.5 in 1982-1987 (64). The average incidence of hemophilia A and B in the hemophilia surveillance system in the US for the 10-year period 1982-1991 was estimated to be 1 in 5032 live male births (88). Our numbers are comparable with the reported incidence estimates from other countries, however, in estimating the incidence in our population one has to account for underestimating cases of delayed diagnosis.

This study provides an extensive assessment of hemophilia treatment practice patterns in patients 0-17 years of age in Lithuania for the period 2007-2011. At the time of the start of the study, all PWH in Lithuania received replacement therapy on-demand for their bleeding episodes from the time of diagnosis (discussed later). However, since the advent of the region specific guidelines for the treatment and care of PWH in 2007, treatment of hemophilia in children <18 years of age has changed enormously. It has focused on the use of long-term preventative treatment and resulted in an expansion of the use of long-term prophylaxis in approximately two-thirds (71%) of individuals with severe hemophilia during the last five years (2007-2011). The use of recombinant FVIII concentrates was widespread in patients on prophylaxis.

The target prophylaxis rate for pediatric patients with severe hemophilia should ideally be 100%, but this goal is not easily achieved not only for economic reasons, but also for various other reasons: regular venipuncture is difficult for very young children, prophylaxis start can be delayed in families from a low social and cultural background and also some families can be reluctant to start treatment in children who remain asymptomatic. Despite convincing results on superiority of prophylaxis versus on-demand treatment both regarding joint disease (13, 21, 22) and QoL (90), results from different studies confirm wide inter-country variations regarding the availability of

prophylaxis for PWH among countries, even within western Europe, that have close economic, social and cultural relationship. According to a PedNet survey representing the situation in the late 1998 of 20 HTCs in 16 European countries, only 39% of boys (0-18 years) with severe hemophilia received primary prophylaxis, 40% various forms of secondary prophylaxis and 19% on-demand therapy, with 2% of the children remaining as yet being untreated (91). Five years later, this survey has been updated and described changing pattern of care of boys with severe hemophilia in western Europe with regular, continuous long-term prophylaxis being provided in more than 50% and 80-100% of boys being treated this way in 20/22 and 15/22 of PedNet centers, respectively (92). In a survey conducted in 2001 in the Netherlands, 86% of children aged 0-16 years and 90% of adolescents aged 17-25 years with severe hemophilia were reported to be on prophylaxis (70). Data from the Spanish Epidemiological Study in hemophilia carried out in 2006, showed that 71.5% of severe hemophilia A pediatric patients received prophylactic treatment (40.5% primary prophylaxis and 55.4% secondary prophylaxis) (72, 75). A survey conducted in 2009 in 19 European countries revealed these wide variations in the percentages of children receiving prophylaxis between 'none' and up to '76–100%' (28). Blanchette *et al.* (68) reported joint data from American and Canadian pediatric patients where 77% of severe hemophilia A patients in the age group of 0-18 years were on some sort of prophylaxis treatment consistent with 71% from our recent study.

It seems that since publication of region specific guidelines and results of prospective RCT, the situation of hemophilia treatment and care has improved in Lithuania and Lithuanian patients, more specifically individuals with severe cases, are at the same level as most developed countries with no factor limitations regarding the numbers of patients on the long-term prophylaxis. In addition, as the concept of prophylaxis took several years to be perceived and adopted in our country, this statistics also reflect an increasing awareness of the benefits of factor prophylaxis and prevention of joint disease by patients themselves. However, despite overall, prophylactic treatment could be available for all children <18 years, it has not been adopted by all children with severe hemophilia. Finally, other important standards of hemophilia care (27) are not generally applied in Lithuania: aspects of centralization, national organization of care, use of registries.

The level of health care provided to patients with hemophilia is primarily related to the availability of CFCs to replace congenitally defective coagulation factors, which has a great effect on both the level of disability and life expectancy of PWH (93). As most of the CFCs are used by patients with severe hemophilia, it is important to use the most efficient treatment regimen for these patients. However, the median annual FVIII use in our cohort of patients with severe hemophilia treated on-demand was different across the years and confirmed the great variability in annual factor concentrate consumption by patients on the same treatment regimen found by previous studies (10, 16, 94). In addition, FVIII consumption was persistently increasing over time, which is in accordance from the worldwide survey on usage of CFCs that was reported as increasing since 1999-2000 (95). Over the six-year period from 2003 to 2008 median annual clotting factor consumption in our study cohort increased by 140%. The reported annual FVIII consumption in severe hemophilia patients with a mean age of 22-26 years treated on-demand during the period of 2000-2006 in other studies ranged from 72 000 to 110 000 IU/year (62, 96, 97). However, it was lower in the earlier studies for the

period 1989-1999: 55 000 IU/year (IQR 28 000-91 000 IU) (94). In our cohort median annual FVIII use in IUs per person with severe hemophilia A was 21 000 IUs in 2003 and it means that we reached 1 IU per capita (approximately the 20 000 IUs per patient which the WFH suggested as a minimum clinical target for hemophilia care) at that time (95). In 2001 it was reported to be only 13 063 IUs in our country (98). In 2008 median annual FVIII use in Lithuania increased by 140% which was reported as 50 250 IUs, however, the amount of IUs per patient per year was very different even among the patients of the same age. In some patients it reached IUs/year similar as for prophylaxis (i.e. 157 500-265 000) (99). This could reflect better availability of FVIII concentrates, improved perception of the disease and the overall improvement of the quality of care of PWH in Lithuania. In addition, factor usage above the level of 20 000 IU may produce other benefits, for example, decreased complications from joint disease (95), however, our study could not evaluate the effect of clotting factor consumption on clinical outcomes because of the lack of documented data on annual frequency of joint bleeds or joint scores at that time. However, as arthropathy, measured by the HJHS was apparent in a cohort of patients treated on-demand, we can speculate that there was no significant improvement in joint health over the time despite increasing use of clotting factors. Moreover, we found few patients with reported very low annual factor concentrate consumption levels in some years that sometimes deviated considerably from what would be expected (given the age and the weight of the patient). These observations probably could be explained by the lack of coordinated hemophilia care in our country that could result in the use of too low FVIII dose per kg, lack of compliance and lack of awareness of the disease.

Furthermore, wide variations in FVIII usage might also indicate the lack of evidence-based practice with regard to on-demand treatment strategy (100). How can the use of CFCs be optimized to maximize clinical outcomes and patient QoL? Can we expect to gain additional benefits having more intensive treatment on-demand on joint status and, if yes, to what extent? A major difficulty in this process, however, is the lack of adequate data correlating long-term musculoskeletal outcome with dosage, which might not exist giving on-demand treatment strategy.

As the treatment of hemophilia is very expensive, further analysis might help to understand differences in FVIII consumption patterns among the persons treated on-demand and identify the consumption's associated factors. In contrast, as it is well documented that there is the only evidence-based treatment for children preventing from the development of arthropathy (prophylaxis) (13, 22), despite it is more expensive for pediatric patients (16, 101), probably it would be more reasonable to adopt prophylaxis for all children in our country and have predictable consumption of CFCs per year. There is evidence of longitudinal data that annual clotting factor consumption per kg bodyweight for prophylactic treatment is highest in very young children at initiation of treatment, decreasing until the age of 20 years and stabilizes in adulthood (6, 20, 102). The decreasing clotting factor use with age in patients using primarily prophylactic treatment might be caused by the increase of clotting factor half-life with age (103), preservation of joints and different (more quiet) lifestyle compared to children. In contrast, clotting factor use may continue to increase in patients who do not receive prophylaxis during early years of life. This increasing of clotting factor use with age in patients treated on-demand may be due to the higher level of arthropathy, as clotting factor consumption has been shown to increase with increasing joint damage (104). In

addition, cost-effectiveness can only be evaluated in adult patients, as the expected benefits of preventing blood-induced joint damage in hemophilia become apparent later in life, i.e. in adulthood.

Evaluation of factor consumption in our study has some limitations. It includes the lack of standardized collected data on factor consumption per patient in hospital. We had to rely on data from the National Health Insurance Fund, despite some information from hospital setting may be missing. An independent data source of factor consumption per patient per year would be valuable in order to get further insights into the accuracy of its use. In conclusion, accurate database and analysis of FVIII and FIX consumption could be valuable information not only to physicians but also to budget decision makers of our country to improve level of hemophilia care according to the European Principles (27).

6.2 Utility of the HJHS in boys treated on-demand

Measurement of the musculoskeletal condition applying standardised physical joint-assessment tools, such as HJHS, is critically important in continuous surveillance of individuals with hemophilia, especially in children. Data are important in describing the signs of chronic changes related to recurrent joint hemorrhage over time and evaluating as well as comparing the efficacy of various treatment principles (21, 105).

The purpose of this study was to investigate and characterize musculoskeletal damage in severe hemophilia using a new physical scoring tool in Lithuanian boys treated exclusively by on-demand treatment practices. Additionally, the intention of the study was to investigate the progression of hemophilic arthropathy during childhood and puberty with particular focus on the age of remarkable changes based on the HJHS scores. The HJHS is a recently developed international scoring tool for assessing joint impairment in boys with hemophilia from 4 to 18 years of age. This new measure was developed by the multinational expert groups (42) with the goal of picking up the subtle early signs of joint damage and also illustrate the differences among patients with mild, moderate, or severe hemophilia. The new instrument was also considered appropriate for screening children on prophylactic treatment as well as on-demand regimens.

Our study utilized the HJHS version 2.0 in patients with severe hemophilia who had been treated exclusively on-demand. As the HJHS is quite a new international instrument for the assessment of joints in children with hemophilia, this study provided the first set of data on musculoskeletal assessment scores using the HJHS in patients receiving exclusively on-demand treatment.

As it mentioned before, one of the goals of this new instrument was to screen children on both prophylactic treatment and on-demand regimens, however, exact scores or range of scores that characterize patients in each of these groups have not been determined as yet. The mean total HJHS score in our study cohort was 24.5 with a range from 5 to 50. The interpretation of these scores today is possible only by comparing the HJHS scores with the published data from several studies quantifying musculoskeletal damage in hemophilia patients using the HJHS instrument in patient cohorts who were typically treated with prophylaxis (Table 13).

The first published data on the six-joint HJHS scores using the HJHS version 1.0 comes from the HJHS reliability study (42). This study reported on eight boys on prophylaxis treatment with mild-to-moderate or severe clinical signs of joint damage, and mean HJHS of 15, ranging from 3.5 to 35, on day 1 and 14, ranging from 2 to 27.5, on day 2 out of possible maximum of 148 in the HJHS version 1.0. Three out of four boys >8 years of age in this study had the median scores above 20 (range 24-35) whereas for boys <8 years of age the median scores ranged from 2 to 12. Despite the HJHS version 1.0 having a bit different scoring from the HJHS version 2.0, each of the six index joints were numerically scored from zero to 20 by both versions. Given the fact that in Lithuania practically all children with severe hemophilia received replacement treatment with FVIII and FIX on-demand (93.4%) that does not prevent spontaneous joint bleeds and subsequent development of hemophilic arthropathy (106), it seems foreseeable that our study cohort had somewhat higher mean scores compared to scores in a population on prophylaxis. The health status of the joints in children with hemophilia using the HJHS version 1.0 was also assessed in a study by Engelbert *et al*. (107). Raw scores of the HJHS in 47 Dutch children with hemophilia (21 boys with severe hemophilia receiving factor replacement prophylaxis, seven boys with moderate hemophilia and 19 with mild hemophilia receiving on-demand treatment) showed that these patients had no or minimal joint impairment (raw scores between 0 and 6, of a maximum 148 in the HJHS version 1.0). Impairment was caused by slight decrease in range of joint motion of knee and ankle joint and the presence of crepitus during movement. Comparing the study of Engelbert *et al*. with our study results, differences in the HJHS scores of the two studies are most likely explained by the fact that we analyzed only patients with severe hemophilia and treatment on-demand. Joint evaluation using the HJHS instrument was also performed in a comparative study by Christoforidis *et al*. (108). The study involved 26 patients with hemophilia A and mean decimal age 12.08 years (SD 4.44). 17 patients had severe hemophilia A (residual factor activity <1%) and 9 had moderate hemophilia A. Mean HJHS for moderate hemophilia was 4.83 (SD 5.27, range 0-15), for severe 8.27 (SD 6.11, range 0-23) (p=0.24). The utility of the HJHS in assessing health status of the joints was also tested on 20 Chinese hemophilia children (age 5-17; hemophilia A/B: 18/2; severe/ moderate/unknown: 5/13/2) (109). The HJHS score ranged from 1 to 35 (mean 13.1, median 12, SD 9.03). Investigators of the latter report stated that the score was significantly higher in older than in younger children, but it was not specified exactly from which age the score was noticed to be higher. Despite using the same treatment regimen (on-demand), our study detected higher mean total HJHS scores as compared with those in Chinese hemophilia children, most likely because the majority of the children in the latter study had moderate hemophilia. Our study, characterizing musculoskeletal damage in severe hemophilia using the HJHS tool in boys treated exclusively by on-demand treatment practices, indicated new scores (110). The mean total HJHS score in that study cohort (*n*=20) was 24.5 with 50% of patients who had HJHS scores of 25 and higher. Our findings documented a higher total as well as a higher six-joint HJHS score in patients with severe hemophilia and treatment on-demand as compared with all above-mentioned studies in which the HJHS instrument was used. Results from the recent study by Khawaji *et al*. (99) confirmed low HJHS (median score of 3 of a maximum of 148) in patients who started prophylaxis at the age of ≤3 years (range 1-3) and much more higher HJHS scores (median score of 40),

comparable to patients with the treatment on-demand, for patients who started prophylaxis at the age of >3 years (range >3-78) ($p<0.001$) (Table 13).

There is evidence that the process of hemophilic arthropathy commences even after a limited number of recurrent joint haemorrhages in childhood (111). However, neither the number of bleeding episodes required to cause irreversible damage of the joint is known, nor the number of joint bleeds that can be tolerated to restrict irreversible hemophilic arthropathy (1, 112, 113). Despite the fact that joint bleeds typically start between one and three years of age in children with hemophilia (114), it is striking that gross clinical signs of hemophilic arthropathy usually appear more than a decade later (13, 105). In our study, which investigated the progression of hemophilic arthropathy during childhood and puberty with a particular focus on the age of remarkable changes based on the HJHS scores, worsening of joint scores could also be detected amongst older children, from the age of 10 onwards. We found a significant difference between mean total HJHS scores in younger patients of group I that was 11.6 *vs*. older children of group II who already showed considerable joint damage with a mean joint score of 31.5. Data indicated that worsening in the HJHS score was noticed with the increasing age and over 50% (8 of 13) of the patients ≥10 years of age presented with values higher than 30 ($p=0.0002$). The comparison of the mean total HJHS score in two age groups resulted in detection of significant difference between the patients younger than 10 years of age and those older, showing that the most risky period and most aggravating development of hemophilic joint damage starts at the age of 10 and onwards. These results were supported recently by researchers from the UK (115). In 39 boys with severe hemophilia (mean age 10 years, range 4-18) and primary prophylaxis at 25-40 IU/kg at least twice weekly for hemophilia B, and three times for hemophilia A, HJHS scores ranged between 0-22, with a tendency to increase progressively with age. Mean values ranged from 1 in boys aged 4-6 years to 4 in boys aged 14-18 years ($p=0.08$). HJHS scores were less than 8 in all boys aged 8 or less. This delay in appearance of hemophilic arthropathy must be taken into account in choosing the right time of the treatment initiation.

All patients in our study had multiple joint involvements. The most frequently affected joints were ankles, followed by knees and elbows. Elbows were the least affected joints and differed significantly from the joint damage in knees and ankles. These frequencies are consistent with those reported by Plug *et al*. (70), Aznar *et al*. (97) and Molho *et al*. (96), in which the most commonly affected joint was ankle. Recent study with 68% of patients with severe hemophilia and 91% of them on prophylaxis, reported HJHS for each joint pair was highest for the ankles at 2 (IQR=0-6, range 0-23), followed by knees at 1 (IQR=0-3, range 0-14) and elbows with a median score of zero (IQR=0-1, range 0-18) ($p<0.01$) (116). Earlier findings reported the knees (117) and elbows (118) to be the most affected joints. Despite changing pattern of affected joints have been reported in prophylaxis patients (119), no robust data exist on the efficacy of variable on-demand treatment regimens on joint pathology. As the trends towards using increasing doses of clotting factors were reported (20), it is possible to speculate that bleeding pattern and frequency of affected joints might also change. A somewhat surprising finding in our study was the low values for one nearly 17-year-old boy who had a total HJHS score of 7. Despite the fact that home treatment and home delivery facilitates immediate and effective treatment at the earliest sign of bleeding and generally related to better musculoskeletal outcome (27), the results of our study showed no significant difference in musculoskeletal outcome of patients who had on-demand

home treatment *vs.* those only on in-clinic treatment. In our study the total HJHS score was even higher in patients with home treatment than in-clinic treatment showing that home treatment in our study population might not be adequate. It should be mentioned that the patient with quite low total HJHS in group II had an access to prompt and early on-demand factor replacement therapy at home from the time of the diagnosis, which was not the case with other patients of our study. The favourable outcome in that particular patient might also be explained by an unusually mild individual clinical phenotype, as has been reported in a few cases amongst children with severe hemophilia (120).

The HJHS scores found in our study illustrated that scores are higher in patients using treatment on-demand as compared with prophylaxis, but more studies including higher numbers of patients were necessary in order to understand the influence of different treatment regimens or management effectiveness. In this context, a 2-year, multi-centre Validation Study of the HJHS was completed (44, 121). 226 patients (mean age 10.8) from five centres with mild (17%), moderate (15%) and severe (68%) hemophilia were utilised in this study. In patients with severe hemophilia and primary prophylaxis, the median HJHS was 5, those treated with secondary prophylaxis had median HJHS of 9, those treated on demand 11.5 (Table 13). It is important to note that findings reported from the reliability and validity studies might be considered in the light of possible limitations since physiotherapists participating in the studies were highly experienced and personally involved in the development of the HJHS. On the other hand, the HJHS has now been taught in workshops worldwide and appeared to be easily adopted, even by physiotherapists with limited experience in hemophilia (44).

Results from studies published so far indicate that the new joint assessment tool enables detection of subtle and early signs of joint damage in intensively treated boys and also illustrates the differences among patients with mild, moderate, or severe hemophilia based on the HJHS scores. This was demonstrated mainly in the studied subjects with severe hemophilia on prophylactic treatment in countries where factor concentrates were widely available. It is a reasonable group to study since the HJHS was designed specifically to be sensitive for mild joint changes in patients with prophylaxis. However, the application of the HJHS tool in a study of patients with severe hemophilia who exclusively received treatment on-demand, demonstrated that the HJHS also is a useful and effective tool in evaluating musculoskeletal outcome following an on-demand based treatment approach in patients with existing joint damage. This shows that new instrument might be equally appropriate for screening children on prophylactic treatment as well as on-demand regimens. Findings from the studies showed that scores are higher in patients using treatment on-demand as compared with prophylaxis. However, in order to understand the influence of different treatment regimens or management as determined by the HJHS, studies including higher numbers of patients are required. It remains to be seen how the HJHS scores should be interpreted in different treatment populations with hemophilia.

Findings based on the HJHS scores also suggested that the HJHS tool may be sensitive to the progression of joint disease with age in hemophilia. A HJHS utility study in episodically treated boys with severe hemophilia (110) confirmed that joint damage is developing slowly over decades. HJHS scores increased as a sign of progressing hemophilic arthropathy that seemed to occur from the age of 10 and onwards.

Table 13. Overview of studies that used the HJHS for assessing musculoskeletal status in patients with haemophilia

Author, year	**Number of patients**	**Type of hemophilia**	**Treatment regimen**	**Mean/median age (range)**	**Median/mean HJHS score**
Hilliard *et al.* 2006 (42)	8	Severe	Prophylaxis	8.7 (4–12)	15 (1st day) 14 (2nd day)
Chen *et al*. 2008 (109)	20	Severe/moderate/ unknown: 5/13/2	On-demand/sporadic/none	NS (5–17)	12
Engelbert *et al*. 2008 (107) Groen et al. 2011 (122)	47	Severe/moderate/ mild: 21/7/19	Prophylaxis/on-demand: 25/22	12.5 (8–18) (SD 2.9)	0 (range 0–6)
Christoforidis *at al.* 2011 (108)	26	Severe 17/moderate 9	Prophylaxis	12.08 (4.94–18.0) (SD 4.44)	8.27 (range 0–23)(severe) 4.83 (range 0–15)(moderate)
Bladen *et al*. 2010 (115)	39	Severe	Primary prophylaxis	10 (4–18)	1 (in 4–6 years of age) 4 (in 14–18 years of age)
Feldman *et al.* 2011 (44) Groen *et al*. 2011 (116)	226	Severe (68%)/moderate/mild	Prophylaxis (65%)/ on-demand (35%)	10.8 (SD 3.8)	5 (IQR 1–12, range 0–43) 5 (IQR 1–12) (severe, primary prophylaxis) 9 (severe, secondary prophylaxis) 11.5 (severe, on-demand)

Author, year	**Number of patients**	**Type of hemophilia**	**Treatment regimen**	**Mean/median age (range)**	**Median/mean HJHS score**
Saulyte Trakymiene *et al.* 2010 (110)	20	Severe	On-demand	11.5 (4–17.2)	24.5 11.6 (in <10 years of age) 31.5 (≥10years of age)
Khawaji *et al.* 2012 (99)	81	Severe	Prophylaxis Group A: prophylaxis at the age of ≤3 yr (*n*=30); Group B: prophylaxis at the age of >3yr ((n = 51).	Group A: 27 (18–45) Group B: 50 (22–78)	Group A: 3 (0–19) Group B: 40 (0–64)

NS – not specified

6.3 Impact of prophylaxis versus on-demand therapy

The study compared clinical joint status based on the HJHS between cohorts of age-matched boys with severe hemophilia managed either by an on-demand or by a prophylactic strategy. Based on the HJHS, joint outcome was significantly better for the prophylactic regimen in comparison with the on-demand strategy. This finding is in accordance with the results from several other studies that compared physical joint status in two different treatment groups and demonstrated superiority of prophylaxis over on-demand treatment strategy in reducing the rate of joint deterioration (6, 10, 16, 21). However, a direct comparison of clinical joint scores among studies is not possible since the HJHS, used in the present study, is a new and recently validated tool (42, 44).

The HJHS for the on-demand group demonstrated a tendency to progression with age. Greater scores in older patients indicate that the severity of joint pathology progresses over time with on-demand treatment. Furthermore, despite the clinical observation that joint status in young children is rather good as well as knowledge that pathologic process leading to hemophilic arthropathy requires several years to produce clinically evident signs (52), the significance of difference in joint status comparing different treatment strategies was equally strong both in older and younger patient groups. The HJHS scores for younger patients were significantly lower in the prophylaxis group compared to the on-demand treatment strategy: 2.2 *vs.* 12.5 ($p=0.0002$). Starting from the age of 10 years and onwards, the difference was even more prominent with the mean HJHS at 3.9 for the prophylaxis group *vs.* 36.3 for the on-demand group ($p<0.0001$). Analysis of the HJHS scores in patients on prophylaxis showed a slight tendency to increase with age, however, the difference between age groups was not significant. This could indicate that prophylaxis protects from regular joint damage although a few bleeds may occur. It is likely that an ongoing joint bleeding during prophylaxis may result in blood-induced subtle musculoskeletal changes that could be detected using a sensitive tool like the HJHS.

The strength of significance in the differences of clinical joint outcomes between treatment groups and age groups results from treatment strategy. However, the difference in physical joint health based on the HJHS found by us, may be more prominent in comparison with recent joint outcome studies (13, 22) because another differences between the care practices in DK and LT patients may have influenced the outcome. Patients in the study were not selected to receive one of the treatment strategies; rather, the clinical practice of the country was the reason for receiving one rather than another of the treatment regimens. DK patient cohort had free access to clotting factors from birth onwards and early home therapy whereas LT patients were treated on-demand with factor restrictions and unavailability of home therapy in half of the patients. Home treatment is an important determinant of outcome and may represent an additional benefit in prevention of joint disease. Differences in outcome might also be influenced by differences in follow-up strategies. Prophylaxis group was followed-up regularly and treated at a hemophilia center according to the state of the art while on-demand group patients were visiting primary care centers and follow-up visits at a regular hemophilia center were very rare. Furthermore, no standards existed for hemophilia care in Lithuania at that time. It is important to note that patients treated on-demand did not

report on the bleeds they experience and were less observant to bleeding-related events in comparison with the more alert patients belonging to the prophylaxis group. In consequence, important demographic data such as age at first bleeding, number of annual joint bleeds were not available in support of improved treatment decisions. Finally, with the real life treatment on-demand like in our study, it is likely there was much heterogeneity in the management of acute hemarthrosis, which we were unable to evaluate. In addition, we are uncertain of how many bleeds went untreated, or were treated too late to prevent arthropathy. No robust data exist on the efficacy of variable on-demand treatment regimens on joint pathology. In our study variability in annual clotting factor usage per year was quite impressive in the on-demand group – from 271 to 2206 IU/kg/year.

A key factor for joint preservation is reduction of numbers of joint bleeds (13, 22). HJHS in 16 DK children showed that these patients had no or minimal joint impairment with a median annual joint bleed frequency at 0. In the JOS study the prophylaxis group sustained an average of 0.63 joint bleeds per year (13). The study on the trends in bleeding pattern during prophylaxis supported few or no joint bleeds (median 0-1.1) (123). Other studies reporting a low bleeding frequency and very low joint scores on the HJHS, add to the already ample evidence that prophylaxis is very successful in altering the natural bleeding pattern of severe hemophilia (44, 108, 115). However, both RCT (13, 22), and our study demonstrated that hemophilia patients could not avoid all bleeds and might develop some degree of joint deterioration even when receiving prophylaxis. The scores in the prophylaxis group were very low and impairment was primarily caused by the presence of mild crepitus in one or two joints. These findings indicate that HJHS is a sensitive tool, being able to pick up subtle changes in the joints, and discriminate the patients on different treatment strategies from early age. In contrast, the analysis of joint damage according to the Pettersson scores (older score) demonstrated the differences in outcome between treatment strategies in patients with severe hemophilia become apparent only after 15 to 20 years.

This is the first study that used standardized clinical outcome measure to compare joint outcome in different treatment groups. Findings from the studies that used the HJHS tool showed that scores were higher in patients using treatment on-demand as compared with prophylaxis (44, 108, 115). However, despite the fact that the joint score was significantly higher in patients treated on-demand, the mean score was only slightly less than one-fourth of the maximum score, indicating that joint status still was not substantially impaired. In order to understand the influence of different treatment regimens as determined by the HJHS, studies including higher numbers of patients are required. It remains to be seen how the HJHS scores should be interpreted in different treatment populations and what numeric point based on the HJHS constitutes an unacceptable score. Although a cohort study such as ours has some limitations, we believe it provides some new knowledge for the interpretation of the HJHS scores in nearly normal and damaged joints.

The results of the comparative study further demonstrate the unequivocal effect of prophylaxis on joint status based on the HJHS. Joint damage remains an inevitable complication in patients treated for bleeding episodes only. Our study gives an insight into early and late manifestations of joint impairment based on the HJHS in hemophilia patients with treatment on-demand compared to joint changes that may develop over the time with the preventive treatment. The time until arthropathy becomes clinically evident

may be quite long. However, joint evaluation using a sensitive tool like the HJHS, may show that a primary prophylactic treatment strategy leads to a better outcome already at a young age. It supports the usefulness of the HJHS in utilization in clinical practice to monitor joint status in children with prophylaxis.

7. Conclusions

1. Comprehensive data on demographics, diagnosis and treatment of all PWH <18 years of age resulted in unified database of high quality and facilitated implementation of a new treatment and monitoring strategy for children with hemophilia in Lithuania.
2. Steady increase has been made regarding annual usage of FVIII from 2003 to 2008, however, great variability in FVIII usage in patients with severe hemophilia treated on-demand might indicate lack of coordinated hemophilia care in our country and lack of evidence in giving optimal treatment.
3. During the last 5 years, treatment of hemophilia for children <18 years in Lithuania improved dramatically. It has focused on the use of long-term preventative treatment and resulted in an expansion of the use of long-term prophylaxis in approximately two-thirds of individuals with severe hemophilia.
4. Assessment of musculoskeletal damage in severe hemophilia patients using the HJHS method showed that:

a) HJHS is a useful and effective tool in evaluating musculoskeletal outcome following an on-demand based treatment approach in patients with existing joint damage;
b) based on the HJHS, the most risky period and most aggravating development of hemophilic joint damage starts at the age of 10 and onwards;
c) despite the time until arthropathy becomes clinically evident may be quite long, joint evaluation using a sensitive tool like the HJHS, may show that a primary prophylactic treatment strategy leads to a better outcome already at a young age.

Suggestions for clinical practice and research

1. This study provided first knowledge combining demographic characteristics and assessment of clinical joint status in children with hemophilia in Lithuania. It is important that the generated relevant data be maintained in a centralized database or registry. Furthermore, tese data could help to form more coordinated haemophilia care in Lithuania.
2. Careful monitoring of clotting factor consumption in a specialized hemophilia treatment center by physicians as well as health authorities could help to optimize factor use, anticipate future demands and be helpful for the national healthcare planning.
3. As prophylactic treatment, albeit much more expensive, is the only evidence-based therapy leading to superior outcomes compared to on-demand therapy, it should be recommended for all children with severe hemophilia and approved as a standard of care on a national level. With that knowledge we have to convince policymakers of the benefits of prophylactic treatment, at least for children.
4. If prophylaxis for adults is not a realistic dream for our country, there is still ample room for further research in finding optimal way to give treatment on-demand and address all determinants of the effective treatment on-demand that are really lack of evidence today. In addition, with the quantity of CFCs that is already available in our country there is a potential to offer secondary prophylaxis at lower doses to adult PWH because it could be beneficial in controlling haemorrhage and imroving quality of daily life without increasing consumption of CFCs (over that for on-demand treatment). If a man of 50 kg start with 10 IU/kg twice or three times a week, this will require a total of about 1000 or 1500 IU/kg/year, respectively.
5. As a result of the introduction of effective replacement therapy (prophylaxis) for children, we need to establish a new approach to optimal and comprehensive follow-up. Preservation of good joint status is a crucial component of hemophilia care, therefore we would recommend patients with severe hemophilia should be assessed 2-4 times per year; mild and moderate patients can be reviewed once per year or two years.
6. A consistent and standardized method of measurement and of joint health status in PWH is very important for assessing deterioration or progression of joint condition, detecting failure of current treatment and determining whether alternative management options are required (41). Implementation of the HJHS into clinical practice along with physiotherapy service as a standardized and regular monitoring of musculoskeletal status outcome could be key step in the future for initiation of the development of specialist and muldicisdiplinary approach of hemophilia care in Lithuania according to the WFH recommendations and the European principles of hemophilia care (27). In the future it could provide the longitudinal data in children as well as possibly introduced into adult setting. There are first promising reports on the HJHS utility for adults (296).

8. Summary in Lithuanian

Hemofilija – tai reta paveldima kraujo krešėjimo liga. Jos gydymas yra brangus ir kompleksinis (54). Lietuvoje 2007 m. hemofilija sergančių žmonių, ypač vaikų, medicininė priežiūra buvo neadekvati ir nekoordinuota lyginant su šiuolaikiniais, kitose Europos šalyse taikomais standartais (27, 28). Be to, norint pradėti taikyti klinikinėje praktikoje 2007 m. išleistas hemofilijos diagnostikos ir gydymo rekomendacijos Baltijos šalyse, reikėjo surinkti tikslius ir išsamius visų Lietuvos pacientų iki 18 m., sergančių hemofilija, demografinius, gydymo ir objektyvius klinikinės išeities duomenis.

Pakaitinė terapija VIII ir IX kraujo krešėjimo faktoriais kraujavimo epizodų metu buvo standartinis gydymas, taikomas Lietuvoje hemofilija sergantiems vaikams. Sąnarių pažeidimas neišvengiamas asmenims, kurie gydyti tik kraujavimo epizodų metu. Tačiau kokiame amžiuje pasireiškia artropatijos požymiai, nėra žinoma.

Tyrimo tikslas – įvertinti Lietuvos vaikų, sergančių hemofilija, demografinius duomenis, gydymą bei nustatyti kaulų ir raumenų sistemos pažeidimą siekiant pagerinti šių pacientų ištyrimą, gydymą ir stebėseną. Tyrimo metu siekta sudaryti Lietuvos iki 18 m. pacientų, sergančių hemofilija, duomenų bazę apimant demografinius, ligai specifinius pagrindinius laboratorinius požymius 2008–2011 m. ir sergamumą 1992–2011 m., įvertinti sunaudojamą krešėjimo FVIII kiekį pacientams, sergantiems sunkia hemofilijos forma 2003–2008 m., ir gydymo pokyčius nuo 2007 m. Taip pat taikyti naują standartizuotą hemofilijos pažeistų sąnarių būklės įvertinimo metodą (angl. *Haemophilia Joint Health Score,* angl. santr. HJHS) (40, 41), nustatyti jo panaudojimo tinkamumą epizodiškai gydytiems pacientams bei įvertinti dėl kraujavimo išryškėjusį sąnarių pažeidimą vaikystėje ir brendimo laikotarpiu atsižvelgiant į amžių, kai vystosi kaulų ir raumenų sistemos pokyčiai, ir taikomą gydymo metodą.

Tyrimas atliktas 2008–2011 m. dviejuose centruose: Vaikų ligoninėje, VšĮ Vilniaus universiteto ligoninės Santariškių klinikų filiale, ir Aarhus universiteto ligoninėje (Danija). Tyrime dalyvavo 0–17 m. pacientai, sergantys hemofilija (n = 64). Į hemofilija sergančių pacientų duomenų bazės sudarymą įtraukti 48 Lietuvos pacientai. Sąnarių pažeidimas naudojant HJHS metodą vertintas 4–17 m. sunkia hemofilijos forma sergantiems pacientams Lietuvoje (n = 20) ir Danijoje (n = 16). Pagal tyrimo hipotezę pacientai suskirstyti į dvi grupes: 4–9 m. (I grupė) ir 10–17 m. (II grupė). Statistinė analizė atlikta naudojant STATA 10 versiją.

2011 m. gruodžio 31 d. Lietuvoje sirgo 48 (0–17 m. hemofilija A ir B) pacientai. Hemofilijos A ir B santykis – 41/7. Sunki hemofilija A ir B sudarė 60 proc. nuo visų hemofilijos atvejų. Vidutinis pacientų amžius – 10,3 m. 48 proc. visų hemofilija sergančiųjų buvo vyresni nei 12 m. Sunkia hemofilijos A forma sergantiems pacientams dažniausiai pasitaikanti mutacija buvo introno 22 inversija (50 proc. nuo visų mutacijų), tuo tarpu nesunkios hemofilijos atvejais beveik visus genų defektus sudarė taškinės *missense* tipo mutacijos (71 proc. nuo visų mutacijų). Visiems hemofilija B sergantiems pacientams nustatytos taškinės *missense* tipo mutacijos. Per tyrimą FVIII inhibitoriai nustatyti 13,6 proc. sunkia hemofilijos A forma sergančiųjų, t. y. 7,3 proc. nuo visų hemofilija A sergančių pacientų iki 18 m. Naujų hemofilijos atvejų dažnis 1992–2011 m. Lietuvoje buvo santykinai įvairus ir jo vidurkis yra 1,57 / 10 000 gimusių berniukų. Tyrime pagal amžių apskaičiuotas hemofilijos paplitimas buvo 15,3 atvejų 100 000 0–17

m. pacientų. Metinis sunaudotas FVIII kiekis (TV/metus) taikant epizodinį gydymą sunkia hemofilija A sergantiems pacientams padidėjo 140 proc. nuo 2003 iki 2008 m., tačiau buvo labai įvairus net tarp to paties amžiaus pacientų. Kai kuriems pacientams skirta tiek faktoriaus kaip ir gydant profilaktiškai. Tai patvirtino koordinuotos hemofilijos priežiūros Lietuvoje, taip pat ir įrodymais pagrįstos medicinos stoką taikant šį gydymą. 2007 m., paskelbus mūsų regionui specifines hemofilija sergančių žmonių gydymo ir priežiūros rekomendacijas, jaunesnių nei 18 m. pacientų gydymas labai pagerėjo. Jis buvo sukoncentruotas į ilgalaikio apsauginio gydymo (profilaktikos) įdiegimą ir todėl per ateinančius penkerius metus (2007–2011 m.) apytiksliai 2/3 (71 proc.) sunkia hemofilija sergančių pacientų pradėta taikyti ilgalaikė profilaktika (40 proc.– pirminė profilaktika, likusiems 60 proc. – antrinė profilaktika) plačiai naudojant rekombinantinį FVIII.

HJHS tinkamumo ir naudingumo tyrimas, atliktas įtraukiant epizodiškai gydytus sunkia hemofilija sergančius berniukus, parodė, kad HJHS metodas yra tinkamas hemofilija sergančių pacientų, kuriems yra taikomas epizodinis gydymas, sąnarių būklei vertinti. Vidutinis HJHS balų skaičius vertinant šešis pagrindinius sąnarius ir eiseną buvo 24,5 iš 124 galimų balų. Jis svyravo nuo 5 iki 50. Labiausiai buvo pažeisti čiurnų sąnariai, mažiau keliai ir alkūnės. Vidutinės alkūnių HJHS vertės reikšmingai skyrėsi nuo kelių ($p<0,02$) ir čiurnų ($p<0,001$) sąnarių verčių balais. Vidutinis bendras HJHS balas I amžiaus grupėje (4–9 m.) buvo 11,6, o II grupėje (10–17 m.) ženkliai didesnis – 31,5 ($p = 0,0002$). Abiejų kelių, čiurnų ir alkūnių reikšmės vertinant pagal HJHS skalę buvo didesnės vyresniems pacientams. HJHS balų didėjimas vertintas kaip progresuojančio sąnarių pažeidimo dėl kraujavimo požymis, kuris prasideda apie 10-uosius gyvenimo metus ir vėliau. Remiantis šiais HJHS skalės vertinimo rezultatais, galima daryti prielaidą, kad HJHS metodas gali būti jautrus nustatant sergančiųjų hemofilija sąnarių pažeidimo progresavimą su amžiumi.

Skirtingų gydymo grupių sąnarių būklės palyginimas, atsižvelgiant į gydymo metodą, parodė, kad pacientams, kuriems buvo taikytas epizodinis gydymo metodas, nustatyti ženkliai didesni balai HJHS skalėje (27,4) lyginant su profilaktiškai gydytais pacientais (3,3) ($p<0,001$). Dviejų gydymo strategijų skirtumas buvo matomas jau ankstyvajame amžiuje abiejose grupėse – I grupėje (4–9 m.) ir II grupėje (10–17 m.). Pacientams, gydytiems profilaktiškai, buvo nustatyti reikšmingai mažesni balai nei tiems, kurie buvo gydomi epizodiškai: atitinkamai 2,2 lyginant su 12,5 ($p = 0,0002$) ir 3,9 lyginant su 36,3 ($p<0,0001$). Šio palyginamojo tyrimo rezultatai parodė akivaizdžią profilaktinio gydymo naudą sąnarių būklei remiantis HJHS vertinimo metodu. Nors dėl kraujavimo išryškėjęs sąnarių pažeidimas kliniškai pastebimas negreit, sąnarių pažeidimo vertinimas, naudojant jautrius sąnarių būklės vertinimo metodus, tokius kaip HJHS, rodo, kad kraujavimų profilaktika užtikrina geresnę sąnarių pažeidimo išeitį jau ankstyvajame amžiuje. Tai patvirtina HJHS metodo klinikinę naudą stebint sąnarių būklę vaikams, kuriems taikoma profilaktika.

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10. List of publications

This Ph.D.thesis is based on the following publications:

1. Saulyte Trakymiene S, Clausen N, Hvitfeld Puolsen L, Ingerslev J, Rageliene L. Progression of hemophilic arthropathy in children: a Lithuanian – Danish comparative study. Haemophilia. 2012 Nov 20. doi: 10.1111/hae.12058. Epub ahead of print.
2. Saulyte Trakymiene S. Musculoskeletal pathologies in children with hemophilia as evaluated with a standardized physical joint scoring systems to assess disability. European Oncology & Haematology, 2011;7(1):76-80.
3. Saulyte Trakymiene, S Ingerslev J, Rageliene L. Utility of the Hemophilia Joint Health Score in study of episodically treated boys with severe hemophilia A and B in Lithuania. Haemophilia. 2010 May; 16(3):479-86.
4. Saulyte Trakymiene S, Rageliene L. Optimal treatment for children with hemophilia: a review. Acta Medica Lituanica. 2009. Vol. 16. No. 1. P. 3-10.

Abstracts and posters:

1. Saulyte Trakymiene S, Daugelaviciene V, Rageliene L. "Analysis of differences of annual FVIII consumption among severe hemophilia A persons treated on-demand in Lithuania". Hematology Conference Spotlight on Hemophilia care management. 27-29 September, 2012 Dresden, Germany. Poster.
2. Saulyte Trakymiene S, Janovich V, Rageliene L, Jusinskaite V, Gelumbauskiene B, Leveckiene L, Gerbutavicius R, Kryzauskaitė L." Physiotherapy capacity building for patients with hemophilia in Lithuania". 8th Baltic Conference of Hematology. 12-14 April, 2012 Tallinn, Estonia. Abstract.
3. Saulyte Trakymiene S, Daugelaviciene V, Stankeviciene S, Ramanauskiene E, Kiudeliene R, Rageliene L. "Changing pattern of hemophilia management in Lithuania". 5th Annual Congress of the European Association for Hemophilia and Allied Disorders. 22-24 February, 2012 Rome, Italy. Abstract and poster.
4. Saulyte Trakymiene S, Daugelaviciene V, Stankeviciene S, Ramanauskiene E, Urbanoviciene A, Rageliene L. "Recent progress in the development in hemophilia care in children in Lithuania". Hematology Conference Achieving progress in a changing world. 13-15 October, 2011 Budapest, Hungary. Poster.
5. Saulyte Trakymiene S, Ingerslev J, Rageliene L. "Hemophilia Joint Health Score in study of episodically treated boys with severe hemophilia A and B in Lithuania". XXIXth International Congress of the World Federation of Hemophilia Buenos Aires, Argentina, 10-14 July, 2010. Poster.
6. Saulyte Trakymiene S, Daugelaviciene V, Nemaniene R, Kiudeliene R, Rutkauskiene G, Rageliene L. "Recent advances in the adoption of prophylaxis into clinical practice in Lithuania". 7th Baltic Conference of Hematology 20-22 May, 2010 Tartu, Estonia. Abstract.
7. Saulyte Trakymiene S, Rageliene L, Kovalova Z, Pruunsild K, Orgulas K."Hemophilia Joint Health Score in study of boys with hemophilia A and B in

Baltic countries". 7th Baltic Conference of Hematology 20-22 May, 2010 Tartu, Estonia. Abstract.

8. Saulyte Trakymiene S. "The Hemophilia Joint Health Score (HJHS) in study of episodically treated boys with severe hemophilia A and B in Lithuania". Hematology Conference Bridging the gap between science and practice. 17-19 September, 2009 Riga. Poster. Winner of the scientific poster session.
9. Saulyte Trakymiene S. "Lithuanian study on joint health and health-related quality of life of pediatric hemophilia patients". 6th Baltic Conference of Hematology 8-10 May, 2008 Vilnius. Abstract.

11. Curriculum vitae

Sonata Saulyte Trakymiene was born on 23 October 1973. In 1998 she obtained her Doctor of Medicine at Vilnius University, and in 2003 completed post-graduate training, including a general pediatrics, and, pediatric hematology and oncology residency, at Vilnius University Children's Hospital. Since 2004 she has been employed as a consulting pediatric oncologist and hematologist at Vilnius Antakalnis Children's Outpatient Clinic. Since February 2006 she entered the Centre for Pediatric Hematology/Oncology at Children's Hospital, Affiliate of Vilnius University Hospital Santariskiu Klinikos. Since 2007 her main interest has been in hemophilia and other coagulation disorders in children. Her training in the field of coagulation disorders included several international courses and an internship in the Division of Haematology/Oncology at the Hospital for Sick Children, Toronto, Canada. In 2010, she completed training in the field of bleeding disorders and thrombosis at the Centre for Hemophilia and Thrombosis, University Hospital Skejby in Aarhus, Denmark under the Mentorship of Professor J. Ingerslev. She has published several papers in professional journals and many abstracts in the proceedings of international or national scientific conferences.

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