

Blood Coagulation, Fibrinolysis and Cellular Haemostasis

Effects of primary and secondary prophylaxis on the clinical expression of joint damage in children with severe haemophilia A

Results of a multicenter non-concurrent cohort study

Rosemarie Schobess¹, Karin Kurnik², Frauke Friedrichs³, Susan Halimeh⁴, Anne Krümpel⁴, Christoph Bidlingmaier², Ulrike Nowak-Göttl⁴

¹Department of Pediatrics, University Hospital Halle, Halle, Germany; ²Department of Pediatrics, University Hospital Munich, Munich, Germany; ³Leibniz-Institute for Arteriosclerosis Research, University of Muenster, Muenster, Germany; ⁴Department of Pediatric Hematology and Oncology, University Hospital Muenster, Muenster, Germany

Summary

Patients with severe haemophilia A (HA) can either be treated by regular FVIII infusions twice or three times per week (prophylaxis), or only in case of bleeding episodes (on-demand). Whereas prophylaxis reduces the number of bleeding episodes and may therefore prevent the development of haemophilic arthropathy, there is still a lot of controversy surrounding recommendations on age and dose at start of prophylactic regimens. The present database study was performed to investigate the role of primary versus secondary prophylaxis in HA children. The outcome variable was imaging-proven haemophilic joint damage. Forty-two children were initially treated with primary prophylaxis following the first bleeding episode, and were frequency-matched (year of birth, catchment area) to 67 pa-

tients receiving “on-demand” therapy with an early switch to “secondary prophylaxis”. In multivariate analysis adjusted for the HA mutation type and the presence or absence of thrombophilia, the Pettersson score investigated at a median age of 12.5 years in joints with at least one documented bleeding episode was not significantly different between the two patient groups ($p=0.944$), and no statistically significant differences were found in patients with target joints ($p=0.3$), nor in children in whom synovitis had occurred ($p=0.77$). No conclusion can be drawn from the data presented herein whether primary prophylaxis or an early start of secondary prophylaxis is superior with respect to joint outcome in children with severe HA.

Keywords

Severe haemophilia A, on-demand, primary prophylaxis, joint damage

Thromb Haemost 2008; 99: 71–76

Introduction

The treatment of children with severe haemophilia A (HA) has been profoundly improved by the recent availability of adequate quantities of viral-safe factor VIII concentrates, advanced techniques to perform venipuncture at home treatment, and the routine application of protocols to prevent bleedings (1–5). Today, in developed countries patients with severe haemophilia A (factor (F) VIII activity < 0.01 U ml⁻¹) can either be treated by regular FVIII infusions two or three times per week (prophylaxis), or

only in case of bleeding episodes (on demand). Whereas prophylaxis reduces the number of bleeding episodes and may therefore prevent the development of haemophilic arthropathy, there is still some controversy surrounding the age and dose at start of treatment regimens (5). These controversies are mainly based on the side effects associated with the use of central venous lines, which are needed for frequent injections in young children. In addition, given the high cost and effort associated with long-term prophylaxis to prevent joint damage, research into cost effectiveness is needed (5–8). Furthermore, since patient/family satisfac-

Correspondence to:

Prof. Dr. U. Nowak-Göttl
Dept. of Pediatric Hematology/Oncology
University Children's Hospital of Muenster
Albert-Schweitzer-Strasse 33
48149 Muenster, Germany
Tel.: +49 251 834 4936, Fax: +49 251 834 7828
E-mail: leagottl@uni-muenster.de

Financial Support:

This study was supported by unrestricted grants from Bayer Vital GmbH, Leverkusen, Germany, CLS Behring GmbH (Hattersheim, Germany) and Octapharma GmbH (Langenfeld, Germany)

*RS and KK contributed equally to this study.

Received June 18, 2007

Accepted after major revision November 15, 2007

Prepublished online December 5, 2007

doi:10.1160/TH07-06-0417

tion with treatment modalities is an important determinant of adherence to difficult treatment regimens in chronic diseases, an individual communication about the disease and its possible consequences within the family depending on personality and life stage is mandatory before initiating adequate long-term therapy (9). Readiness to receive information is very variable within affected families. Based on the fact that the information recipient may sometimes comprehend much less at HA onset, primary prophylaxis may not be able to be performed immediately after diagnosis of severe HA.

The purpose of the present non-concurrent (database) cohort study was to investigate the role of “primary” versus “secondary” prophylaxis with regard to the clinical expression of the disease, i.e. the occurrence and severity of imaging-proven haemophilic joint damage.

Methods

Ethics

The present database study in consecutively recruited pediatric patients with HA was performed in accordance with the ethical standards stipulated in a relevant version of the 1964 Declaration of Helsinki, and was approved by the Medical Ethics Committee of the University of Muenster, Germany.

Definition of terms used in the present survey

In the present non-concurrent cohort study, primary prophylaxis was defined as factor infusions given to prevent bleeding before the third, but usually starting after the first, bleed (10). In addition, patients who did not suffer more than one symptomatic joint bleed into the same joint within a six month period before the start of long-term continuous treatment were classified as primary prophylaxis patients (modification to ref. 11). Secondary prophylaxis was defined as long-term continuous factor replacement therapy not fulfilling the modified criteria for primary prophylaxis. Traumatic or spontaneous joint bleed was defined as an episode characterized by pain, thought to represent intra-articular bleeding necessitating factor replacement therapy. Significant soft-tissue bleed was defined as a bleed into a muscle or confined space which may include neural tissues. A target joint was defined as three or more bleeding episodes occurring into the same joint within a three month period. In addition, synovitis, i.e. the inflammation of the synovium, was diagnosed when synovial hyperplasia and signs of haemosiderin were present in the magnetic resonance imaging (MRI) (12).

Inclusion criteria

Caucasian, previously untreated patients (PUP) with severe HA aged neonate to 16 years, who had been admitted to the University Children's Hospitals of Frankfurt, Halle, Munich, and Muenster, Germany, at the first symptomatic and spontaneous onset of the disease including all sources of bleeding episodes were included in the study.

Exclusion criteria

Pretreated pediatric patients with HA, affected brothers/relatives with HA, and children in whom HA was diagnosed prior birth were not included in the present review.

Outcome measures

The occurrence rate and severity of imaging-proven haemophilic joint damage (primary aim), the development of target joints and the maximum annual bleeding frequency (ABF) during the initial treatment regimen (secondary aim) were defined as outcome measures of interest. As outcome predictors the initial therapeutic regimen applied, and the haemophilic gene mutations were defined.

Study population

From October 1985 to December 2001, 147 consecutive Caucasian pediatric PUPs with a first symptomatic onset of HA were recruited from different geographic catchment areas in Germany. Of the 147 consecutively enrolled patients, children with severe HA who were treated with primary prophylaxis following the first symptomatic bleeding event leading to diagnosis were frequency-matched (year of birth ± 1 year; catchment area of study center) with PUPs receiving secondary prophylaxis (13). The final study cohort included 109 PUPs with severe HA, 42 children with primary prophylaxis, and 67 children in whom secondary prophylaxis was the first line HA treatment following the first symptomatic bleed.

Treatment regimens performed

At the discretion of the participating centers and according to the standard of care in the years of patient enrolment (1985–2001) children were either treated with primary prophylaxis or with secondary prophylaxis (4, 14, 15). The opportunity of primary prophylaxis was offered to all newly diagnosed patients irrespective of age at presentation, including neonates and infants. According to the definitions given, treatment in this non-concurrent cohort study (16) was classified as primary prophylaxis or as secondary prophylaxis at the time of data analyses (10, 11). The treatment regimens were maintained as the standard over time at the participating study centers. An intensified treatment protocol was introduced in the mid 1990s for patients who presented with severe soft tissue bleeding at HA onset. Such children received a primary prophylactic treatment regimen following the first symptomatic haemorrhage. In the children reported herein, imaging-proven haemophilic joint damage (in the following termed “haemophilic arthropathy”) was classified according to Pettersson (X-ray score): joints with a Pettersson score ≤ 1 were classified as normal (17). In addition, patients with repeated joint bleeding despite adequate prophylaxis and children with suspected synovitis were investigated with magnetic resonance imaging (MRI). The MRI classification was performed according to Nuss (MRI-score: [12]). In addition, for each participating child the maximum ABF, based on reviews of patient infusion logs and the family's report, was recorded at comprehensive face to face clinical visits.

Laboratory analysis

The plasma levels of factor VIII were determined by one-stage clotting assays purchased from Behringwerke/Marburg, using standard laboratory methods. Mutation analysis for HA and thrombophilia screening was done as described elsewhere (18, 19).

Statistics

All statistical analyses were performed using the Stata (version 8.0, College Station, TX, USA) and StatView 5 software packages (SAS Institute Inc.). Continuous data were presented as median (minimum-maximum: min-max) values and evaluated by non-parametric statistics using the Wilcoxon-Mann-Whitney U test. To compare the frequency distributions of adverse outcome, univariate analysis was performed using the chi-square test or, if necessary, Fisher's exact test. In addition, the effect of the treatment regimens and the mutation types on haemophilic joint damage and ABF were assessed by multivariate analyses: Odds ratio (OR) and 95% confidence intervals (CIs) were calculated. Since we have shown that the presence or absence of thrombophilia influences not only the onset but also the severity of this bleeding disorder, multivariate analyses have been adjusted additionally for this condition. The degree of agreement beyond chance between local and central raters (Pettersson score; Nuss score) was measured with the kappa statistics (Stata 8.0). P-values < 0.05 were considered statistically significant.

Results

Patient characteristics

According to the clinical standard of care with respect to the year of patient enrollment (1985–2001), 42 of the consecutively recruited pediatric patients with severe HA were initially treated with primary prophylaxis (two to three times weekly: median (min-max) dose of 40IU/kgbw [18–60]) and were frequency-matched to 67 patients who received early secondary prophylaxis with substitution of a median (min-max) dose of 40IU/kgbw (30–60) factor VIII concentrate (Table 1). When using the modified classification derived from references (10) and (11) none of the patients in the primary prophylaxis group had more than a total of three symptomatic bleeding episodes and no more than one symptomatic joint bleed. In patients treated with secondary prophylaxis more than three clinically relevant bleeds, including soft-tissue, muscle or joints, or two or more bleeds into the same joint within a 6 month period have been recorded before starting long-term continuous therapy. Factor replacement therapy was performed without the knowledge of the haemophilic mutation status. In addition, there was no significant difference with respect to the source of factor VIII concentrates used (p=0.12).

Table 1: Patient characteristics.

	Primary prophylaxis Number [%] 42 [39]	Secondary prophylaxis Number [%] 67 [61]	P-value*
Age at first bleeding: median [min.-max] values (years)	1 [0–3.5]	1 [0–9]	0.679
Annual bleeding frequency joint bleedings only (before start of prophylaxis)	2 [0–20**] 1 [0–2**]	5 [0–36**] 3 [0–20**]	0.028 0.026
Factor concentrates used [%]			0.121
– pdFVIII	47	22	
– rFVIII	42	52	
– vW&FVIII	16	26	
Start of early prophylaxis median [min.-max.] values (years)	1.7 [0.1–6.7]	2.5 [0.8–16]	1.0
Joint scores target joint: median [min.-max.] values:			
– Pettersson score	1 [0–11]	1 [0–13]	0.819
– Nuss score	4.5 [0–10]	4.0 [0–9]	0.908
Overall Pettersson score: (including joints with at least one bleeding episode)	0 [0–11]	0 [0–13]	0.944
Median [min.-max.] age in years	13 [5–18]	12 [3.1–18]	0.459
Distribution of target joints: total [%]	55	46	0.299
– ankle	47	42	
– knee	22	33	
– elbow	17	13	
– hip	-	6.5	
– knee & ankle	13	6.5	

univariate analysis; ** inhibitor patients

Mutation spectrum	Primary prophylaxis Number [%]	Secondary prophylaxis Number [%]	P-value*
Inversion 22	24 [60]	30 [49]	0.390
No inversion 22	16 [40]	31 [52]	
Missense**	8 [20]	18 [30]	
Nonsense – stop exon 13 – stop exon 14 – stop exon 18	3 [8]	4 [7]	
Large deletion – exon 14 – exon 22	3 [8]	1 [2]	
Splice – Intron 11 IVS11 +5G>A	-	1 [2]	
Frameshift – exon 14 2781 del T	-	1 [2]	
Chromosomal abnormalities – translocation (11;X)(q13;q22)	-	1 [2]	
Not identified so far	2 [5]	5 [8]	
Not available	2 [5]	6 [10]	
Total	40	61	0.440

* univariate analysis; ** Exons 4,6,7,8,9,11,12,16,19,20,21,23,25,26.

Table 2: Spectrum of mutations in children with severe HA with respect to treatment protocol.

Mutation profile

The HA mutation spectrum was no different between PUPs treated with primary versus secondary prophylaxis (Table 2). In addition, when comparing patients with inversion 22 with children, without this mutation a statistically significant association was not found with respect to the treatment regimens ($p=0.390$).

Joint score with at least one documented bleeding

The overall Pettersson score of the total number of joints with at least one documented bleeding episode was no different between the two treatment regimens ($p=0.944$) with a median (min-max) Pettersson score of 0(0–2) for children without a target joint.

Target joints

Sixty-five of 109 PUPs (59.6%) developed at least one target joint with no statistically significant difference between the two patient groups with respect to the severity of haemophilic arthropathy: The target Pettersson score obtained in the patients was found to be comparable in children treated with secondary prophylaxis compared with PUPs receiving primary prophylaxis (OR [CIs]: 0.6[0.2–1.5]; $p=0.3$: univariate analysis). In multivariate analysis adjusted for the presence or absence of thrombophilia and the HA mutation type the Pettersson score in patients with a target joint was no different between the treatment groups (OR [CIs]: 0.9[0.7–1.2]; $p=0.3$).

Development of synovitis

No statistically significant difference was found between the two treatment regimens and the development of synovitis in eight out of 109 children affected (OR [CIs]: 0.2.0[0.16–24.5]; $p=0.58$: univariate analysis). Following adjustment for the presence or absence of thrombophilia and the HA mutation type in multivariate analysis the odds ratio was further reduced (OR [CIs]: 1.47[0.1–21.8]; $p=0.77$).

The degree of agreement beyond chance between local and central raters (Pettersson score; Nuss score) was measured with the kappa statistics: Results obtained showed a substantial agreement (87.14%) beyond that expected by chance alone (42.4%) between local and central readers in the patients tested ($\kappa=0.77$; $Z=17.27$; $p < 0.001$).

Annual bleeding frequency

Before starting any prophylactic regimen the bleeding frequency in children with primary prophylaxis was significantly lower compared with patients treated with secondary prophylaxis (OR [CIs]: 0.90[0.82–0.98]; $p=0.015$: univariate analysis). In multivariate analysis adjusted for the presence or absence of thrombophilia and the HA mutation type, the primary prophylactic regimen again showed a reduced ABF (OR [CIs]: 0.89[0.81–0.99]; $p=0.042$) after a median follow-up of 12.5 years. Interestingly, however, is that although the majority of children (61%) were treated with secondary prophylaxis following a diagnosis of severe HA, there was no statistically significant difference with respect to the start of at least one prophylactic regimen (primary or secondary): Within a median (min-max) time of two (0.1–15.9) years following HA diagnosis all PUPs were switched over to a definitive prophylactic factor replacement therapy.

Discussion

There is a general consensus amongst physicians who treat haemophilia patients that a program of prophylaxis started early on in life and before the onset of joint damage should be considered the optimum therapy for children with severe haemophilia (1–5). Since the development of the golden standard primary prophylaxis regimen, i.e. the Malmö approach (1), treatment modifications starting primary prophylaxis with once-weekly infusions via peripheral veins with rapid escalation based on bleeding frequencies to full dose prophylaxis are being increasingly administered in developing countries. However, recommendations regarding age and dose at start are still a matter of debate (5, 15, 20). In a recently published randomized treatment trial (prophylaxis versus enhanced episodic treatment in bleeding cases) Manco-Johnson and colleagues were able to clearly demonstrate that early prophylactic treatment using 25 IU factor VIII per kilogram of body weight every other day was superior with respect to prevention of bone and cartilage joint damage compared to episodic treatment in case of bleeding episodes (21).

The aim of the present multicenter database study was to investigate the role of primary with secondary prophylaxis with respect to the clinical expression of the disease. The data obtained from this frequency-matched cohort study clearly demonstrate that children who were treated either with primary prophylaxis following the first symptomatic bleeding onset, or with secondary prophylaxis did not differ with respect to the severity of imaging-proven haemophilic joint damage after a median follow-up time of 12.5 years: No significant associations between treatment regimens and the development of target joints were found, and the Pettersson score of joints with at least one documented bleeding was also comparable between the two groups. Interestingly, however, we have to note that despite target joint development the overall joint scores were within the lower range reported, confirming MRI and radiographic findings shown by Manco-Johnson et al. that the Pettersson score alone may not be sensitive enough to pick up subtle changes (21). The long-term follow-up joint status in the present study with a median Pettersson score of zero for all joints with at least one bleeding episode, and of one in children with a target joint is acceptable in a cohort of HA children in whom the parents were not aware of the diagnosis prior to the onset of the disease. However, mainly due to the observed maximum ABF of 20 and 36 under primary and secondary prophylaxis, both observed in children with inhibitors, the total rates of target joints was high: As shown by Kreuz et al. in 1998, the latter could be improved by initiating early and frequent prophylaxis, i.e. at least three times a week (15). In this study, Kreuz and coworkers clearly demonstrated that seven out of eight patients in whom prophylaxis had been started at least prior to a second joint bleed had constant X-ray and orthopedic scores of zero or one during the follow-up at a median follow-up period of 11 years compared with children with more than two joint bleeds prior to prophylaxis start. In this prospectively followed cohort FVIII was administered in doses of 30–40 IU and in some cases up to 50 IU per kilogram three times weekly. Of note, in a retrospective Cohort study, Gouw et al. reported very recently that the incidence of inhibitor development appeared to be associated with treatment intensity, i.e. peak treatment moments

in case of bleeds (20). In this study, regular prophylaxis was associated with a 60% lower risk of inhibitor development than observed in children with on-demand treatment (RR 0.4; CI 0.2–0.8). Thus, the authors concluded that early prophylaxis may protect patients with haemophilia against the development of inhibitors. On the one hand, our findings differ from previous studies carried out in Norway, Sweden and the Netherlands [6, 7, 22, 23]. However, these differences are mainly attributable to the fact that HA children in the present cohort were switched over to prophylactic factor substitution very early, i.e. when two or more episodes of bleeding into the same joint had occurred within a 6 month period. Of note, all of the children were finally treated with prophylaxis within the study observation period. As expected, however, prior to the start of a definite long-term prophylactic regimen the annual bleeding frequency was significantly lower in children with primary prophylaxis. On the other hand, however, our findings are in line with European reports that prophylaxis in severe HA is justified when started at an early age but may be individualized: Carlsson et al. showed in a Norwegian-Swedish cohort that the cut-off risk of premature death in adult patients with severe HA was significantly higher for “on-demand” therapy compared with prophylaxis (7). Astermark and colleagues from Sweden reported in 1999 that a significant decrease in the overall number of joint bleeds per year was found after shortening the infusion interval (2), and that those who started prophylaxis before the age of three had a better outcome than those who started at a later age. In 2001, Van den Berg and coworkers from the Netherlands showed that an individually tailored long-term intermediate dose prophylactic regimen could prevent arthropathy (22), and Fischer et al. clearly pointed out in a multicenter cohort derived from Sweden and the Netherlands, that after a two-decade follow-up period the median Pettersson score was eight points higher for every year that prophylaxis was postponed after the first joint bleed (24). Thus, apart from the better clinical outcome, the individualization of prophylaxis adjusted for the bleeding frequency opens the opportunity for improved communication with family members of affected newborns or infants, thus increasing compliance with adherence to difficult treatment regimens to be performed over a long life span period. In cases where a primary prophylaxis is impossible, the procedure to switch from “on-demand” to early secondary prophylaxis can be achieved in the majority of young children with severe haemophilia without the immediate need for central venous access devices. In addition, the needs of parents around the time of diagnosis of severe HA could be better addressed.

Limitations of the present survey are mainly based on the study design (5), the long patient recruitment period, and the non-randomization of the treatment protocols. In addition, since MRI was not performed at the beginning of prophylaxis in all patients, the possibility that changes in joint architecture were not found at the end of the follow-up period has to be discussed as a further shortcoming of the study. Patients who were enrolled from the different pediatric haemophilia treatment centers in Germany have been treated over the entire study period by the same medical teams using the same treatment protocols. The patients enrolled have been on treatment regimens that have remained unchanged with respect to treatment indications and the criteria chosen to treat a bleed. Finally, the frequency-matching

according to the year of birth and geographic catchment area contributed to better comparability between the patients enrolled. However, since the follow-up of the patients enrolled in this database will continue, we will have the unique opportunity to re-evaluate the joint scores within the next ten years to clarify whether the results presented herein will be confirmed.

Thus, no conclusion can be drawn from the data presented here whether primary prophylaxis or an early switch from on-demand to secondary prophylaxis is superior with respect to joint outcome in children with severe HA. However, compared to randomized trials in which highly selected patients are studied, data presented here reflect the “real life” situation in HA children and their families in Germany. Results of the present study allow us at least to discuss that long-term continuous treatment perform-

ed as primary or early secondary prophylaxis should be initiated as early as possible, i.e. before a second symptomatic joint bleed into the same joint within a six month period will occur. To overcome the limitations of the present and other retrospective cohort studies, prospective large-scale and long-term studies in previously untreated haemophilic children are required in the near future to further address the topics of interest.

Acknowledgements

The authors thank all of the technicians from the participating laboratories, in particular Sabine Thedieck, Annette Sander and Heike Ringkamp for excellent technical assistance. In addition, we thank Gwyneth Schulz for help in editing this manuscript, and Carmen Escuriola Ettingshausen and Wolfhart Kreuz for helpful discussions.

References

1. Nilsson IM, Berntorp E, Löfqvist T, et al. Twenty-five years' experience of prophylactic treatment in severe hemophilia A and B. *J Intern Med* 1992; 232: 25–32.
2. Astermark J, Petrini P, Tengborn L, et al. Primary prophylaxis in severe hemophilia should be started at an early age but can be individualized. *Br J Haematol* 1999; 105: 1109–1113.
3. Fischer K, van der Bom JG, Mauser-Bunschoten EP, et al. Changes in treatment strategies for severe haemophilia over the last 3 decades: effects on clotting factor consumption and arthropathy. *Haemophilia* 2001; 7: 446–452.
4. Ljung R. Paediatric care of the child with haemophilia. *Haemophilia* 2002; 8: 178–182.
5. Blanchette VS, Manco-Johnson M, Santagostino E, et al. Optimizing factor prophylaxis for the haemophilia population: where do we stand? *Haemophilia* 2004; 10/4: 97–104.
6. Fischer K, van der Bom JG, Molho, et al. Prophylactic versus on-demand treatment strategies for severe hemophilia: a comparison of costs and long-term outcome. *Haemophilia* 2002; 8: 745–752.
7. Carlsson KS, Höjgard S, Glomstein A, et al. On-demand vs. prophylactic treatment for severe haemophilia in Norway and Sweden: differences in treatment characteristics and outcome. *Haemophilia* 2003; 9: 555–566.
8. Kern M, Blanchette V, Stain AM, et al. Clinical and cost implications of target joints in Canadian boys with severe hemophilia. *J Pediatr* 2004; 145: 628–634.
9. Rahi JS, Manaras I, Tuomainen H, et al. Meeting the needs of parents around the time of diagnosis of disability among their children: evaluation of a novel program for information, support, and liaison by key workers. *Pediatrics* 2004; 114: e482.
10. Berntorp E, Astermark J, Björkman S, et al. Consensus perspectives on prophylactic therapy for haemophilia: summary statement. *Haemophilia* 2003; 9: 1–4.
11. Ota S, McIlmont M, Carcao MD, et al. Definitions for haemophilia prophylaxis and its outcomes: The Canadian Consensus Study. *Haemophilia* 2007; 13: 12–20.
12. Nuss R, Kilcoyne RF, Geraghty S, et al. MRI findings in haemophilic joints treated with radiosynoviothetesis with development of an MRI scale of joint damage. *Haemophilia* 2000; 6: 162–169.
13. Thomas DC, Greenland S. The relative efficiencies of matched and independent sample designs for case-control studies. *J Chron Dis* 1983; 36: 685–697.
14. Kreuz W, Escuriola Ettingshausen C, et al. Prevention of joint damage in hemophiliac children with early prophylaxis. *Orthopäde* 1999; 28: 341–346.
15. Kreuz W, Escuriola-Ettingshausen C, Funk M, et al. When should prophylactic treatment in patients with haemophilia A and B start? The German experience. *Haemophilia* 1998; 4: 413–417.
16. Mayer D. Essential evidence-based Medicine. Cambridge University Press 2004; 58–60.
17. Pettersson H, Nilsson IM, Hedner U, et al. Radiologic evaluation of prophylaxis in severe haemophilia. *Acta Paed Scand* 1981; 70: 565–570.
18. Bogdanova N, Markoff A, Pollmann H, et al. Spectrum of molecular defects and mutation detection rate in patients with severe hemophilia A. *Hum Mutat* 2005; 26: 249–254.
19. Escuriola Ettingshausen C, Halimeh S, Kurnik K, et al. Hemophilia phenotype is dependent on the presence of prothrombotic risk factors. A multicenter cohort study. *Thromb Haemost* 2001; 85: 218–220.
20. Gouw SC, van der Bom JG, van den Berg HM, et al. Treatment related risk factors of inhibitor development in previously untreated patients with hemophilia A: the CANAL cohort study. *Blood* 2007; 109: 4648–4654.
21. Manco-Johnson MJ, Abshire TC, Shapiro AD, et al. Prophylaxis versus episodic treatment to prevent joint disease in boys with severe hemophilia. *N Engl J Med* 2007; 357: 535–544.
22. Fischer K, Astermark J, van der Bom JG, et al. Prophylactic treatment for severe haemophilia: comparison of an intermediate-dose to a high-dose regimen. *Haemophilia* 2002; 8: 753–760.
23. Van den Berg HM, Fischer K, Mauser-Bunschoten EP, et al. Long-term outcome in individualized prophylactic treatment of children with severe haemophilia. *Br J Haematol* 2001; 112: 561–565.
24. Fischer K, van der Bom JG, Mauser-Bunschoten EP, et al. The effects of postponing prophylactic treatment on long-term outcome in patients with severe hemophilia. *Blood* 2002; 99: 2337–2341.