Nordic Hemophilia Guidelines

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Hemophilia in the five Nordic countries

Background

In hemophilia A and B, coagulation factors VIII (FVIII) or IX (FIX), respectively, are absent or have deficient function. This impairs the ability of the blood to coagulate leading to increased risk of serious and life-threatening, often delayed, bleeding. Factor XI deficiency (hemophilia C) and other coagulation and factor deficiencies as well as rare acquired forms of hemophilia are not the topic of this chapter.

Both hemophilia A and B are inherited in an X-linked recessive manner and, therefore, almost exclusively males have the phenotype. Homozygosity and lyonisation may leed to hemophilia in females.

The clinical severity of hemophilia A and B closely correlates with the level of activity of FVIII or FIX, respectively. Severely affected individuals have <0.01 kIU/L activity level in plasma (<1% of normal); moderately affected 0.01-0.05 kIU/L (1-5% of normal); and mildly affected >0.05-<0.04 kIU/L (>5%-<40% of normal) (1). If inadequately treated, severe hemophilia inevitably will cause spontaneous painful bleeding into joints and muscles. Iron deposition in the cartilage will lead to inflammation and hemophilic arthropathy caused by degeneration of the cartilage and gradual wearing and tearing of bone structure.

Gradually severe disability will develop due to arthopathy, muscle waisting and contractures. Serious bleeds also can occur in internal organs, e.g. the brain, with and without trauma or following surgery. Intracranial hemorrhage can have grave consequences, including paralysis and death. In mild hemophilia abnormal bleeding occurs following operations or trauma, whereas the clinical severity of moderate hemophilia varies from mild to severe.

A family history of hemophilia is often the reason for referral but 30-50% of new cases have no prior family history (2). When hemophilia is not known in the family, severe hemophilia may be suspected due to abnormal bleeding from the umbilical stump, following circumcision or when unusual bruises or hematomas are noted in infant boys, sometimes leading to wrongful suspicion of child abuse. When the boy begins to crawl and walk, abnormal unexplained bruising or limping may occur due to hemarthrosis. Intracranial hemorrhage may also occur during infancy. When no

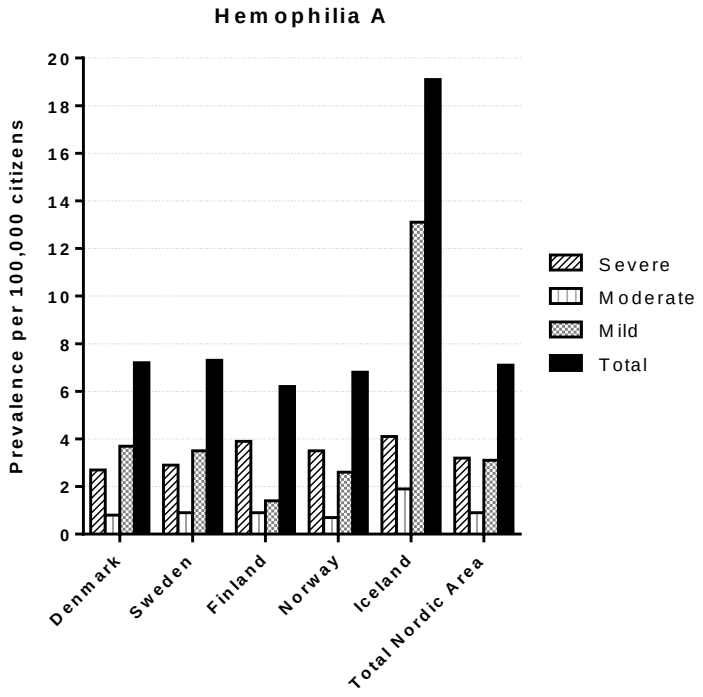
family history is present, however, moderate and, in particular, mild hemophilia may not be diagnosed until adult life following a hemostatic challenge such as wisdom tooth extraction. In mild patients trauma and other bleeds may not awake the necessary attention and the outcome may become serious. As discussed above, the clinical severity and complication rate are strongly related to the factor concentration. Occasionally, female carriers are mildly symptomatic, i.e. if the FVIII less than 0.30-0.40 kIU/L (3).

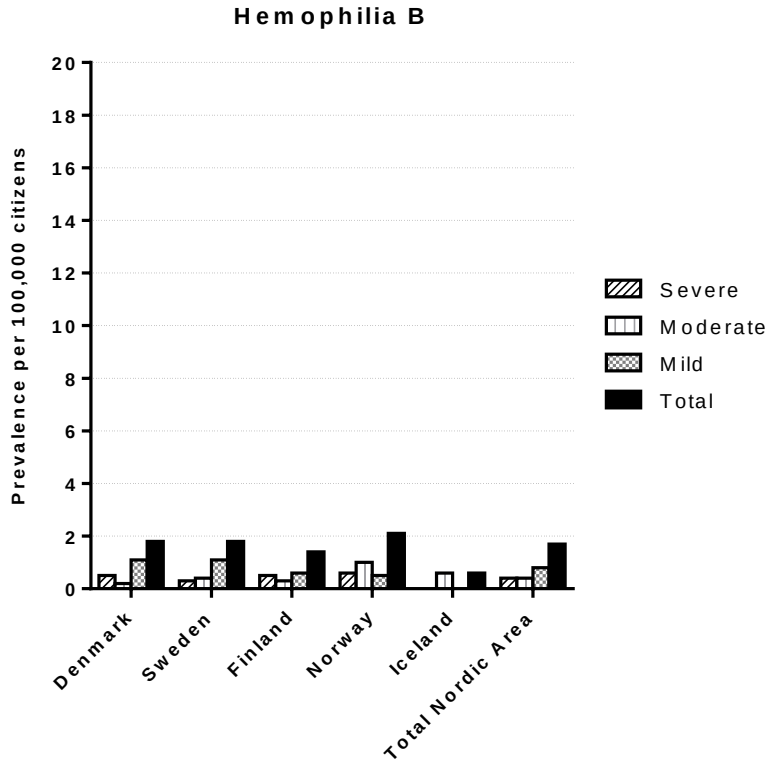
Prevalence of hemophilia in the Nordic countries

Hemophilia A affects approximately 1 in 5,000 males world-wide whereas hemophilia B is less common (4). The Nordic Hemophilia Council (NHC) surveyed the hemophilia centers in the five Nordic countries at the end of year 2012 and found 1,851 patients with hemophilia A, i.e. severe 833, moderate 221 and mild 797 in a total population of 25.9 million (previously unpublished results). The prevalence of hemophilia A per 100,000 inhabitants therefore is 7.1 (total), 3.2 (severe), 0.9 (moderate) and 3.1 (mild). This corresponds to one in every 7,042 males, 45% of whom have severe hemophilia A.The prevalence of severe hemophilia is similar at all the centers. The prevalence of hemophilia, in particular mild hemophilia is unusually high in Iceland, see Figure 1.

Hemophilia B was present in 1.7 per 100.000 inhabitants with 0.4 (severe), 0.4 (moderate) and 0.8 (mild) per 100,000 inhabitants, see Figure 1. This corresponds to one in every 29,411 males, 25% of whom has severe hemophilia B.

Figure 1: Prevalence of hemophilia A and B per 100,000 inhabitants in the Nordic countries at the end of year 2012





Hemophilia treatment in the Nordic countries

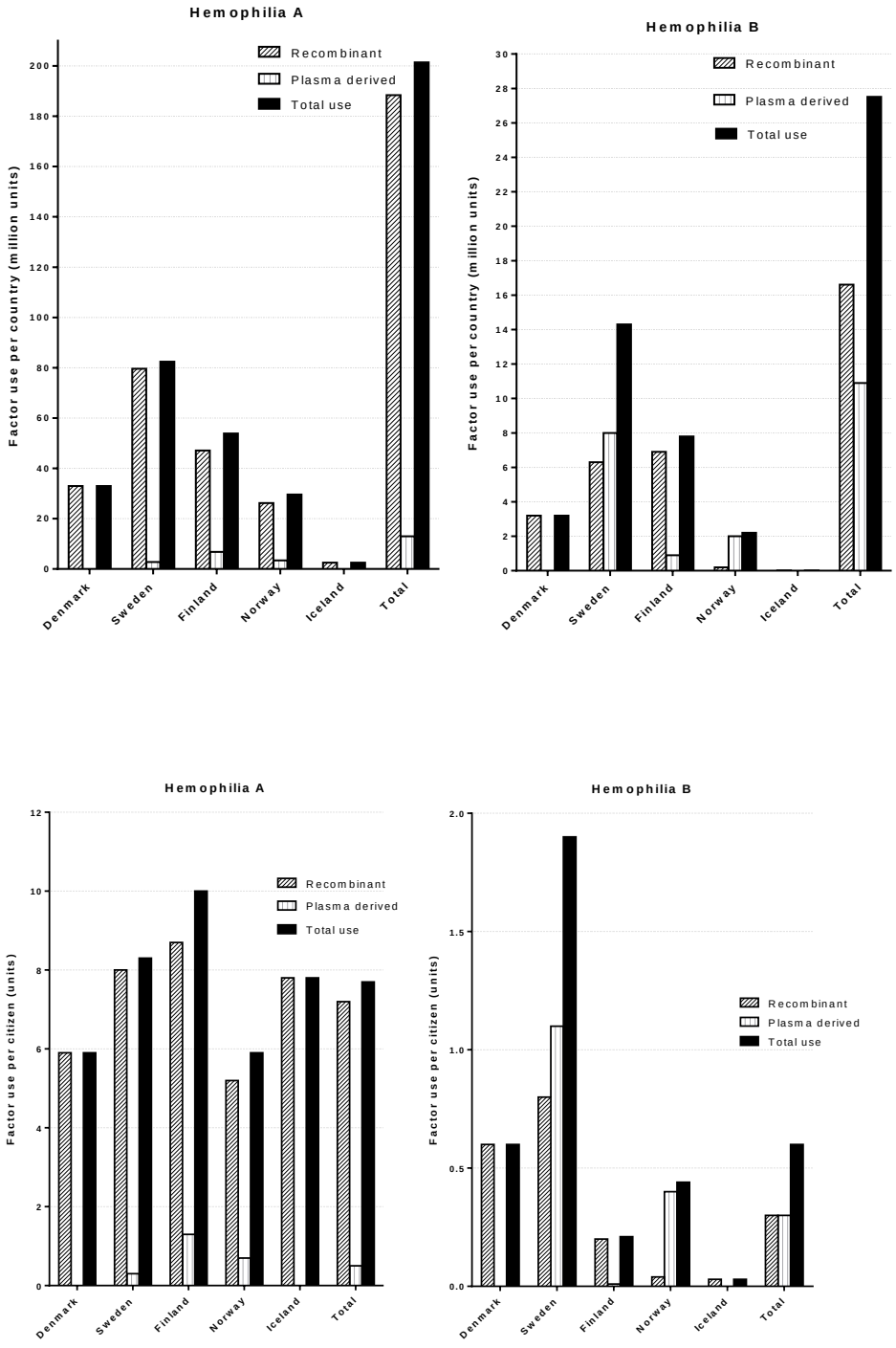
Prior to the availability of effective therapy, patients with severe hemophilia had a mean life expectancy of only about 16 years. However, since the late 1950's the life expectancy of a newborn severe PWH receiving some form of replacement therapy has increased steadily (5). In 1960 the average life expectancy had risen to 23 years in Sweden and it is now approaching normal in the Nordic countries, all of which now practice early and continuing prophylactic factor replacement therapy.

A plasma protein fraction correcting coagulation in hemophilia blood was first described in 1937 but only later termed coagulation factor VIII (6). In the 1950s, Margareta and Birger Blombäck at the Karolinska Institute in Stockholm while working on a method to purify fibrinogen by treating Cohn's Fraction I with a glycine solution found that fibrinogen and Factor VIII (and also as it later turned out, von Willebrand factor) remained as precipitates, while prothrombin, plasmin and other proteins were washed off. Together with Inga Marie Nilsson, a young scientist and physician from Malmö General Hospital, Margareta found that factor VIII could be almost completely recovered from this fraction designated "Cohn's fraction 1-0" (7). A sterile preparation of fraction 1-0 was injected for the first time to Inga Marie's patient in May 1956 at the Malmö General Hospital. The patient was a young female patient with life-threatening menstrual bleeds and a prolonged bleeding time (i.e. with severe von Willebrand disease). The girl's bleeding stopped promptly, her Factor VIII activity increased to a high level and her bleeding time was normalised. After this, the Blombäcks began preparing Fraction 1-0 from plasma for PWHs with impressive efficacy. Industrial production of Fraction 1-0 by Kabi pharmaceuticals was started in 1964. Calling the product AHF (antihemophilic factor), Kabi became one of the two first commercial producers in the world of Factor VIII concentrates. Although this first AHF concentrate was of low purity and contained large amounts of fibrinogen, it was used for many years to treat hemophilia and, as it also contained von Willebrand activity, for treating von Willebrand disease. Indeed, the introduction of Fraction 1-0 led to effective hemophilia care in Sweden, a decade earlier than in most other countries. It was only about 10 years after Inga Marie's initial injection that effective therapy started elsewhere using cryoprecipitate. For more detailed description on the history of factor VIII discovery and production see also Ahlberg et al (8).

Again, the Swedish group pioneered in hemophilia treatment by starting prophylactic therapy in young boys in 1958. During the 1970's and 1980's increasingly more concentrated products were produced, and when the injection volume decreased the freeze-dried factor concentrates became available for home treatment. Although prophylactic therapy caused a dramatic improvement in the othopedic outcome of PWHs in Sweden and the Nordic countries (9), the value of costly prophylactic therapy was not generally recognized outside the Nordic area until many decades later when a prospective randomized trial finally conducted demonstrated the markedly improved clinical outcome of boys receiving early prophylaxis (10). Recently, data from Malmö has shown that not only the joint score but, importantly, the overall quality of life of PWHs treated with prophylaxis in Malmö has close to normalized, in particular in those patients who have been treated with primary and continuing prophylactic therapy (11).

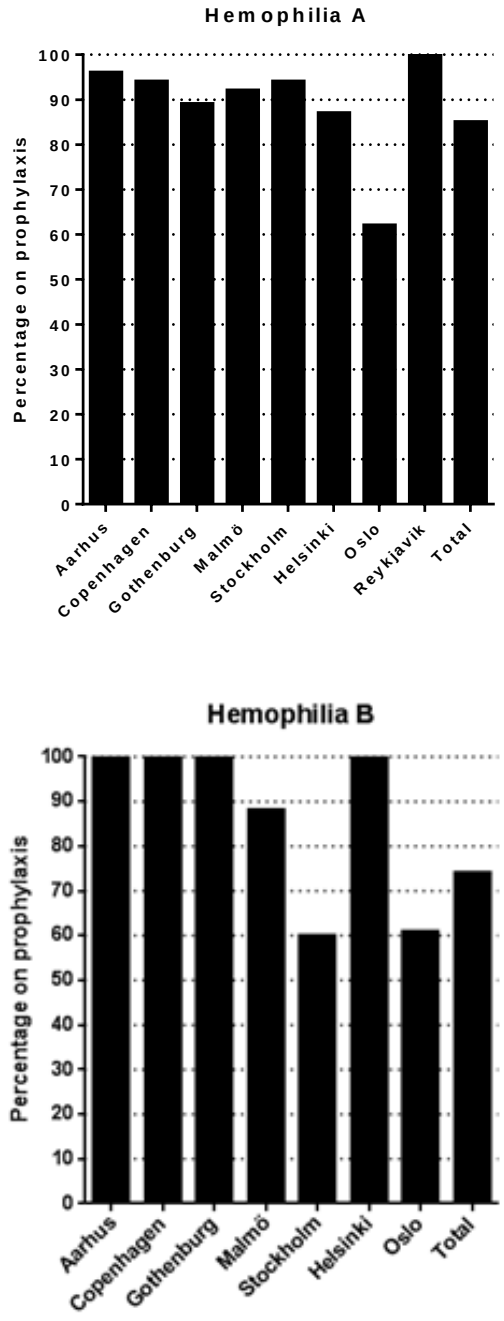
Currently, all the five Nordic countries practice primary prophylaxis in severe hemophilia A using preferably recombinant products (Fig. 2). Variation in factor usage can be explained by differences in treatment policy regarding prophylaxis, number of major surgeries, number of patients undergoing ITI during the year studied.

Figure 2: Factor VIII and factor IX concentrate consumption in the Nordic Countries in 2012. Factor use per country is shown in upper panels and factor use per citizen in the lower panels



Factor replacement is often started before the child starts walking. According to our survey, in 2012, 85% of patients with severe hemophilia A and 74% with severe hemophilia B received regular prophylactic replacement therapy (Fig. 3).

Figure 3: Percentage of non-inhibitor patients with severe hemophilia A and B on regular prophylaxis in the Nordic countries. Prophylactic therapy comprises about 80-85% of all factor use



Complications of hemophilia treatment

Until year 1985, as cryoprecitate and later plasma-derived coagulation factor concentrates were not virus inactivated, there was a high rate of hepatitis B and C and, in the late 1970's and early 1980's, of HIV transmission in PWHs. Most PWHs infected with HBV, some with HCV, and none with HIV cleared the virus. Close to 90% of severe PWHs receiving factor concentrates before year 1986 in the Western worlds were infected with HIV and AIDS was a major cause of morbidity and mortality in PWHs in the 1980's and 1990's before effective treatment was available. Hepatitis C has become curable with drug treatment in many cases. Due to the use of locally produced plasma dervived factor VIII concentrates in Norway only 14 patients were infected with HIV, in Finland only two patients and in Iceland none were infected. However, hepatitis C was transmitted to about 30-60% of patients in Norway, Finland and Iceland. Figures in Sweden reached just above 80%. Since 1986 all available plasma derived and recombinant concentrates have been virus inactivated preventing transmission of the above encapsulated viruses and, fortunately, no hepatitis B, C or HIV transmission has occurred after their introduction. Nevertheless, patients and care-givers alike remain concerned that the current measures to eliminate viruses will not entirely prevent transmission of known and unknown non-encapsulated viruses and prions, e.g. variant Creutzfeldt-Jacob disease (12).

A second, major remaining problem associated with factor concentrate use is the development of inhibitory antibodies to the transfused coagulation protein that is alien to patients with severe hemophilia and induces an alloimmune response in around 30% of them (12). This will be discussed in more detail in a later section of the NHC guidelines. Based on the 2012 NHC survey, in the Nordic population 112 out of 833 severe hemophilia A patients (13.4%) and 14 out of 113 severe hemophilia B patients (12.4%) have a history of current or suppressed inhibitor (Fig. 4).

Figure 4: Current and previous inhibitor patients at the Nordic centers (severe hemophilia)

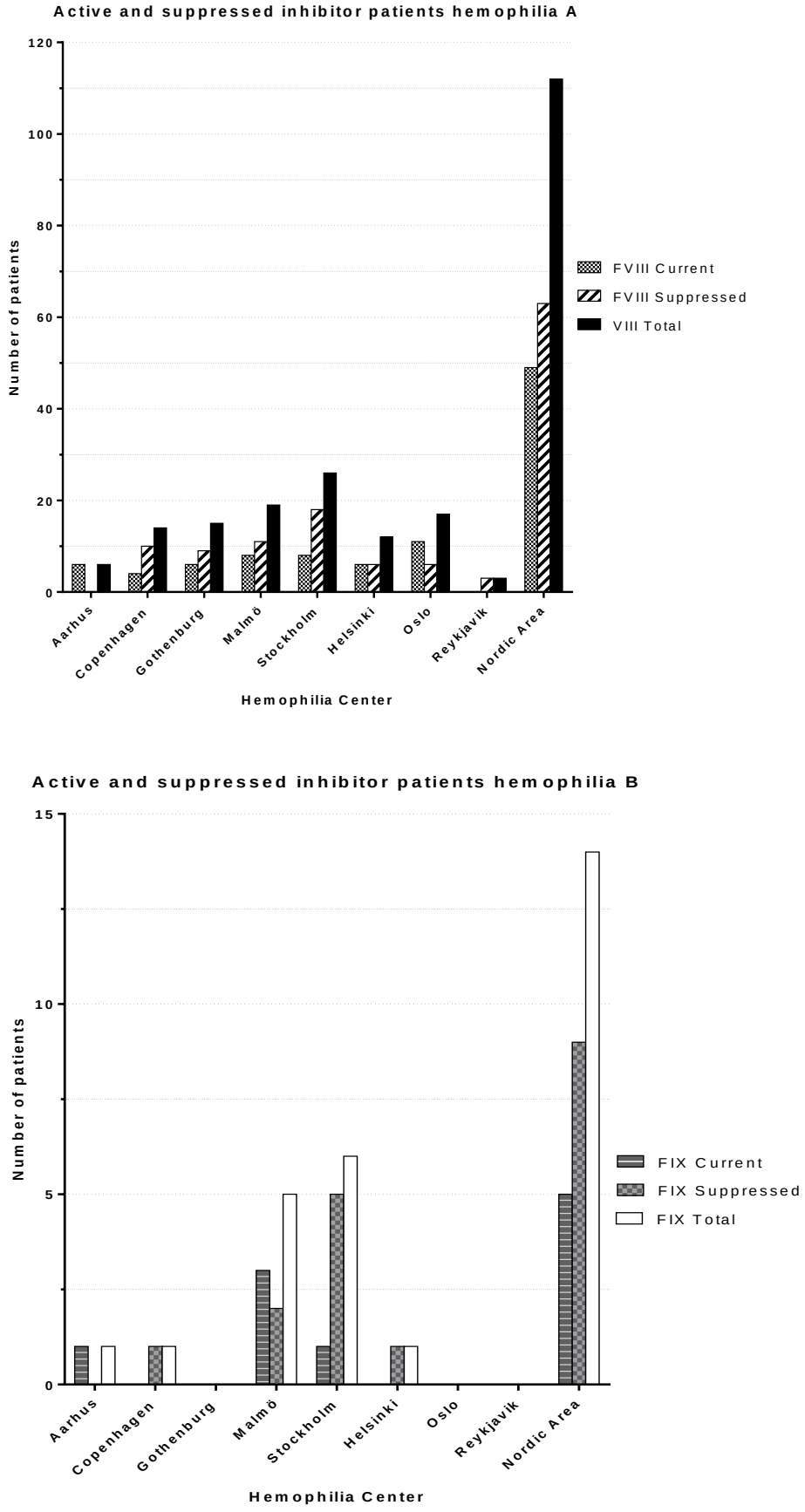


Table 1: NHC 2012 survey: Number of hemophilia A/B patients, referral populations of each center and country populations according to year 2012 official census of patients registered at Nordic Hemophilia Centers at end of year 2012

	Center	Severe A/B	Moderate A/B	Mild A/B	Total A/B	Approximate referral population size	Country population size (census)
DENMARK		152/30	45/9	209/63	406/102	5,585,509	5,580,000
	Aarhus	83/9	33/5	131/33	247/47	3,048,020	
	Copenhagen	69/21	12/4	78/30	159/65	2,537,489	
SWEDEN		283/30	91/35	340/102	714/167	9,600,000	9,556,000
	Gothenburg	54/5	10/5	73/26	137/36	1,900,000	
	Malmö	103/24	26/19	92/40	221/83	3,300,000	
	Stockholm	134/33	57/10	178/37	369/80	4,400,000	
FINLAND	Helsinki*	212/25	46/16	75/30	333/71	5,400,000	5,422,000*
NORWAY	Oslo	173/28	33/52	131/24	337/104	5,000,000	5,034,000
ICELAND	Reykjavik	13/0	6/2	42/0	61/0	320,000	320,000
NORDIC AREA		**833/113**	**221/114**	**797/219**	**1851/446**	**25,905,509**	**25,912,000**

Organisation of hemophilia care – the EUHANET criteria

Since the start of the treatment of hemophilia and other bleeding disorders the aim of the management has been to transform the severe disease form to a moderate or mild one. The expert hemophilia care, i.e. regular replacement therapy or prophylaxis to avoid unnecessary bleeding complications, is best tailored by the comprehensive care centers (CCC). CCC organizes different disciplines around the patient's medical needs throughout the life of the patient. On call services at 24/7 necessity secures the expert management during emergency. Provision of early diagnosis, pediatric and family care, through the adolescent years and transition clinics, genetic counseling, including attention to carrier and obstetric issues (see chapter "Carriers of hemophilia"), leads to the optimal comprehensive management to all patients and families with this genetic disease.

In the future, new medical challenges among the ageing hemophilia population will call upon specific attention. The treatment of age-related diseases, such as cancer and cardiovascular disease, creates circumstances which may modify the bleeding disorder and therefore need to be adopted according to the requirements of hemophilia (13). In Europe it is the current national responsibility to organize the centralized care of rare diseases overall, and in the case of hemophilia great benchmarks already exist. The local policies, support from the authorities and national bodies should be engaged to foster the above aims. The EUHANET project is an EU-funded effort to harmonize hemophilia care in Europe.

The historical role from the first injection of a FVIII concentrate given in Sweden to the developed modern care paved the way for the hemophilia treatment worldwide (9). The fundamentals are built on the close, continued interaction between the laboratory and clinics. This interaction has established the diagnosis, provided opportunities to tailor prophylaxis, treatment of bleeds and management of major surgery with proper dosing of coagulation factor and appropriate follow-up. Also, the diagnosis of the significant complications of hemophilia, i.e. inhibitors and infections, are based on laboratory medicine. In fact, the laboratory services are needed for 24 h on 7 days to cover emergency services and are implemented in the new EUHANET criteria.

Until now the official criteria for a hemophilia center have not existed, but after the experience of European Hemophilia and Allied Disorders Surveillance System (EUHASS), the follow-up project EUHANET aims at unifying the treatment quality in Europe and beyond (14). The suggested criteria of EUHANET (Table 2) accord well with the current operative functions in our Nordic centers. Our national or center-related patient numbers belong to the small center category, but the patient numbers treated in a center as such will not limit the quality targets and provision of CCC activities. The provision of care needs constant surveillance and development to secure optimal operations and support. Under the current economical constraints the driving force of center leaders and practical staff motivates the community to establish, maintain and strengthen the discipline according to the local needs.

The two categories of centers according to EUHANET include activities both of European Hemophilia Comprehensive Care Centers (EHCCC) and Hemophilia Treament Centers (EHTC). As our small Nordic populations are concentrated in the large cities, networking activities within the countries are needed. The national guidelines for the management of hemophilia have so far been available only in Sweden, but the Nordic Hemophilia council platform will update the current uniform recommendations for the diagnosis and management of coagulation disorders. As examples, the Council activity has already provided three guideline documents, i.e. acquired hemophilia (www.nordichemophiliacouncil.org), von Willebrand disease and heparin-induced thrombocytopenia (15, 16). The Nordic cooperation has been ongoing since decades and was formalized in 1999.

Our local national backgrounds vary somewhat, but culturally the Nordic countries are very close. However, during the HIV catastrophe our respective countries were confronted with the unexpected viral transmissions with different incidences. The patient populations in the Nordic countries were affected by varying rates of HIV.

Multidisciplinary activities

According to the recommendations of World Federation of Hemophilia and European Association of Hemophilia and Allied Disorders (EAHAD and EUHANET) multidisciplinary activities should be readily available for patients with hemophilia (17, 18). The CCC activities have been shown not only to reduce mortality but decrease morbidity and days of absence from school and work due to the efficacious treatment

(18). The patients need constant opportunities to consult the Center in any practical daily life and acute problems. Many times these consultations associate with rehabilitation after a major bleed or surgical intervention, but may affect any discipline related to hemophilia care. Algorithms for emergency care aim at securing immediate management options to avoid worsening complications and increasing treatment costs due to delayed replacement therapy.

The key route of proper management includes the intravenous injections, which may become problematic during the years. The pediatric mode of administration varies between the Nordic countries. In Denmark and Finland at the age of around one year a Port-a-cath is inserted to all children. It is managed with a meticulous strategy to avoid infections and other complications. The Port-a-caths may cause collateral formations in the vasculature in the central venous system, the clinical impact of which is unknown. On the other hand, the peripheral vein utilization may subject the patient to repeated subcutaneous exposure of the clotting factor which may be immunologically harmful. High dose prophylaxis is uniformly the method of choice in the Nordic centers.

In case of sudden inhibitor urgent bypassing therapy with either FEIBA® or NovoSeven® should be offered to control bleeding tendency, and immune tolerance induction (ITI) should be undertaken to regain rapid tolerance to the traditional therapy with FVIII. Scandinavian centers have actively studied inhibitor development and participated to international ITI programs, such as ProFeiba and ObsITI to gather wide international experience.

Registries

Surveillance of treatment safety and health economics is of utmost importance in hemophilia. The traditional inhibitor frequency may alter, new concentrates with their short pre-registration follow-up enter the market and new viral entities may appear, demanding continued surveillance. All Nordic CCCs have reported to EUHASS, which monitors mortality and the main health hazards including incidence of inhibitors, infections and thrombotic complications associated with treatment of hemophilia and allied disorders.

The register capturing should be developed uniformly in Europe to be able to compare the treatment across centers and to participate to clinical studies without any large changes to the daily routines to ease the patient recruitment (19).

Outcome analysis, QoL and health economy

The physical and outcome evaluation of the patient should occur based on an established protocol including a functional self-assessment (HAL) and objective performance and joint status should be evaluated and data collected to a register for comparisons. The basic SF-36 quality of life assessment tool or agreed methods of assessing the quality of life should be implemented to the patient management to provide an objective tool to evaluate the impact of the replacement therapy.

The regular prophylaxis is expensive but gains good quality of life in comparison with on demand-type of treatment. The treaters should raise active awareness of the costs of the treatment and look for the most cost-efficient individual solutions. Active individualisation of the dose is needed both in patients who do not experience any bleeds and among thos who experience more than two bleeds yearly. Once daily injection may be an option, high dose injections once a week may qualify in hemophilia B, and the newly developed molecules with extended half-life should be carefully evaluated for their health economical value. These kinds of data can only be captured on the registries.

Table 2: EUHANET criteria (20). Recommended application of the center status CCC and HTC

- Delivery of hemophilia care
- Standard and general requirements
- General policy and objectives, policies and procedures
- Record and data collection
- Organisation, personnel appraisal and continuing education
- Supply and management of therapeutic products, reagents and medical devices
- Quality planning, evaluation and improvement
- Participation in registries related to inherited and acquired bleeding disorders
- Participation in clinical research
- Awareness, information and education of patients and their families
- Diagnosis of hemophilia and other related bleeding disorders and all forms of acquired hemophilia
- Therapy of hemophilia and other related bleeding disorders and all forms of acquired hemophilia
- Treatment programme, prophylaxis, home treatment plan
- Treatment of acute bleeds and prevention, emergencies, treatment outside normal working hours
- Elective surgery
- Treatment of patients with inhibitors, including immune tolerance
- Treatment of patients with chronic viral infections
- Treatment of patients with acquired hemophilia and acquired vWD
- Periodic clinical and multidisciplinary review
- Genetic services
- Outcome indicators
- Advisory service
- Network of clinical and specialised services in conjunction with the hemophilia team

Laboratory diagnosis

Pre-analytical aspects of hemophilia testing

The pre-analytical phase is often equal with the time from the blood collection to the point when the sample is analyzed in the laboratory. Errors at this phase are often explained by incorrect specimen collection, transportation or storage. Other important aspects may be related to the patient itself i.e. anticoagulant medication that may interfere with the assay or an abnormal hematocrit, which may lead to an improper blood to citrate ratio in the test tube.

In order to reduce the pre-analytical error rate it's important to understand the sources of variability and mechanisms that may lead to false assay results. It is also important to understand that coagulation tests are exceptional susceptible to suboptimal sample quality as the sample collection itself will initiate a hemostatic response. Thus, improper sample collection technique and/or incorrect handling prior to analysis will increase the risk of having the coagulation system activated to the extent that screening as well as specific factor assays can lead to mismanagement of the patient. This is particularly true for hemophilia testing as FVIII is one of the most labile coagulation factors and is degraded with time *in vitro*.

There are several published guidelines, written by experts in the field, how to assure sample integrity during the pre-analytical phase. An often referred general guideline how to collect, transport and process blood samples prior to coagulation testing is published by the Clinical and Laboratory Standards Institute (CLSI) (21). According to the book "Quality in Laboratory Hemostasis and Thrombosis" from 2009, the ideal plasma sample for hemostasis testing is obtained if the following determinants are carefully considered (22):

- Venipuncture: Ensure atraumatic phlebotomy with minimal tourniquet use.
- Collection tube and order of draw: Draw 3.2 % light blue stopper first or only after a non-additive tube.
- Fill tube adequately (no less than 90% fill).
- Adequately thoroughly mix with anticoagulant.
- Transport promptly at room temperature.
- Centrifuge within 1 hour of phlebotomy to obtain platelet poor plasma.

- Test plasma in primary tube or aliquot into a non-activating secondary tube immediately following centrifugation.

Screening of hemophilia

General screening assays, i.e. APTT and PT, are important for the initial laboratory evaluation of patients with bleeding disorders. If congenital or acquired hemophilia A or B is present the APTT will be prolonged and the PT remains within normal limits. Furthermore, in congenital hemophilia the APTT will be corrected on mixing patient plasma 1:1 with normal plasma. If mixing does not correct the prolongation it may indicate the presence of an inhibitor (or other anticoagulants present in the plasma). Unfortunately, there are plenty of commercially available APTT reagents that vary in their sensitivity for coagulation factor deficiencies and all are not suited for detection of mild hemophilia A or B. It is also important to understand that the APTT is a global plasma assay that depends on the sum effect of 10 different coagulation factors and under certain conditions low FVIII or FIX levels, compatible with mild hemophilia A or B, may be masked by increases of one or several of the other factors resulting in a normal APTT. It's not possible to give detailed information about the possibilities and limitations with various APTT reagents in these guidelines. Thus, it is important that the treating physicians are aware about the local screening methodologies, reference intervals and their specific properties. Upon a possible discrepancy between clinical manifestation/suspicion of hemophilia and screening test results it is recommended to measure FVIII or FIX with factor-specific assays (see below).

Specific FVIII and FIX assays

Plasma FVIII:C or FIX:C level represents the functional (coagulation) activity of the factors and can be measured using either coagulation-based or chromogenic assays. It is also possible to measure the mass concentration of the FVIII or FIX antigen with immunologic assays. However, immunoassays are not performed in clinical routine and will not be further discussed here. The FVIII:C and FIX:C assays should be calibrated with material that has traceability to current international standard for FVIII or FIX in plasma (23). In this way the unit is given in international units (IU) and one IU is the factor activity present in one mL normal plasma. In the Nordic countries the results are given in kIU/L or IU/mL but in the Anglo-American sphere it is common to use IU/dL, which can cause confusion (IU/dL is the same as percentage in absolute

numbers). Venous blood samples are drawn into evacuated tubes with 3.2% citrate as anticoagulant. When a family history is present, umbilical cord blood is tested in male infants at birth to determine FVIII or FIX levels. For pre-natal diagnosis, see chapter “Carriers of hemophilia”.

Factor VIII:C assays

The *one-stage assay* is the dominantly used assay principle in the world. The main feature of the one-stage assay is that it is based on the APTT test with the difference that the sample is pre-diluted in FVIII-deficient plasma before analysis. In this way, a test system is created that works with the simplicity of the APTT reaction but the pre-dilution procedure makes the FVIII activity in the sample the limiting factor and thus determines the final coagulation time. The ability of the sample to correct the APTT of a FVIII-deficient plasma can be expressed as the FVIII:C activity if the assay is calibrated with a plasma with known concentration of FVIII:C.

The performance of the one-stage assay is affected by the type and quality of the APTT reagent and FVIII-deficient plasma used. The FVIII-deficient plasma can be obtained from a patient with severe hemophilia A (<0.01 kIU/L and no antibodies) but today this plasma is usually purchased from a diagnostic company as an immunodepleted and lyophilized plasma. It is important to check new lots of FVIII-deficient plasmas that it is free from FVIII (<0.01 kIU/L) as this otherwise will compromise the test. According to the discussion about the APTT reagent above, the choice of APTT reagent will have an impact on the general assay characteristics. It is important that the laboratory choose an APTT reagent that has proven capacity to detect all hemophilia categories i.e. mild to severe hemophilia A (24).

Another version of the FVIII:C assay is the *two-stage assay*. The name comes from the assay procedure that involves two separate reactions in a way that makes FVIII:C in the sample being the rate-limiting factor in a variant of the PT-assay. In brief, the first step involves generation of FXa with the help of FVIII from patient plasma (the prothrombin is removed by aluminium hydroxide adsorption in order to inhibit fibrin formation) and in the second step normal plasma is added as a standardized source of prothrombin and fibrinogen and the coagulation time is monitored. Compared to the one-stage assay, the two-stage assay is more demanding to perform and is

therefore only performed in a few specialized laboratories. However, there are several commercial kits available for a *chromogenic assay* of FVIII:C that basically are variants of the two stage assay although the end product is color development instead of fibrin formation. In general, the chromogenic assay is more sensitive to mildly lowered FVIII:C and more specific than the APTT-based one-stage assay and, due to the high dilution factor of the sample, interfering substances influence it less. The chromogenic assay involves two steps; in the first step the diluted sample (or standard) is mixed with a reagent cocktail with purified factors IXa, X and phospholipids, leading to the formation of FXa, and in the second step a specific chromogenic substrate for FXa is added. Cleavage of the substrate yield a color formation in the reaction chamber that is recorded spectrophometrically. The amount of color development is directly proportional to the FVIII:C activity in the sample. This assay is common among the Nordic hemophilia centers. The chromogenic assay is also used by the pharmaceutical industry when the potencies of FVIII concentrates are assigned.

The different FVIII:C assays should give similar result in most cases. However, patients with certain mutations in the *F8* gene causing mild hemophilia A may be missed using the one-stage APTT based FVIII assay. In some cases the one-stage assay result may be several times higher than the chromogenic assay (or the two-stage clotting assay) higher and this phenomenon is called assay discrepancy. In general, the results of the chromogenic or two-stage assay reflect the clinical phenotype in hemophilia A better compared to the one-stage assy. However, in later years it has become evident that there are some genotypes causing inverse assay discrepancy, with lower one-stage assay results in mild hemophilia A. Thus, mild hemophilia A may be challenging to identify correctly in the laboratory, if only one of the assay principles are used. Therefore, it is advisable to have new cases with suspected hemophilia investigated with both one-stage and chromogenic assays in the laboratory work-up (25, 26).

Reference interval: Usually between 0.50-2.00 kIU/L. Local differences may apply.

Interpretation: PWHs A and VWD have low FVIII:C levels. Levels <0.01 kIU/L are seen in severe hemophilia A. Moderate deficiency is characterized with FVIII:C levels

between 0.01-0.05 kIU/L and patients with mild deficiency have higher levels (up to 0.40 kIU/L). Carriers of hemophilia A have usually approximately 50% of the normal activity but occasionally have levels in the mild hemophilia range leading to increased bleeding. Observe that FVIII is an acute phase reactant and its levels may increase several folds under certain conditions (e.g. trauma, infection, etc).

Factor IX:C assays

Until recently, there were only one-stage assays commercially available for clinical use. These assays work in similar ways as described for the one-stage FVIII:C assays above with the only difference that the sample is diluted in FIX-deficient plasma before analysis instead of FVIII-deficiency plasma. Thus, the main FIX:C assay principle is a test system based on the APTT with dilution of the sample (patient or standard plasma) in a plasma lacking FIX, which means that the coagulation process will be limited by the content of FIX in the sample. The assay is calibrated with a standard that is traceable to the current international standard of FIX:C in plasma and results expressed as IU (see FVIII:C above). Venous blood samples are drawn into vacuum tubes containing 3.2% citrate as anticoagulant additive.

Recently, chromogenic FIX:C assays have become commercially available and could be an alternative to the one-stage assay. However, these assays have not yet been fully validated or approved by regulatory bodies (e.g. FDA) for detection or monitoring PWHs B. Nevertheless, local evaluations in Nordic laboratories and others are encouraging and it is likely that these assays will display analytical advantages over the one-stage assays in the same way as been shown for chromogenic FVIII:C assays. If assay discrepancy, caused by mutations in the *F9* gene, is seen in (mild) hemophilia B is not yet known.

Reference interval: Dependent on the assay used, usually around 0.60-1.50 kIU/L but local differences may apply.

Interpretation: Congenital deficiency of FIX is the cause of hemophilia B. Acquired hemophilia B, caused by specific inhibitors exists but is less frequent than the rare acquired hemophilia A. The degree of the deficiency defines the different forms:

severe with FIX:C <0.01 kIU/L; moderate deficiency with FIX:C levels between 0.01-0.05 kIU/L mild deficiency with higher levels up to 0.30 kIU/L. Carriers of hemophilia B express about 50% of the expected normal FIX:C activity. Deficiency of FIX can be observed with nephrotic syndromes.

Antibodies against FVIII or FIX

The hallmark of neutralizing anti-FVIII or anti-FIX antibodies (=inhibitors) is a prolonged APTT and normal PT with a persistent prolongation of the APTT following mixing the patient sample with an equal volume of normal plasma. Most are alloantibodies that have a fast and dose-dependent antigen-antibody reaction but care must be taken in cases of time-dependent autoantibodies, usually seen in acquired hemophilia A. For this reason, it is recommended to incubate the samples up to two hours during a mixing experiment in order to allow the antibody to have effect. FIX inhibitors have faster kinetics and it is usually not necessary to perform longer incubation time than 10 minutes in order to reach completion. For FVIII antibodies it is also important to use buffered normal plasma (imidazole or HEPES) as this stabilizes the FVIII activity during the incubation and will reduce the risk of obtaining false positive results of low titer.

The recommended test procedure for quantitation of the inhibitor titer is the Bethesda-Nijmegen mixing test (inhibitor assay) (27). In brief, the test involves mixing of equal volumes of test plasma with normal plasma of known activity and then measure the residual activity in the plasma mixture. As a control the normal plasma is mixed with an equal volume of FVIII-free plasma. Both test and control samples are incubated for 2 h (shorter time is possible for FIX antibodies) and then the factor activities in both samples are determined. Any residual activity in the sample between 25 and 75% can be used for calculations of inhibitor titer. By definition, one Bethesda unit (BU) is the inhibitor titer that neutralizes 50% of the factor activity in one mL plasma. If the residual activity is less than 25% it indicates an inhibitor titer above 2 BU/mL. Hence, these samples are prediluted in FVIII deficient plasma before analysis until a residual activity within the 25-75% range is reached. The final inhibitor level is then calculated by multiplication with the dilution factor. If several dilutions result in residual activities in the 25-75% range then the dilution that is closest to 50% is chosen for calculation of the inhibitor titer. The Bethesda procedure can also be

performed with porcine FVIII, spiked into FVIII-deficiency plasma, in rare cases when a patient may be treated with FVIII of porcine origin. It is recommended to obtain the inhibitor assay when there is a washout of the use of a concentrate.

Reference interval: The cut-off for a positive result was originally set at 0.4 BU/mL, as the recommendation was not to use any residual activity above 75% (75% residual activity corresponds to 0.4 BU/mL). Later recommendations favor the use of 0.6 BU/mL as the cut-off for positivity as the test is less reliable in the low titer range (reduced risk of false positive results).

Interpretation: The presence of inhibitors may be suspected in patients with unexpected bleedings despite regular prophylaxis. This is also strengthened if the patient display reduced recovery and half-life of the substituted factor.
Note: The Bethesda assay is usually performed on patients with severe type of hemophilia containing no measurable FVIII:C (or FIX:C) activity. If the patient have an activity of 0.10 kIU/L or higher this must be taken into consideration when the inhibitor titer is calculated. It is also possible to remove the endogenous activity by heat-treating the plasma sample at 58˚C for 90 min before analysis.

Genetic diagnosis

Genetic diagnosis is clinically useful to predict the risk to develop inhibitor and for carrier- and prenatal diagnosis. For a detailed description of the genetic diagnosis we refer to the “Practice Guidelines for the Molecular Diagnosis of Haemophilia A or the UKHCDO document "Clinical Genetics Services for Haemophilia" (ISBN 901787 07 9) of the UK Haemophilia Centre Doctors’ Organisation (UKHCDO) (28). See also (29, 30). Depending on the experience and competence of the hemophilia team and the local organisation of genetic services, a clinical geneticist or counsellor can be part of the hemophilia care team.

Genetic diagnosis of severe hemophilia A starts with screening for the intron 22 inversion of the F8 gene which is caused by homologous recombination involving intron 22 and related sequences outside the F8 gene (31). Approximately 40% of cases of severe hemophilia A is caused by intron 22 inversion. Similarly, an inversion involving intron 1 has also been discovered in 1-2% of severe cases which can also be screened for with a PCR technique. In the remaining cases of severe hemophilia

A as well as all other cases the whole F8 gene, 26 exons, must be sequenced since most patients have their own unique mutation. Mutations such as nonsense and deletions, “null-mutations”, will obviously cause severe hemophilia since the DNA reading frame will be altered, mRNA aberrant and no protein will be synthesized. A missense mutation will usually produce a dysfunctional protein with reduced clotting activity but may also result in a “neutral mutation” or a polymorphism. In such cases it is important to know if the same mutation has been reported previously in patients with hemophilia. The FVIII Mutation Database, at present (March, 2015) includes 5,472 individuals with 2,015 unique mutations (32). In a few percentage, no mutations will be found despite sequencing of the whole gene, some of these cases having a more complex genetic background.

In hemophilia B, the 8 exons of the F9 gene are sequenced and in almost all cases the mutation will be found. No inversions are present in the F9 gene but some patients have complete gene deletions, a strong predictor for development of inhibitors. The Factor IX Mutation Database, at present (March, 2015) includes 3,713 individuals with 1,095 unique mutations (33, 34).

Carrier diagnosis in sporadic case of hemophilia A or B, which encompasses around 50-60% of all newly diagnosed cases, may be a problem. In about 80% the mother of a sporadic case also carries the mutation and is thus a carrier. In the remaining 20% cases no mutation can be found and these women may be true non-carriers or being gonadal mosaics, *i.e.* it is not possible to conclude if she is a non-carrier or carrier.

Prenatal diagnosis (PND) can be achieved by chorionic villus sampling during the 11 to the 13th week of gestation when karyotype analysis can be performed in order to determine fetal sex and PCR can be used to diagnose the mutation within 2-3 working days. The main reasons for PND may be to prevent the birth of a hemophilia affected boy by termination of the pregnancy, to prepare the obstetrical procedures or, for the parents-to-be, to psychologically prepare having a child with hemophilia.

Later in pregnancy amniocentesis can be used as source of fetal DNA. Fetal sex determination can also be made by Y-chromosome analysis in blood from the pregnant woman very early in pregnancy and thus avoiding invasive diagnostic procedures in pregnancies with female foetus. This technique does not yet have sufficient sensitivity for routine clinical use. Lately, pre-implantation genetic diagnosis (PGD) enabling the implantation of female or unaffected male embryos has become

possible (35, 36). PGD is a demanding procedure which however may be indicated in selected cases.

Differential diagnosis

Once a decreased FVIII level has been confirmed, the differential diagnosis includes congenital hemophilia A, acquired hemophila A, severe and moderate von Willebrand disease (VWD) and type 2N VWD (Normandy) (“autosomal dominant hemophilia”). Appropriate investigation to sort this out includes the case history and inheritance pattern, ruling out the presence of inhibitors, measuring VWF:RCo, and, when appropriate, the VWF:FVIII binding which determines the FVIII binding capacity of patient's VWF (37, 38). Definite diagnosis may be dependent on sequencing of the F8/9 and vWD genes.

Concentrate treatment including prophylaxis

Background

Treatment only when acute bleeds occur is called treatment *on demand or episodic treatment.* Even if the bleeding is stopped, pain subsides, and mobility improves, blood remains in the joint, having harmful long-term effects on the articular cartilage. Unnoticed minimal bleeding could occur during on-demand treatment as well as during prophylaxis, causing damage to joints where patients have not had any symptomatic bleeding.

Replacement therapy in reference to hemophilia has been called *prophylactic treatment*. The goal of prophylactic treatment is to prevent bleedings, primarily into the joints, with subsequent development of arthropathy. Importantly, prophylactic treatment will protect also for other serious bleeds such as intracranial bleeds, muscle bleeds and intra-abdominal bleeds. Prophylaxis may be given as primary or secondary, or as episodic. The idea with primary prophylaxis is to start replacement prior to initiation of joint disease. As we do not exactly know how many joint bleeds it takes before cartilage destruction starts, as the bleeding phenotype differs among individual patient and as even subclinical bleeds may occur, it is not surprising that the definition of prophylaxis differs among countries. However, international bodies have tried to define prophylaxis and recently the SSC of the ISTH published their definition (39), Table 3. Cohort studies, especially from Sweden and the Netherlands, clearly show the long-term benefit of prophylaxis (40, 41) and prospective, randomized studies in children comparing prophylaxis and treatment on demand with a follow up time around 5 years are available (10). They show a much better outcome when receiving prophylaxis, even with this relatively short follow-up time. In a comparison between on demand treatment and the Swedish prophylactic strategy, outcome was superior with prophylaxis but to a much higher cost (41).

Lack of knowledge

In the recent Swedish health technology assessment a background report of the topic was published as a systematic review (42). It was concluded that concentrate treatment is efficacious and that prophylaxis is superior to treatment on demand in

terms of number of bleeds occurring. Prophylaxis starting from early age has a protective effect for development of hemophilic arthropathy. These conclusions are strongly supported by recent randomized clinical trials (10). Below are listed some items that often are discussed among treaters and where opinons differ.

Prophylaxis

- When to start
- When/if to stop or taper down
- Dose regimens
- Long term outcome
- Quality of life
- Health economy

Treatment on-demand

- Optimal dosing
- Long term outcome
- Quality of life
- Health economy

Assessment

Physical score

Physical score are performed mainly by physiotherapists and the physical score that is recommended is HJHS (hemophilia joint health score) which takes into consideration both function, pain and signs of arthropathy. HJHS was developed to study early joint disease in hemophilia and has been validated in children up to the age of 18 years (43). Studies in adults are ongoing. Other scores as the Gilbert score is not sensitive enough in patient with no or just minimal joint damage but is still used in some clinical trials.

Quality of life

To evaluate quality life standardized quality of life formulas can be used where the simplest is EQ-5D but also SF-36 is used in many centers.

Hemo-QoL is a validated, disease specific QoL instrument useful in children which exist in different versions depending on the need. As generic instruments, SF-36 may be used.

Imaging technique scores

Different imaging techniques exist and MRI is the most sensitive method to detect early signs of joint damage. The first recommended method to use when signs of joint damage occur is X-ray of the joint and in selected cases MRI is an alternative. Due to the high cost MRI cannot be routinely recommended. MRI is also used in clinical studies. Ultrasound to detect joint disease is a coming diagnostic alternative that needs to be further evaluated.

MRI and US are complementary imaging techniques depending on the circumstances. Validated scoring systems exist for plain X-ray (Pettersson score), MRI (IPSG score and several others) and are being developed for US (44-46).

Bleeding frequency

The patients should be instructed to document bleedings and their injections with clotting factor concentrate in a diary in a prospective way. This can be done in different ways (paper, electronically) and all achievements to encourage compliance are highly needed. Reporting can be stimulated and should be actively asked for at outpatient visits.

Mortality

With an increasing age of the hemophilia population it is very important to document causes of death. Varying result from different retrospective or case-control studies report different results regarding the risk for cardiovascular disease. Historically bleeds have been an important cause of death causing the reduced expected survival time for PWHs. Still many patients in the world do not get appropriate treatment and therefore bleeding is a major cause of mortality and death in many countries. Cancer also has to be considered as an important cause of death due to previous infectious complications with hepatitis C and HIV. Data can be drawn from

patient records or from National Registries if available. Currently, at European level EUHASS registry provides the causes of death for the past four years.

Economic data / cost and outcome

Total annual consumption of clotting factor concentrate (CFC) is an important measurement of treatment cost. Hemophilia-related absenteeism from school or work is health-economic important data for the individual patient or for the family with a hemophilia child/children as well as cost for surgery, adaptations of domiciles etc.

Pediatric issues

The main pediatric issues for prophylaxis are:

- When to start
- Venous access
- Dosing
- Inhibitors

The items are addressed under Recommendations and in the section on inhibitors.

Clinical experience over decades and numerous retrospective and, recently, also prospective studies clearly demonstrate that prophylactic treatment, albeit much more expensive, is superior to on-demand treatment regardless if outcome focus is on number of joint- or life-threatening bleeds or arthropathy, evaluated by radiograph (X-ray) or magnetic resonance imaging (MRI) (9, 10). Opinions vary widely between countries and treatment centers but, in general, the trend is towards an early start, i.e. primary prophylaxis. In principal, there are four different protocols for prophylaxis as outlined in Table 4.

Several studies on record divide prophylaxis into ‘high-dose’ and ‘intermediate-dose’ categories. ‘High-dose protocols’ such as those used in Sweden, are designed to permit individuals with hemophilia to be able to live as normal a life as possible. This translates into guidelines to try to maintain a Factor VIII concentration (FVIIIC) >1% of normal at all times so as to avoid breakthrough bleeds. To do so usually requires the administration of FVIII 10-15 IU/kg/daily or 20-40 IU/kg every second day or at least three times weekly for patients with hemophilia A and every third day or twice weekly for patients with hemophilia B. ‘Intermediate-dose protocols’ are exemplified by protocols developed in The Netherlands, use lower doses administered slightly

less frequently (e.g. 15-25 IU/kg 2-3 times per week). A recent publication compared the Swedish and Dutch regimens (40). The Swedish patients experienced less annual joint bleeds, a greater proportion of patients who experienced no joint bleeds and those with a Pettersson score of zero compared to the 'intermediate dose' group. However, clinical scores and quality of life were rather similar between the two groups. The long-term difference between the two dosage regiments is not yet known.

The trend in Europe and other well off countries (Canada, Australia, etc) has been towards primary prophylaxis, i.e. start before the age of 2 or after the first joint bleed. The rationale behind an early start is that even a small number of joint bleeds can result in irreversible damage, as well as that damage may progress despite prophylactic therapy. It has also been shown that the time point at which prophylaxis is begun is an independent factor for good joint outcome (47). However, it must not be forgotten that the aim of prophylactic treatment is to avoid not only arthropathy but also other serious bleedings such as intracranial hemorrhage (48). It has been shown in a few recent publications, in which start of treatment with a prophylactic approach, in comparison to on demand, seem to decrease the frequency of inhibitors (49, 50). Recent publications also support that FVII should be introduced avoiding concomitant "immunological danger signals" (50). In most cases, an early therapeutic approach is initiated by giving a dose of approximately 25 IU/kg once or twice a week via a peripheral vein, with the aim of increasing the frequency of administration as soon as possible (51). The ultimate goal is to reach full-scale primary prophylaxis, which usually involves the following: in hemophilia A, factor VIII is administered at a dose of 20-40 IU/kg/day every second day or three times weekly; in hemophilia B, factor IX is given at a dose of 30-40 IU/kg/day every third day or twice weekly. However, both the dose and the dose interval have to be individually tailored for each child owing to bleeding phenotype and pharmacokinetic differences between patients. In older children with hemophilia A it is possible to optimize the cost–benefit ratio of treatment by daily injections of FVIII (10-20 IU/kg) (52). The level of the lowest concentration is probably more important than the peak level after injection. However, it is the clinical outcome, not the achieved trough levels, that determines whether the given dose is adequate. Most children can be treated at home by their parents and from the age of 10-12 the child can usually start self-injections. In the future, prophylactic treatment

should be individualized more than it is today. How should this be accomplished is a matter of ongoing research.

The first choice of venous access should be a peripheral vein and in most cases this will be successful. However, venous access can be very difficult and it may be necessary to consider a central venous access device (CVAD) – usally Port-A-Cath. In fact, current practice differs and in e.g. Finland all patients get ports. Introduction of a CVAD entails risks that must be weighed against the potential benefits for individual patients. The most frequent complications with CVADs are infections, mechanical problems and catheter related thrombosis (usually clinically silent). Implantation of a central venous catheter solely on psychological grounds should be discouraged. A child who is merely afraid of venous puncture needs to be helped by other means (53, 54).

Recommendations

- Prophylactic treatment should start at the age of one before joint bleeds occur. It should ideally be administrated once per week, at a dose of 25 IU/kg, in a peripheral vein so the child and parents get used to the new therapy. During the first 20 exposures, intensive treatment and treatment during inflammatory states should be avoided if possible. In hemophilia B treatment should be done in a hospital setting due to the risk of anaphylactic reactions. As soon as venous access allows, the frequency is increased to every second day. If it is not possible within a reasonable time or technically and psychologically difficult to administer home treatment in a peripheral vein, a central venous access device may be considered. These are usually easy for the parents to use but introduce the risk of complications such as infections or central venous thrombosis (usually without clinical symptoms).
- Recombinant rather than plasma derived FVIII/IX products should be used when available. The aim of prophylactic treatment is to enable the child to live a life as normal as possible without hemorrhages and to avoid overprotection.
- Patients with moderate hemophilia with a factor level of 1-2% should usually also have primary prophylaxis. Dose and dose intervals are suggested at 20-40 IU/kg body weight (BW) given every other day in hemophilia A and 2-3 times per week for hemophilia B. Dose is tailored according to clinical

response and dose per kg body weight can often be lowered with age. PK analysis using the Bayesian method should be used to describe and help to optimize treatment. At routine check up the previous factor infusion is registered in detail (time point, dose) and one sample is typically taken at check up for PK calculation. Normally young children with severe or moderate hemophilia are monitored every 6 months and older children and adults every 12 months (mild every 3rd year). If bleeding occurs during prophylaxis, the same dose is given to treat an acute bleed. When the patient is on lower doses during regular prophylaxis, a higher dose is given if bleeding occurs.

- The introduction of products with prolonged half-life may change treatment routines but before these products are introduced more generally in clinical practice, robust study data must be available.
- Patients on prophylaxis or treatment using on-demand as well as patients not requiring concentrate treatment, e.g. mild hemophilia A responsive to desmopressin, will be assessed using national database registries where treatment data are captured. Web based apps together with other tools, should be used to facilitate reporting from patients.
- Assessment should be done using hemophilia joint health score, bleeding frequency, and quality of life instruments i.e. SF-36 or EQ-5D allowing health economic evaluation. Resource use within and outside the health care sector is to be registered such as concentrate consumption, surgical procedures, absence from school or work and early retirement. Joint disease is followed by HJHS. The uses of MRI and /or ultrasonograhy in the routine follow up probably have a potential but need further studies.

Table 3: Definitions of replacement therapy with clotting factor concentrates (39)

Factor replacement therapy	Definition
Episodic 'on demand' replacement therapy	Replacement therapy given at the time of clinically evident bleeding
Regular replacement therapy	Replacement therapy given to prevent bleeding
Primary prophylaxis	Regular continuous* replacement therapy started in the absence of documented joint disease, determined by physical examination and/or imaging studies, and before the second clinically evident joint bleed and age 3 years
Secondary prophylaxis	Regular continuous* replacement therapy started after two or more joint bleeds but before the onset of joint disease documented by physical examination and/or imaging studies
Tertiary prophylaxis	Regular continuous* replacement therapy started after the onset of joint disease documented by physical examination and plain radiographs of the affected joints
Intermittent 'periodic' prophylaxis	Replacement therapy given to prevent bleeding for periods not exceeding 45 weeks in a year

*Continuous is defined as the intent to treat for 52 weeks/year and receiving a minimum of an *a priori* defined frequency of infusions for at least 45 weeks (85%) of the year under consideration.

Table 4: Main dosing strategies for long-term prophylaxis in hemophilia A (55)

Regimen	Dosing principle	Convenience	Efficacy	Cost
Dutch regimen	15-25 IU/kg Start early after occurrence of joint bleeds	+/-	+	-/+
Traditional Swedish (high dose)	20-40 IU/kg Start before joint bleeds	+/-	++	--
Pharmacokinetic (Swedish)	Individualised from high-doseby reducing dose interval and total dose	-	+++	+++
Canadian (dose escalation)	50 IU/kg weekly Intensify stepwise depending on bleeding frequency Start early after occurrence of joint bleeds	+	+	+

+=superior; -=inferior (subjective rating by E Berntorp).

Adolescence

Adolescence is the time of rapid physical, social and cognitive development which occurs during the transition from childhood to adulthood, usually between the ages of 10 and 24 years.This is a challenging time for any teenager and evenmore so for those with a chronic disease. For them it is often harder to break family ties, harder to feel accepted by their peer group and to be realistic about their future. Young teenagers need to move towards independence and for people with hemophilia this includes achieving self-management, maintaining adherence to therapy and coping with the impact of hemophilia on lifestyle (56).

The developmental tasks of adolescence include emotional separation from parents and establishment of autonomy. Peers have a central role in building up the personality. Adolescents seek new experiences and higher levels of rewarding stimulation, and often engage in risky behaviour without considering future outcomes or consequences. Poor compliance with hemophilia therapy during adolescence in combination with risky behaviors, may result in serious and recurrent bleeding episodes with impact on future outcomes. The teenager may for the first time question their medical regimen and be ashamed of the diagnosis (57).

In a global survey of treatment strategies in hemophilia A involving 147 hemophilia treatment centers, compliance was rated according to age. Compliance with all types of prophylactic therapy was the highest in children up to 12 years of age, with 90% high or very high adherence. This number dropped to 54%, however, in adolescents aged 13-18 years (58).

A Scandinavian survey in young men with severe and moderate hemophilia showed that the average age for a patient to take over responsibility for their treatment was 14 years, but 25% required parental assistance in hemophilia-related care until a mean age of 17.2 years. A majority (68%) treated bleeds immediately and 60% used extra infusions when needed. Thus one-third of them put themselves at risk for complications by an unwillingness to recognize the need for treatment. Over 40% had at some time failed to follow the treatment regimen (59).

Caregivers can support adherence by education, encouragement, and by providing positive feedback to the patient.

The perception that treatment is a normal part of life is shown to increase adherence to therapy in adolescents and treatment individualized to patients' bleeding pattern and lifestyle can improve compliance.

The challenges faced by the adolescent should be addressed in the years before transition to the adult clinic. Arranging efficient end caring transfer for young people with hemophila is one of the great challenges in the coming century.

Transition programs are necessary even when pediatric and adult services are in the same hospital, as geographical closeness often does not translate into a close professional relationship. A joint pediatric-adult clinic is very useful to introduce adolescents to adult physicians and to hand over clinical issues. Joint clinics between pediatric and adult health-care teams can improve the transfer and help young people to communicate with the new team.

Inhibitors

Introduction

The development of antibodies is a serious complication of factor replacement therapy. The antibodies bind to the factor VIII or IX molecule and in many cases neutralize (inhibit) the hemostatic efficacy. The incidence of inhibitory antibodies in patients with severe hemophilia A is about 30%, whereas less common in patients with a milder form of the disease. Among persons with hemophilia B, inhibitors are less frequent and usually <5%. The presence of an inhibitor is confirmed using the “Bethesda inhibitor assay” with Nijmegen modifications and classified according to the peak titer into “high” (>5 BU/mL) or “low responding” (<5 BU/mL). The antibodies usually appear within the first 50 treatment doses, but may occur throughout life.

Inhibitory antibodies at low titer can be overcome by saturating levels of the deficient factor, whereas bleedings in patients with high titer need to be treated with “bypassing agents”. These agents will not be affected by the factor VIII or IX inactivating antibodies but induce hemostasis. There are two bypassing agents currently available in Nordic countries; one plasma-derived activated prothrombin complex concentrate (aPCC) and one recombinant coagulation factor VIIa (rFVIIa). These agents are also used in inhibitor patients for the cover of surgical procedures and in the prevention of bleeds (prophylaxis). The most favorable option for inhibitor patients is the eradication of the inhibitor by immune tolerance induction (ITI) therapy. In this therapy, regular infusions of factor concentrates (factor VIII or IX) are administered (usually daily and at high doses) for weeks to years with or without immune-modulating drugs. We recommend that the ITI should be performed according to an international protocol and the patients should be recruited to international studies whenever possible.

Bypassing agents for the treatment of bleeds

Most of the studies of rFVIIa and aPCC are retrospective and observational with low scientific value if one applies strict scientific criteria, but both agents have shown to be effective in the majority of cases. One drawback using these drugs is the cost. Therefore, the treatments with rFVIIa and aPCC need to be optimized to the extent possible. Two randomized head-to-head-studies have been conducted showing a

similar high hemostatic effectiveness with both products. However, a difference in efficacy was observed with the respective products in one and the same patient, suggesting that predictive markers for the treatment response need to be identified (60, 61).

The randomized study by Young et al (61) compared not only rFVIIa with aPCC, but also two treatment doses of rFVIIa in a blinded design. The results suggest in accordance with other case series and cohort studies, that rFVIIa can be administered at a dose of 270 µg/kg on a single occasion, instead of three doses of 90 µg/kg, without reducing the efficacy or exposing the patient to risk (62).

The mechanisms of action differ between aPCC and rFVIIa. Therefore, a sequential or combined use of them has been studied and suggested to improve efficacy (63). The risk of thromboembolic complications however always needs to be taken into account (64), in particular in patients with a central venous access device, and the parallel use of them used cautiously and for the time being only in resistant cases. An algorithm for the use of aPCC and rFVIIa has been defined (65).

Recommendations

FVIII and FIX should be used as the first option in patients with a current low inhibitor titer, in order to saturate the inhibitor and reach a hemostatic factor level. In the case of life-threatening bleeds, irrespective of inhibitor response, FVIII/IX:C should be monitored at least daily. The risk of allergic reactions associated with FIX concentrates should be taken into consideration.

The use of bypassing agents at the doses of aPCC 50-100 IU/kg every 6-12 h or rFVIIa 90-120 µg/kg every 2-3 h is indicated for patients with inhibitor levels >5 BU/mL for treatment of any bleed and in those with high-responding inhibitors but a current low level (<5 BU/mL) in case of a non-life-threatening bleed. Children may need higher doses up to 270 µg/kg of rFVIIa as an initial dose followed by lower doses depending on the hemostatic effect.

rFVIIa is preferred in patients with a known anamnestic response prior to start of ITI, as well as in patients previously not being exposed to plasma products.

Antibody removal by immunoadsorption might be considered in patients with high inhibitor titers in order to allow treatment with FVIII/IX concentrates.

Concurrent use of tranexamic acid should always be considered with rFVIIa treatment, but also in association with aPCC to improve the hemostatic effect.

Higher doses of rFVIIa (up to 270 µg/kg) and/or shorter intervals (<2hrs) should be considered in young children and in the case of treatment failures.

The daily dose of aPCC should routinely not exceed 200 IU/kg.

In hemophilia B patients with inhibitors, rFVIIa is preferred. FIX-containing agents e.g. aPCC should not be routinely used.

In the case of bleeds resistant to monotherapy with each bypassing agent, a sequential use in the order of aPCC (50-75 IU/kg) and rFVIIa (90-100 µg/kg) with an interval of ≥2 hrs or a combined use of aPCC (20-30 IU/kg) and rFVIIa (30-60 µg/kg) may be considered. The risk of thromboembolic complications however always needs to be taken into account.

Bypassing agents to prevent bleeds

Prophylactic treatment with bypass agents is a costly treatment, but may be considered in persistant inhibitor patients and/or phenotypic bleeders to protect against harmful bleeds while waiting for the inhibitor to become eradicated. A head-to-head comparison between the two currently available bypassing agents has not been performed, but available data suggest that both drugs can be used prophylactically to reduce the number of bleeds (66, 67). In patients with low-responding inhibitors, prophylaxis with the deficient factor can be used to prevent against bleeds as well as potentially induce tolerance.

Recommendations

Prophylaxis with rFVIIa (90 µg/kg) once daily or aPCC (50 IU/kg) every other day should be considered in patients with severe and/or frequent bleeds i.e.:

1. One severe/life-threatening bleed
2. Three significant bleeds in the same location within a six month period
3. Significant bleeds requiring by-pass therapy ≥ once monthly

The number and severity of bleeds during prophylactic treatment with bypassing agents should be carefully monitored.

A hemostatic improvement should be required defined as a reduction in the number of significant bleeds with ≥50% within a 2 month period.

Immune tolerance induction (ITI) therapy

ITI treatment with the intent to induce tolerance was described in the 1970s and should be the ultimate goal when possible in all patients with a persistent inhibitor to reduce the risk of harmful bleeds. Successful treatment also has a cost-saving potential. The principle mainly consists of a repeated exposure for the deficient factor with or without the concomitant use of immunosuppressive agents. Several different regimens have been described, many of which seem to have a similar outcome. A decline of the pre-ITI titer to low levels and a low peak before or during ITI seems to mirror a beneficial immune response. One randomized study has so far been conducted - in patients with “good risk” severe hemophilia A and high titer inhibitors comparing high (200 IU/kg/d) and low dose (50 IU/kg 3 times/week) FVIII. No difference in success rate (about 70% in the intention-to-treat analysis) between the treatment arms was seen. However, the time to achieve a negative titer, i.e. the phase with most frequent bleedings, was significantly shorter with the high dose regimen (68).

The other non-randomized studies reported in the literature are difficult to compare since the agents, doses, dose intervals, and definitions of tolerance vary. However, most of the retrospective analyses show tolerance to be induced in up to 60-80% of the cases regardless of the type of agent and dose (69). A higher efficacy rate of von Willebrand-containing FVIII products to induce tolerance compared with more highly purified products has been suggested in patients with unfavorable prognosis. However, additional studies and data are needed to confirm these findings.

ITI and mild/moderate hemophilia

In hemophilia A, up to 25% of new inhibitors occur in patients with mild or moderate disease and changes the bleeding phenotype from mild/moderate to severe (70). Inhibitors most commonly arise following an intensive episode of replacement therapy for surgery or major trauma. The risk of inhibitor development also appears to be associated with some high-risk factor VIII gene mutations. The limited data available in patients with non-severe hemophilia A suggests that when treatment is used, strategies that modulate the immune system, such as the use of rituximab may

have greater benefit than ITI performed with only the deficient factor, but additional studies are needed to confirm these findings. Importantly, the inhibitors might be transient and disappear spontaneously. Therefore, the necessity of eradication treatment should be critically examined for each individual patient (71).

ITI and hemophilia B

ITI treatment in hemophilia B seems to be associated with a less successful outcome compared with hemophilia A. The reasons for this are not known. In addition, the procedure is, in some cases, jeopardized by the occurrence of an allergic reaction and nephrotic syndrome. The use of ITI in these patients therefore needs careful monitoring and should initially be provided in the hospital setting. To reduce the exposure for the deficient factor IX molecule, lower dose and immunosuppressive drugs should be considered, such as the use of steroids, rituximab, cyclophosphamide, cyclosporine, mycophenolate mofetil and/or other agents (72, 73).

Recommendations

All children with confirmed low-responding inhibitor should continue on regular replacement therapy to induce tolerance.

Adults with a low-responding inhibitor should if persistent and, preferentially if bleeds are not successfully treated on demand with the deficient factor, be offered regular replacement therapy to induce tolerance.

Children with high-responding inhibitor, but no bleedings may wait with ITI until decline of the inhibitor - preferentially below 10 BU/mL. In case of bleedings, ITI should be started immediately.

Adult patients with high-responding inhibitors should be offered ITI as for children.

A high factor dose seems to reduce the time to reach a negative inhibitor titer, and since bleeds mainly occur during this period, a dose of 100-200 IU/kg/d should be first-line option whenever possible. Lower dose may however be used with a similar final outcome – at least in so called good risk patients.

No consistent data indicate the beneficial use of one type of product over the others, but in patients who fail the initial attempt of ITI with high purity FVIII, a VWF-containing FVIII concentrates should be considered.

Switch of ITI protocol or discontinuation of ITI should be considered when no further significant decline (approximately 50%) or improvement in clinical phenotype / PK has occurred for 4-6 months.

In resistant cases and in poor risk patients, the combined use of the deficient factor and immunosuppression should be considered – even as first-line treatment in adult patients.

Immunosuppression may be considered as a first-line option in patients with hemophilia B and a causative gene defect such as a gene deletion and/or nonsense mutation.

After successful tolerance the dosing should be tapered to regular prophylactic treatment.

In patients with mild/moderate hemophilia, the possibility of spontaneous remission (≈ 20%) should be taken into consideration and a watch and wait strategy might be advisable before treatment. If persistent, the combined use of the deficient factor and immunosuppression should be considered as a first-line option.

Surgery in hemophilia - practical guidelines

Preoperative planning

Surgical and invasive procedures can be performed safely in PWHs. Due to the increased risk of bleeding complications during surgery, thoroughly planning should be performed prior to surgery. Coordinated standard pre-, intra and postoperative assessment and planning are mandatory (intended) to optimize surgical outcome and utilization of resources, while minimizing the risk for bleeding and other adverse events during and after surgery. Because of the concentration of expertise and experience, it is recommended that any surgery in patients with hemophilia and especially inhibitor patients are planned and executed in conjunction with a hemophilia treatment center (HTC) (74).

The patient's expectations regarding surgical outcome and recovery are also important to explore upfront of an orthopedic procedure. The hematologist should provide a written detailed treatment plan including duration and dosage of hemostatic therapies, also covering the rehabilitation phase.

The patient's hemostatic functions should be screened prior to surgery. Laboratory test as:

Platelet count, APTT, prothombin time, FVIII/FIX level, inhibitor test, fibrinogen, blood group including irregular antibodies and recovery test prior to surgery should be performed. It is important that an inhibitor test is performed recently before surgery and that an in vivo response assessment is performed to test the recovery of a standard dose of the factor concentrate selected for substitution during surgery. Data from these tests can be used to plan the substitution program during and after surgery.

Based on the response (recovery), a substitution program should be outlined, giving exact information on the number of units of coagulation factor to be used and the timing of concentrate infusion during surgery and the entire post-operative period and whether repetitive bolus infusions or continuous infusion are preferred.

The substitution schedule should also provide information about the need for prophylactic treatment during the rehabilitation training program both in hospital and home.

Factor FVIII/FIX should be monitored peri- immediately postoperative and at least once daily in the hospitalized period to adjust the factor levels achieved (75).

Due to an increased risk of inhibitor development during the first 20 exposure days surgery should be postponed if possible.

Thromboprophylaxis should not be administered routinely. In patients with previous VTE, with severe risk factors, such as obesity and active cancer, thromboprophylaxis might be considered.

Substitution principles

In clinical management of surgical episodes in patients suffering from hemophilia, two major substitution principles have been adopted: Bolus injections of factor concentrate every 6-12 h and continuous infusion of factor concentrate by means of a pump delivery system.

Continuous infusion

The continuous infusion (CI) principle has been in use in some hemophilia centers for numerous years. One of the strongest arguments favouring continuous infusion is its superiority in providing the patient with a safe and constant level of the coagulation factor in question by balancing input with clearance. At a reasonably constant factor level, the risk of early and late re-bleeding may be diminished or abrogated. Further, continuous infusion may reduce concentrate spending compared to bolus injections, since peaks of factor level are avoided. However, there are some issues concerning CI practices. The bag system most often used with the pumps has the theoretical risk of infection and /or factor concentrate degradation during storage at room temperature. These questions have been extensively studied and appear not to be a problem within 72 h of CI determined by laboratory testing of stability and sterility. Phlebitis at the infusion site was regularly reported using CI, however this problem is nowadays very seldom seen after small amounts of heparin or LMW-heparin was added to the infusion bag. A quite frequently reported complication is related to loss of battery power or other failures of the delivery pump system. Finally, suspicion has been raised that continuous infusion may be associated with development of

inhibitors, especially in non-severe hemophilia, although medical evidence in standard terms are lacking.

Bolus injections

Bolus injections refer to administration of pre-planned doses of factor concentrate infused at scheduled time intervals. The response to bolus injections is dependent of the dose administered. A sufficient factor level in blood is the one that does not go below a predetermined trough level of factor (immediately before the next dose) and that does not cause untoward bleeding. This means that the immediate pre-dose sample should illustrate the minimum target level of factor that ensures, in the clinical situation, adequate hemostasis. While this value is a critical determinant of bleeding risk, the post-dose factor level may vary a great deal.

A clear disadvantage of using bolus injection strategy is the requirements for frequent injections at 8-12 hour intervals. Since the hemostatic efficacy of concentrate with bolus administration is dependent of the through level, a certain degree of spillage may be demanded to maintain that particular level. Another disadvantage of bolus injection methods is related to the substitution program and its costs. The peak value of factor in blood probably represents an overshoot of factor needed, and thus a relative risk of overuse of factor concentrate.

Major surgery including orthopedic surgery

FVIII/IX level 0.7-1.0 kIU/L immediately before a surgical procedure and replacement therapy for 7-10 days after major surgery are to be targeted. Prophylaxis should then be continued. Tranexamic acid (25 mg/kg p.o / 10 mg/kg i.v.) should be combined with factor replacement 3-4 times daily for 7-10 days.

For the bolus infusion: A bolus dose of approximately 50 IU/kg (FVIII) should be administered just before anesthesia. The dose for giving a steady state level is calculated for the next 24 h according to the formula (clearance (CL) x BW x 24) where default values of 3 and 4 can be used as CL for FVIII and IX respectively. Two hours after the bolus dose (see above) it is recommended to give another 2.000 IU to an adult patient and the total dose for the next 24 h according to the formula is then given in 6 hour intervals for FVIII and 8 hour intervals for FIX,

Continuous infusion

Recovery calculation to determine the initial bolus dose:

$$\text{Recovery} = \frac{\text{Increase in factor level (\%) x BW}}{\text{Test dose IU}}$$

$$\text{Bolus dose} = \frac{\text{Desired increase in factor level (\%) x BW}}{\text{Recovery}}$$

Infusion rate = Clearance x desired factor level (IU/kg)

Daily dose = Infusion rate x BW x 24 h

$$\text{Clearance} = \frac{\text{Infusion concentration kIU/L x infusion rate mL/24 h}}{\text{Measured factor level kIU/L}}$$

Clearance (mL/h/kg) often measured. Varies between individuals and products, especially for FIX:

Hemophilia A: Adult: 3, Children: 5

Hemophilia B: Adult: 6

Desired FVIII/IX levels in the patients for continuous infusion and trough levels for the bolus injection group:

Day 1-3: 0.70 kIU/L

Day 4-6: 0.50 kIU/L

Day 7-9: 0.30 kIU/L

Then tapering off - bolus infusions before physiotherapy.

Minor surgery

In general, a factor level of 0.5 kIU/L is recommended before the surgical procedure and replacement therapy for 1-5 days depending on the procedure.

Specific surgery

Dental extraction

For invasive surgical intervention it is recommended to increase the factor level >0.5 kIU/L pre-operatively and use an oral antifibrinolytic agent (tranexamic acid) agent pre-and post operatively in combination with local therapy (76).

Circumcision

A general recommendation for circumcision is a factor level of 0.7-1.0 kIU/L at the start of surgery and a level >0.5 kIU/L maintained for at least 2-3 days (some recommend 7-10 d) together with antifibrinolytics. When performing circumcision in patients with mild hemophilia A desmopressing (DDAVP) 0.3 µg/kg intravenously before the initiation of surgery and an additional dose on the second day can be considered in DDAVP responding patients (77).

Liver biopsy

In patients undergoing liver biopsy, the preoperative factor level should be as for major surgery 0.7-1.0 kIU/L and replacement therapy should be continued for at least 3 days with concomitant use of tranexamic acid as described below (78). Bed rest for 8-12 h after the biopsy is recommended.

Tonsillectomy/Adenotomy

In children undergoing tonsillectomy preoperative factor level should be 0.7-1.0 kIU/L and replacement therapy should be continued for 7-10 days days with concomitant use of tranexamic acid as described below (77, 78).

Prostatectomy

Prostatectomy should be considered as major surgery. However, substitution therapy should be continued for at least 2 weeks due to the increased risk of late bleeding complications.

Mild hemophilia

Surgery in persons with mild hemophilia A can be performed using desmopressin (DDAVP) when FVIII can be raised to an appropriate therapeutic level. Administration of desmopressin (DDAVP) can raise FVIII level adequately (three to six times baseline levels) in patients with mild, and possibly moderate, hemophilia A. Testing for DDAVP response prior to surgery should be performed after one and four hours.

Desmopressin does not affect FIX levels and is of no value in hemophilia B.

- 0.3 µg/kg i.v. or s.c.
- 300 µg i.n. (spray) (150 µg if BW <30 kg)

Intravenously (i.v.): slow injection of DDAVP (diluted in 10 mL saline) during 15 minutes or infusion (diluted in 50-100 mL saline) during 30 minutes diluted in 50-100 mL saline. Peak FVIII/VWF levels are observed at 60 minutes.

Subcutaneously (s.c.): Peak FVIII/VWF levels are reached after about 120 minutes.

Octostim® solution (15 µg/mL) is the most suitable for s.c. administration, due to its high concentration. Often a single 15 µg dose s.c. will suffice in adults.

An additional dose of DDAVP is infused on the second day (12/24h). DDAVP may cause fluid retension, which deserves special attention in the youngest children (<4 years) in whom FVIII concentrate should be considered. A fluid restriction of 1-1.5 L is recommended.

Tranexamic acid

Tranexamic acid is an antifibrinolytic agent. Administration can be oral, intravenous or topical (e.g. as mouthwash). It can be used in combination with DDAVP, FVIII/FIX and rFVIIa. To increase its effectiveness, tranexamic acid should be given prior to elective procedures and with repetitive dosing to ensure concentrations in tissues as well.

- Orally 25 mg/kg 3-4 times daily for 7-10 days
- Intravenously 10 mg/kg 3-4 times daily for 7-10 days
- Mouthwash 10 mL of a 5% solution 4 times daily, which can be swallowed

Limitations

- Contraindicated in the management of upper urinary tract bleeds
- Dose reduction is necessary in patients with renal insufficiency
- Should be avoided, or its usage minimized, in patients with a recent thromboembolism and/or a previous personal or family history of thromboembolic disease
- No data are available on the use of tranexamic acid in newborns

Adverse effects

Nausea, vomiting, diarrhea and abdominal pain.

Postoperative management

Adequate pain control is an important factor in successful postoperative management and rehabilitation. However, in general, neuraxial anesthetic and analgestic techniques (epidural anesthesia) are contraindicated postoperatively due to the risk of bleeds. However, nerve blocks may be used in this patient group (with caution and under replacement coverage). Acetylsalicylic acid and Cyclooxygenase-1 inhibitors should also be avoided since they induce platelet dysfunction and thereby contribute to impaired hemostasis. COX-2 inhibitors are suitable with proton pump inhibitors, unless there is renal insufficiency.

A physical therapy plan to assess pre- and postsurgical rehabilitation is advisable to patients undergoing elective orthopedic surgery and the physical therapist should be

experienced in the management of hemophilia and in frequent communication with the other members of the hemophilia treatment team

Orthopedic aspects

Orthopedic surgery in PWHs is truly a collective effort, involving not only the surgeon but also collaboration with the comprehensive hemophilia center team to address serious considerations. The optimal timing of orthopedic surgery during the lifetime of the hemophilic patient is unknown. However, the more demanding social and professional life of youth also favour the early correction of joint disease. These factors have contributed to the tendency towards early orthopedic intervention, and the focus of such procedures has shifted from relief of pain towards the correction of functional disability.

Table 5: Recommended plasma factor levels before and after surgery

	Hemophilia A and B	
	Desired level kIU/L	Duration (days)
Major surgery		
Pre-op	0.7-1.0	
Post-op	0.6-0.8	1-3
	0.4-0.6	4-6
	0.3-0.4	7-9
Minor surgery		
Pre-op	>0.5	
Post op		1-5 depending on procedure

Surgery in PWHs with inhibitors

Surgery in persons with hemophilia and high–titered inhibitors is a clinical challenge and was for a long time considered as almost impossible. However, surgical experience during the last 10-15 years using bypassing agents have shown that despite increased bleeding risk compared to non-inhibitor patients the results are in general good (79). Consequently, patients with inhibitors should not be denied surgical procedures. Nevertheless, surgery continues to pose a major challenge in these patients, as the costs are significantly higher than in patients without inhibitors in addition to a higher risk of bleeding.

All surgical procedures in patients should be conducted by a specialized surgeon in association with a hemophilia comprehensive care center.

Currently, there are today no standardized laboratory assays to monitor the efficacy and optimal dosing of bypassing products following surgery. However, preoperative evaluation of hemostatic response to bypassing agents using thrombin generation test (TGT) or thromboelastography has been reported as a means to predict and optimize the hemostatic outcome during the peri- and postoperative phase (80, 81).

aPCC and rFVIIa

The bypassing agents aPCC - factor eight inhibitor bypass activity (FEIBA®, Baxter AG, Vienna, Austria), and recombinant activated factor VII (rFVIIa) (NovoSeven®, NovoNordisk A/S, Bagsvaerd, Denmark) are the treatment of choice in patients with if the inhibitor level exceeds 5 BU/mL. Which one to use depends on several factors as the age of the patient, prior history of efficacy to a product, costs and safety. APCC have been used extensively for a long period of time and has the advantage of dosing every 8-12 h, whereas rVIIa must be infused every 2-3 h. rFVIIa offers the advantage of being a recombinant protein, and therefore unlikely to be contaminated with infectious agents, as opposed to aPCC which is plasma derived. However, the risk is minimized as aPCC is now double virus inactivated and no transmission of blood born infectious agents has been reported since these precautions were undertaken. Both products are effective in achieving hemostasis, and one should switch to the other product if the first choice fails. Side effects including venous thrombotic events, disseminated intravascular coagulation (DIC) and myocardial

infarction have been reported using both aPCC and rFVIIa, although at a very low incident rate, if doses within the manufacturers recommended range are used. The main disadvantages of rFVIIa compared to aPCC are high cost and frequent infusions (see chapter Inhibitors).

Management of substitution therapy in the peri- and postoperative phase

In patients with a low-titer (<5 BU) or a low responding inhibitor the use of high dose FVIII or FIX concentrates to overcome the inhibitors might be applicable in the initial phase. However, an anamnestic response may occur and one should be prepared to switch to a bypassing agent at any time.

aPCC - FEIBA®

During the last 15 years more than 200 surgical procedures have been reported in case reports using aPCC as replacement therapy in patients with inhibitors. The hemostatic efficacy in these case series have been reported from 78% to 100%. Variable initial doses, frequency and duration of treatment using aPCC have been reported however, continuous infusion has not been studied.

The Norwegian experience using aPCC for surgery counts 37 surgical procedures, 17 major and 20 minor (79-82). APCC was delivered by short –time infusions (15-20 min) three times daily. A preoperative loading dose of 100 IU/kg was given. The following doses were adjusted to a total daily dose of 200 IU/kg/d. Following the third postoperative day, the dose of aPCC was tapered to a daily dose 150 IU/kg and from the 7th postoperative day tapered gradually to 100 IU/kg. 50 IU/kg every second day was given as post surgical prophylaxis and prior to physical therapy. A good or excellent hemostatic outcome was observed for all minor procedures and in 15/17 (88%) of the major procedures. A few consensus reports for using aPCC as replacement therapy in inhibitor patients undergoing surgery based on the present literature have been published (83, 84). Common in these recommendations are a preoperative bolus infusion of 50-100 IU/kg and then a dose of 75-100 IU/kg every 8-12 h with a maximum daily dose of 200 IU/kg and depending on the clinical condition and type of surgery the dose may be tapered until discharge (Table 6).

rFVIIa - NovoSeven®

Many case series with a small number of patients have reported a good hemostatic outcome using rFVIIa for different surgical procedures in PWHs with inhibitors. However, variable doses and protocols have been reported and only two small prospective randomized studies have been published addressing the dose and mode of administration (85, 86). Shapiro and colleagues compared the effect of two doses of rFVIIa in 29 patients with inhibitors for minor and major operative procedures. The patients were randomized to either 35 µg/kg vs 90 µg/kg every 2 h for 2 days, then every 2-6 h for total 5 days. Concerning major surgery the effectiveness at day 5 was found to be 40% for the low dose whereas 83% for the high dose concluding that rFVIIa 90 µg/kg is an effective first-line option for major surgery in patients with inhibitors. Concerning minor surgery, 70% and 100% of the procedures were found to be effective or partially effective for the low dose and high dose, respectively.

Pruthi and colleagues (86) studied the efficacy and safety of administering rFVIIa after an initial bolus dose of 90 µg/kg and then randomization to either repetitive bolus infusion (BI) (90 µg/kg) every two hours or continuous infusion (CI) 50 µg/kg/h for 5 days in 22 major surgical procedures in hemophilia A or B patients with inhibitors. They found comparable hemostatic efficacy and safety of BI and CI, however the treatment was considered as ineffective in three subjects in each arm.

Valentino and colleagues reported from the Haemophilia and Thrombosis research registry and literature, which also incorporated a small number of medical procedures (n=45) in addition to surgical and dental procedures, and found rFVIIa to be effective in 333 (84%) of the 395 cases represented (87). Thromboembolic complications attributable to rFVIIa were reported in 0.025% of these procedures.

Based on the present literature a few general expert recommendations have been given for using rFVIIa to cover surgical procedures (83, 88) (Table 6). The initial bolus dose should at least be 90 µg/kg given immediately preoperatively and then every 2 h for at least 48 h However, due to observed bleeding complications in a minority of procedures an even higher initial bolus dose of 120-180 µg/kg have been proposed. After 2 days the dosage interval may be increased to 3, 4 the 6 h on days 3, 5, and 8 respectively, and continued until discharge.

Pretreatment with 90 µg/kg is recommended before each physical therapy session.

In case of unexpected peri- or postoperative bleeding episodes using bypassing agent one should increase the dose of already initiated treatment agent to maximum dose for rFVIIa (up to 270 µg/kg) or aPCC (200 IU/kg/d). If hemostasis is still not achieved an alternative bypassing agent should be rapidly implemented similarly to unresponsive severe bleeding episodes (Fig. 5). If monotherapy with either of the products at maximum doses have been ineffective sequential or concomitant treatment with both bypassing agents might be considered for salvage treatment.

Bypassing agents and antifibrinolytics

The antifibrinolytic agent tranexamic acid (TXA) increases clot stability and is used concomitantly with coagulation factor replacement to improve hemostasis in PWHs without inhibitors. It is not contraindicated to combine rFVIIa with TXA to improve hemostasis although it is not systematically studied. In contrast to rFVIIa, aPCC has not been recommended to be given together with TXA unless a time lag of 6 h between administrations of the two drugs. The reason for this caution is safety concerns with an estimated increased risk of thrombotic events and disseminated intravascular coagulation (DIC). However, strong evidence supporting this precaution is lacking. A recently clinical study showed good hemostatic results and no episodes of thromboembolic events or DIC and hypercoagulability in inhibitor patients that had previously been refractory to monotherapy treatment (79, 89). At least whenever possible applied locally either as mouth rinse or moistened dressings the combination of TXA and aPCC is considered as safe. The dose of tranexamic acid commonly used is 10 mg/kg intravenously or 25 mg/kg orally 3-4 times daily for 7-10 days.

Bypassing agents and thromboprophylaxis

Although thrombosis might be a concern using bypassing agents, postoperative anticoagulation (e.g. low-molecular-weight heparin) is not recommended in patients with inhibitors. For the majority of the patients the use of graduated compression stockings and early mobilization are sufficient to prevent venous thromboembolism.

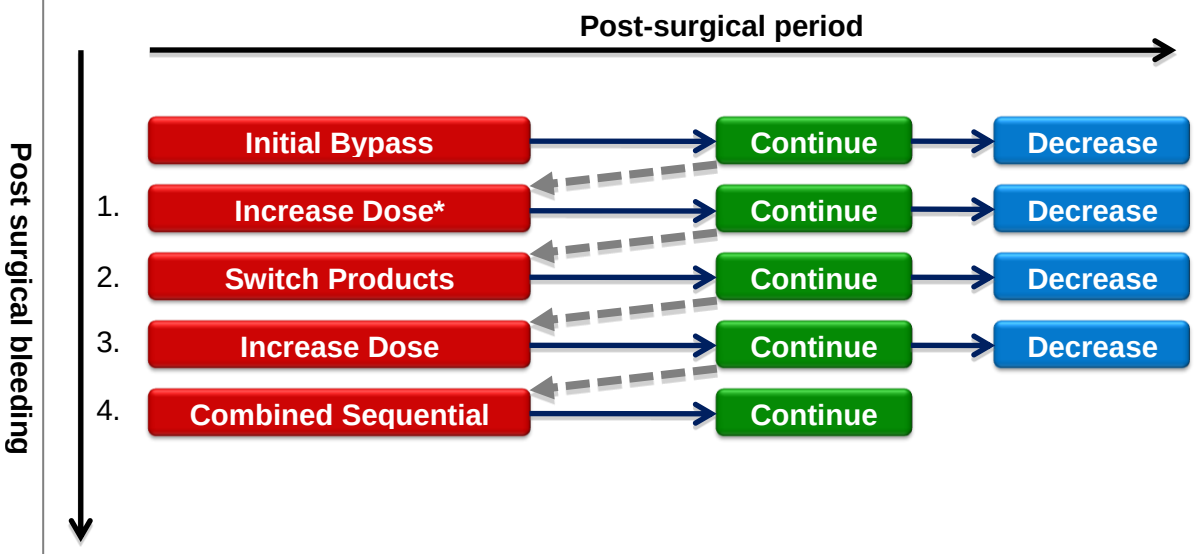
Table 6: Recommended dosage of rFVIIa and aPCC for surgery in patients with hemophilia and inhibitors

		Preoperative dose	**Postoperative management**
rFVIIa			
	Minor surgery	90 µg/kg	90 µg/kg every 2 h up to four times, then every 3-6 h until discharge
	Major Surgery	90-120 µg/kg	90 µg/kg every 2 h the first 48 h, then 90 µg/kg every 3, 4 the 6 h on days 3, 5, and 8 respectively until discharge CI*: 50 µg/kg/h
aPCC			
	Minor surgery	50-100 IU/kg	50-75 IU/kg every 8-12 h until discharge
	Major surgery	75-100 IU/kg	70 IU/kg every 8 h for at least 3 days with a maximum daily dose of 200 IU/kg. Dose may be tapered from day 4 to 50-75 IU/kg every 8 h.

*CI: Continuous infusion

Figure 5:

Algorithm to manage post-surgical bleeding episodes in patients with high–titer inhibitors



*Omit this stage if already at maximum dose.

Modified from suggested treatment strategy of life-or limb threatening bleeding episodes

Comorbidities in the ageing patients with hemophilia

Introduction

Improved treatment has extended life expectancy for PWHs during the last two decades making them susceptible not only to complications of hemophilia, but also to age related co-morbidities same as in the general male population (90, 91). Apart from the initial devastating effects on morbidity and mortality associated with the transmission of viral pathogens during the 1980's and early 1990's, the availability of factor concentrates and improved treatment regimens have had a favourable influence on longevity and quality of life of PWHs.

At present with only scarce evidence based data available, little is known about how to manage these "new" concomitant illnesses in a scientific manner, apart from hemophilic arthropathy and chronic infections with HIV (human immunodeficiency virus) and HCV (hepatitis C virus). Co-morbidities like metabolic syndrome, cardiovascular and renal disease, along with infection related issues and cancer represent a series of new challenges to physicians treating PWHs. Expertise from specialists in e.g. cardiology, neurology, oncology, nephrology and urology need to be included in the multidisciplinary team of physicians treating elderly PWHs in comprehensive hemophilia care centers.

Current status and recommendations / managing suggestions

Joint disease

The most prominent co-morbidity in middle-aged and older PWHs is irreversible joint arthropathy (91, 92). Due to lack of treatment, recurrent hemarthroses result in initial synovial hypertrophy and neoangiogenesis further increasing the risk of bleeding and later on result in degenerative changes of the joint. This leads to limited use of the affected, often weight-bearing joint, causes pain, muscle atrophy, anchylosis (reduces range of motion), contractures and osteoporosis, the latter expressed by a reduced bone mineral density (BMD) or impaired bone structure.

The goal of treatment is to try to improve joint function, relieve pain and assist the patient in resuming to normal activities of daily living. Physiotherapy is an important treatment modality to improve or maintain muscle function and joint motion, may reduce the risk of falls and encourage an interest for an active lifestyle. Appropriate pain management including suitable medication needs to be carried out to prevent further deterioration, but also needs to be monitored closely for side effects (93). Lifestyle changes, e.g. weight loss and regular exercise, would also be beneficial. The use of secondary prophylaxis (regular treatment with factor concentrate after onset of arthropathy) reduces bleeding frequency and facilitates rehabilitation, but does not alter established degenerative changes that worsen with age. Despite adequate treatment and even in the absence of an inhibitor, target joint bleeds require procedures, such as radiosynovectomy to control synovial hypertrophy or at times angiographic embolization to stop joint bleeding from arterial origin (93, 94). To reduce severe pain and disability arthroscopy, arthrodesis, arthroplasty or total joint replacement are efficient interventions.

Osteopenia can be prevented or reduced through a supplement of calcium, vitamin D and exercise, while osteoporosis necessitates specialist treatment with one or several drugs including bisphosphonates, estrogens, calcitonins and monoclonal antibodies (95). Thus, assessment of bone mineral density (BMD) by imaging studies (DEXA scan) and laboratory evaluation are recommended as part of comprehensive hemophilia care.

Chronic joint pain is a common symptom in the ageing PWH. Pain management during acute bleeding episodes, peri- and post-operatively and for long-term needs has not been systematically studied in hemophilia, thus treatment is mostly empirical as there are no consensus guidelines available (96). Pharmacological treatment options include paracetamol (cave liver dysfunction) and COX-2 inhibitors (cave gastro duodenal intolerability, cardiovascular disease) in combination with synthetic or genuine opiates (cave addiction). Non-pharmacological treatment modalities should also be evaluated. A standardized management approach includes a close relationship between pain specialist and the staff at hemophilia treatment centers. In addition to assessing pain, quality of life and disability status need to be evaluated and followed. Psychological aspects and issues with addiction are important to consider. Treatment algorithms/protocols may be helpful.

Infection related issues / complications

With the introduction of HAART (highly active antiretroviral treatment) a substantial decrease in HIV infection related deaths (over time) were seen (95). Also the HIV related occurrence of NHL (non-Hodgkin lymphoma) has declined. HAART treatment increases the risk of metabolic syndrome, diabetes, renal insufficiency and atherosclerotic CVD (cardiovascular disease) in non-bleeding patients. A similar impact is suspected to apply to PWHs (90, 93). Close laboratory monitoring is therefore recommended. HAART has also been reported to increase frequency and severity of hemarthrosis in hemophilia (95).

HCV is the major cause of chronic liver disease since especially genotype 1 responds poorly to standard treatment with subcutaneous Peg-IFN (pegylated interferon) and oral ribavirin. Poor treatment response is also seen in the numerous PWHs who have a HIV and HCV co-infection. Those who are co-infected also have a marked increased risk for progression in their liver disease with a later risk of transformation from liver cirrhosis into HCC (hepatocellular carcinoma (90, 91, 93). Cirrhosis and portal hypertension with development of esophageal varices in combination with hypocoagulable state, including thrombocytopenia, increase the risk of bleeding (93). Periodic fibroscan and ultrasound screening accompanied by measurement of AFP (alpha fetoprotein) may help identify progressive disease earlier (95). New antiviral therapy including HCV protease inhibitors will hopefully achieve sustained virological response rates. Otherwise the only curative option is liver transplantation.

Metabolic syndrome

The term describes a complex of signs that increase the risk for type 2 diabetes, stroke and coronary artery disease. Diagnostic criteria include increased body mass index (BMI) >30 kg/m^2, hypertension, dyslipidemia and hyperinsulinemia. Middle-aged PWHs tend to become obese and inactive due to severe arthropathy. HAART treatment for HIV can result in hypertension, ischemic heart disease and dyslipidemia. Patients need appropriate treatment management, regular clinical and laboratory follow-up, which should also be coordinated with the primary care physician, if needed (97).

Cardiovascular disease

Conflicting data exist on whether hemophilia protects against development of atherosclerosis and cardiovascular events (94, 95, 98, 99). The same risk factors that affect the general population also seem to have impact on ageing PWHs. Increasing age, obesity, smoking, arterial hypertension, diabetes and dyslipidemia and inflammation (detected with high sensitivity-CRP and elevated factor VIII levels in hemophilia B) contribute to cardiovascular disease.

An institutional non-evidence-based Dutch guideline covers acute coronary syndrome (ACS) and percutaneous coronary intervention (PCI), where, after substitution with the deficient factor, the PWH is treated as close to general guidelines for non-PWHs as possible (100). DDAVP (desmopressin) should be avoided as a hemostatic due to non-specific thrombogenic effects. Thrombolysis is not recommended. If necessary, documented in case series, a bare-metal stent should be favoured since only four weeks of dual antiplatelet therapy is needed, or alternatively a coronary artery bypass grafting (CABG) (90, 99). When treating valvular heart disease a material should be chosen that does not necessitate anticoagulation.

Emphasis should be made on not to “overtreat” in the course of replacement therapy especially with bypassing agents to avoid thrombotic events. A way to avoid hazardous peak levels during substitution therapy can be achieved by administering the needed coagulation factor by continuous infusion instead of bolus injections. Conversely, a certain empirical minimum factor level has to be maintained to allow for necessary antithrombotic treatment. In severe PWHs; >5% for aspirin alone and >30% for dual antiplatelet therapy (93).

Virtually no data are available for defining treatment strategies for cerebrovascular and peripheral artery disease (99). This also applies for non-valvular atrial fibrillation and venous thromboembolism. The use of low molecular weight heparin could be considered for short term treatment. Erectile dysfunction can be seen as the first manifestation of vascular disease and endothelial dysfunction. It can accompany the metabolic syndrome or be caused by age-related changes in hormonal, neurological and psychological function (101).

Renal disease

In young PWHs, hematuria often is a benign, transient, often idiopathic event. In older patients this bleeding symptom can be caused by several different conditions and etiology should be evaluated. In chronic renal disease uremia and anemia via platelet dysfunction increase the risk for kidney bleeding. So does hypertension that can be caused by chronic renal disease and at the same time represents a risk factor for development of cardiovascular disease as well as cerebral hemorrhage. HIV-associated nephropathy and immune complex glomerulonephritis, nephrotoxicity of HAART and co-infection with HCV make up a large proportion of causes for renal insufficiency. If dialysis is needed, peritoneal dialysis could be the preferred choice since no anticoagulation is needed. This however could contain the risk for infection and peritoneal hemorrhage especially in patients co-infected with HIV and/or HCV (94, 98, 102). For hemodialysis patients prophylactic factor dosing needs to be carefully tailored for access surgery and to allow the required anticoagulation.

Cancer

If malignancies that are a consequence of viral infection are excluded only a few clinical studies have addressed the issue of cancer in the ageing hemophilia population. It is uncertain whether the incidence of cancer in PWHs differs from that observed in the general middle-aged population (93, 102). Persons with severe hemophilia tend to have a higher rate of virus-related cancers whereas milder forms present an overweight of non virus-related cancer types. At times patients are diagnosed with acquired hemophilia due to unusual bleeding of a cancer. Attention must also be drawn to the importance of prompt evaluation if a middle-aged PWH experiences new, aggravated or recurring bleeding episodes due to a second peak of inhibitor incidence at the age of 60 and above. Despite the increased risk of bleeding investigation and procedures should not be delayed or avoided in PWHs. Relevant hemostatic treatment must be given to prevent bleeds both in the setting of diagnostic interventions and later on as well prior to surgical, chemo-, or radiotherapeutic treatment. One specific cancer type needs mentioning since it is one of the most frequent cancers in men, with increasing frequency up to the age of 70: prostate cancer (103). Prostate specific antigen (PSA) screening has reduced the percentage of disseminated disease at diagnosis more then 20-fold. The diagnosis necessitates needle biopsy. Despite hemostatic treatment bleeding occurs, but is

often mild to moderate and self-limiting. Antifibrinolytics should be used with caution and close observation for thrombus formation in the bladder and in the upper urinary tract with the risk of developing hydronephrosis. Several treatment options are available and seem to have equivalent survival rates.

Conclusion

Ageing PWHs present new challenges to hemophilia caretakers. The EHTSB (European Haemophilia Therapy Standardization Board) has in the absence of studies as a result of consensus meetings generated recommendations for the assessment, monitoring and follow-up of PWHs (104). On-going and future studies will hopefully clarify the most appropriate preventive measures and treatment regimens for co-morbidities, which often create management challenges in view of the hemostatic status of the PWH.

Centralized comprehensive hemophilia care is important throughout the life of PWHs. The challenges with comorbidities developing during aging are best managed in close multidisciplinary collaboration with different medical and surgical specialists and networking with patient's local hematologist and primary care physician.

Treatment of pain

Among PWHs pain is a very common condition affecting quality of life (105). Basic pain treatment can be symptomatic and in some cases also directed against the underlying disorder. When evaluating pain it is important to take the patient's life situation into account. The pain could be acute and/or severe or chronic. Pain from joints or muscles is very common in PWHs especially if the patient has hemophilia arthropathy, in which case the pain often is chronic. Bleeding in a joint or muscle will produce an acute pain and should be treated with relevant hemostatic drug as soon as possible in order to stop the bleeding. The evidence is scarce for the use of ice to reduce bleeding and inflammation due to joint or muscle bleeding in hemophilia.

If the PWH is not on a prophylactic treatment regime with factor concentrate and has a target joint, prophylaxis should be offered to avoid recurrent bleeding, inflammation and pain.

Several instruments exists for the evaluation of pain in PWHs among which are the visual analogue scale (VAS), health related quality of life (HRQoL), McGill Pain Questionnaire (arthritis) and others (96, 106).

In many situations chronic pain should be managed in a multidisciplinary team where the patient is rehabilitated with the help from pain management clinic, physiotherapists, psychologist, orthopedists, social workers, experts in management of pain in addition to the hemophilia doctors and nurses.

Analgetics

Mild analgetics are often used in the treatment of both acute and chronic pain. Paracetamol (acetaminophen) is the basic treatment and can if necessary be combined with tramadol or codeine.

The analgetic effect of codeine is caused by codeines conversion to morphine. In approximately 10% of the white population codeine is without analgetic effect, caused by inability to convert codeine to morphine. Tramadol is a synthetic codeine analogue. Common side effect to treatment with codeine, tramadol and morphine is nausea, constipation, vomiting and drowsiness. Codeine should be used with caution, especially in elderly patients because of the risk of cognitive side effects.

Information about dosage of analgetics to the patients is very important for the prevention of toxicity e.g. liver toxicity in the use of paracetamol in patients with chronic hepatitis or HIV.

Aspirin has an irreversible inhibition on platelet aggregation and should not be used in treatment of pain for PWHs. If the pain is caused by inflammation in a joint COX-2-inhibitors (celecoxib or etoricoxib) can be considered in selected PWHs. COX-2 inhibitors do not inhibit platelet aggregation. However even COX-2 inhibitors can have serious side effects like COX-1 inhibitors and should be used with caution in specific patients. One of the most serious side effects is gastroduodenal ulcers. The risk of gastrointestinal ulcers is lower with COX-2 inhibitors than COX-1 inhibitors and H_2 receptor antagonists or protoni pump inhibitors can be used to minimize the risk of ulcers. Both COX-1 and COX-2 inhibitors can have severe gastrointestinal, renal and cardiovascular (MI, stroke and other arterial thrombosis) side effect.

Among the NSAIDs COX-1 inhibitors e.g. ibuprofen has a reversible inhibition on platelet aggregation. COX-1 inhibitors should generally only be used on strong indication and with caution in the treatment of pain in PWHs due to the increased risk of bleeding and other serious side effects. If there is a strong indication for the use of COX-1 inhibitors in people with hemophilia it is recommended to choose a drug with a short half-life. Ibuprofen has a short half-life and the risk of side-effects (gastrointestinal ulcers and cardiovascular events) is considered low when the daily total dosage is 1,200 mg and below.

Some patients may benefit from using analgetics with prolonged effect especially for treatment of pain at night. Also transdermal formulas can benefit many patients with chronic pain issues.

In the case of severe acute pain morphine could be necessary to use at start, but due to the risk of addiction it should be given for a limited period of time.

Patients with severe complex chronic pain should be managed at a pain clinic. In the treatment of chronic pain gapapentin (medication for epilepsy) or tricyclic antidepressants can have an additive effect on the treatment with analgetics.

It is important to be aware of that children often express pain in a different way than adults. Before injections it is common to apply anesthetic cream to the skin of the child in order to minimize pain.

Pain in PWHs could be managed as described below (107, 108):

Mild pain and/or chronic pain

- Paracetamol alone or combined with
- Codeine **or**
- Tramadol

Pain and joint inflammation (NSAIDs)

- COX-2 inhibitors - celecoxib or etoricoxib
- COX-1 inhibitors – ibuprofen only in special circumstances

Acute severe pain

- Morphine

Orthopedic surgery and treatment by the orthopedist

Treatment by the orthopedic surgeon should always be considered, if the pain is a symptom caused by joint damage. Synovectomy with the removal of the synovial membrane can often be used, if the patient has inflammation without severe cartilage or bone destruction in the joint. If the joint is severely damaged a joint prosthesis is often the best solution to the pain problem. In some cases the physiotherapist or orthopedist can help the patient with orthosis or heightening of shoe heels.

Intraarticular corticosteroid injection in joints with hemophilia arthropathy

Intraarticular injection of corticosteroid for the treatment of inflammation and pain in joints with arthritis e.g. rheumatoid arthritis is a documented and established treatment modality (109).

If the PWH has a joint with inflammation, corticosteroid injection into the joint can be used. It has been demonstrated in a few studies that intra-articular injection of corticosteroids can reduce pain in hemophilia joints with inflammation (110, 111).

A prophylactic dose of factor concentrate should be given prior to the injection of corticosteroid. The intra-articular injection must be given under sterile condition and if possible, effusions can be drawn from the joint. In case of suspicion of infection the synovial fluid must be sent to further investigation to rule out infection and injection of

corticosteroid should not be given. The most serious but also very rare complication to intra-articular corticosteroid is infection.

The dose of corticosteroid depends on size of the joint and the degree of inflammation. The dose of corticosteroid could be e.g. triamcinolonehexacetonide (Lederspan®) 10-40 mg or triamcinolonacetonid (Kenalog®) 20-80 mg.

As it is essential to the effect of the treatment, that the corticosteroid is given into the joint, it is recommended that the injection is given by a physician, trained in giving injections into the joints. If possible the injection could be given guided by ultrasonography to increase the precision of injection.

After the injection the patient must avoid loading of the joint for at least 24 h. When corticosteroid is used in arthritis the effect of the injection stays at least four to six weeks but usually for several months or even longer. Osteoporosis around the joint needs to be managed appropriately.

Mild side effect is experienced in up to 10% of cases as flushing of the face, increased sweating in minutes to hours after the injection. In patients with arthritis approximately 2% can experience worsening of the pain lasting the first 24 h after the injection. Although systemic effects of the corticosteroid injection is minimal, measurements of blood glucose should be done in patients with diabetes mellitus, as the blood glucose in some cases can be elevated in the first days after the injection.

Physiotherapy

Introduction

The physiotherapist's role in care of the hemophilia patients has changed over the years depending on the progress of medical care with the ability to provide prophylaxis treatment with the missing factor (9). However, there is still a need of physiotherapy for patients who have not received preventive treatment during childhood and for those who have developed antibodies to the medication.

Physiotherapy can be divided into prevention, assessment, and treatment/rehabilitation.

Prevention

Patients will at an early age receive prophylaxis with coagulation factor concentrates and can be physically active to the same extent as non-hemophiliac children resulting in normal physical strength and mobility (112). Low physical activity can result in impaired bone mineralization and reduced bone mineral density in children with hemophilia compared with healthy (113). Good function of muscles around the joints has been shown to prevent joint and muscle bleeds. It is therefore essential to train muscle strength, endurance, and coordination at an early age (114). An important part of the role for the physiotherapist is to inform and provide support to parents and teenagers about physical activity and sports that are appropriate for PWHs (115). The physiotherapist can also show parents how to examine the joint mobility of the youngest children for early detection of joint bleeding.

Assessment

Assessment instruments that are disease specific for PWHs have been developed over the past 10 years (116).

The physiotherapist will assess the joint and muscle function during the annual control at the treatment center. This includes joint mobility, muscle strength, pain, joint and muscle contractures, axial changes in the joints, balance and gait functions.

In acute bleeding a physiotherapist can help with differential diagnosis between joint and muscle bleeding and synovitis together with the physician.

The Haemophilia Joint Health Score (HJHS) has been developed for children from 4 to 16 years of age and it is validity and reliability tested. It is used for the evaluation of joints in children (117). For adults and elderly patients the HJHS needs to be complemented with assessment of possible age-related conditions for example problems with the hip and shoulder joints.

Other evaluation instruments that may be present are visual analog scale (VAS) to rate the pain experience in daily activities or at acute trauma/bleeding (118). Haemophilia Activities List (HAL) can be used to get the patient's own perception of their ability in terms of activity (a person carrying out a task or action) and participation (a person's involvement in a life situation) (119).

The physiotherapist works as a part of the team and suggests contact with the occupational therapist when the patients need assisted devices at home for the ADL (activities of daily living). A disease-specific ADL status is developed in India (120) but is not used in the Nordic Countries at the moment due to cultural differences between the countries that makes the manual not suitable for the Nordic conditions.

Intervention

During an acute bleeding the physiotherapist plans an exercise program to restore lost function. Several studies show that mobility and strength exercise leads to faster normalization of the function and also significantly reduces the risk of permanent disability (114). Repeated bleedings in a joint leads to cartilage damage and give a hemophilia-related joint disease (hemophilia arthropathy). Active exercise under the guidance of a physiotherapist in combination with intensive treatment with factor concentrate can break the vicious circle and reverse early hemophilia arthropathy. The results are better the sooner physiotherapy begins (121).

The purpose of rehabilitation at hemophilia arthropathy and after an acute bleeding in the joint or muscle is to reduce pain, restore joint mobility and muscle strength. Treatment may include different types of mobility exercises (active, active unloaded, passive), posture instructions, careful manual extractions for increased mobility and pain relief purposes, strength and endurance exercise, coordination training, etc. Exercise in warm basin can be useful as pain relief like TENS, heat and cool pack. The training should be 3x/week to get the desired result (115).

Patients undergoing orthopedic surgery, for example synovectomy or different types of joint replacement receive physiotherapy exercise both before and after surgery (122). Before surgery it is important to train muscle strength around the joints and maintain the mobility that exists. After surgery the patient trains their mobility and strength according to the actual programs/protocol at the orthopedic clinic for the current operation.

If the hemophilia-related arthritis has caused malalignment, stiffness and pain, the physiotherapist may prescribe orthotics and orthopedic shoes together with the attending orthopedic surgeon depending on the rules in different countries (123).

The physiotherapist also tests out walking aids and recommend other appliance needed in daily life.

Summary of physiotherapy work at treatment centers in the Nordic countries:

- Informs about the joints and muscles function to parents, teenagers and adults.
- Assess physical activity, joint mobility and muscle strength
- Proposes appropriate recreational and sporting activities
- Tests out and practicing assistive devices
- Designs exercise programs after a bleeding disorder
- Patients exercise to increase mobility and muscle strength
- Patients exercise before and after orthopedic surgery
- Treatments for pain relief
- Is a resource for colleagues outside the treatment center

Carriers of hemophilia

Introduction

It has been estimated that there are approximately three potential female carriers for each male with hemophilia (124). Due to X-chromosome inactivation the clotting factor levels in carriers are expected to be about 50% of the level of non-carriers. However, the factor levels may vary from very low to the upper limit of normal values (125). For this reason the factor level in some carriers will be in the range of mild hemophilia and they may require hemostatic support during surgery, trauma and delivery (3).

Diagnostic

The timing of genetic testing needs careful consideration taking into aspects such as age and psychosocial issues. It's a sensitive issue to test healthy children for inherited disorders and it raises ethical considerations (126, 127). Testing clotting factor activity is however recommended before puberty or before surgery. It's important that the girl as well as her family understand that a normal factor level does not exclude carriership, which must be tested with genetic analysis.

Menstruations

One of the first hemostatic challenges a carrier may be facing is menstrual bleeding. Girls with low factor levels should have a treatment plan prior to menarche for the possibility of excessive menstrual blood loss. Excessive bleedings may appear with the first or any following menstrual period during the adolescence. Hemostatic therapeutic options for the management of menorrhagia include antifibrinolytic therapy, DDAVP and clotting factor replacements. Hormonal therapy should be introduced by a gynecologist with knowledge of bleeding disorders in collaboration with the hemophilia treatment center (HTC) (128).

Preconception counseling

Before planning a family the carrier and her partner should be offered an educational visit at the HTC. At this visit the woman and her partner may gain understanding of the carriership. The couple should be offered contact with a genetic counselor at this

point if needed. Counselling should include discussion of the genetic risk and the options of prenatal testing that are available (129).

Prenatal diagnosis

Different options for prenatal testing are available.

Ultrasound assessment in the third trimester is used to determine fetal gender. Knowledge of the gender allows appropriate management of labour and delivery.

Chorionic villus sampling (CVS) is the principal method used for prenatal diagnosis of hemophilia. The procedure is performed at 11-12 weeks of gestation. CVS carries a risk of miscarriage at approximately 1% (130). The clotting factor level of the carrier should be checked and prophylactic treatment for any invasive prenatal diagnostic test should be arranged if the level is <0.50 kIU/L. If later in pregnancy, an amnoitic fluid sample may be used as DNA source for prenatal diagnosis after culturing of cells and DNA extraction.

Analysis of free fetal DNA present in maternal blood to determine fetal sex as well as pre-implantation genetic diagnosis (PGD) may be more available in the future (131).

Pregnancy and delivery

The FVIII levels in carriers of hemophilia A may increase sufficiently during pregnancy to permit safe hemostasis during delivery. In carriers of hemophilia B the FIX level can not be expected to increase to the same extent (132, 133). Factor level should be checked at week 32-34 to allow appropriate management of delivery and to assess the need for prophylactic treatment. A written delivery plan should be drawn up in advance and the delivery should take place in a unit with suitable expertise.

Tranexamic acid may be used during delivery and to prevent post partum hemorrhage. DDAVP may be used in carriers of hemophilia A after the child is born to improve hemostasis further. In carriers with inadequate clotting factor levels (<0.50 kIU/L) prophylactic replacement therapy is recommended to cover labor, delivery and the immediate postpartum period. The treatment should be continued for at least three to four days for vaginal delivery and five to seven days for cesarean section. Epidural anesthesia is permitted when factor levels are >0.50 kIU/L. The risk for delayed post partum hemorrhage in carriers is increased since clotting factors return

to pre-pregnancy levels after delivery (128, 134). Also, it is very important to manage anemia which subjects the patients to additional risk of bleeds.

In case of unknown or male gender the use of scalp electrode, fetal blood sampling, vacuum extraction and instrumental delivery should be avoided. Vaginal delivery is recommended, however Cesarean section should be considered early when needed (135). If the neonate is of male gender a blood sample from the umbilical cord should be obtained for coagulation analysis. Vitamin K should be administrated orally until hemophilia is excluded.

Hemophilia nurse functions

The hemophilia nurse plays a key role in the management of the PWH with bleeding disorders in terms of care, education and support, not only for the patient but also to the family. He/she is a link between the patient and his family, the hemophilia center and society. The functions of the hemophilia nurse may vary in the centers of the Nordic countries.

Ideally the hemophilia nurse coordinates and facilitates the comprehensive team meetings and collaborates within the interdisciplinary team. The hemophilia nurse educates the patient/caregivers in hemophilia and provides information on hemophilia to day-care/school and other health care providers. In some of the Nordic countries the nurses can make home visits if needed.

The nurse plays an important role as a support at the time of diagnosis of hemophilia to help the family to adapt to their new situation and emphasize the normality of the child. In caregivers of inhibitor children emotional stress associated with the disease, financial burden, problems associated with treatment administration and difficulty in dealing with pain are frequently encountered. Knowledge that treatment of bleeds may be less effective for children with inhibitors may lead to increased concerns. Caregivers and the nurse need to be aware of potential problems and be alert to the need for a level of support beyond what is standard routine.

As the population with hemophilia is ageing and co-morbidities will add to the complexity of the disease, the hemophilia nurse coordinator needs to focus not only on hemophilia but also take a more holistic care of the patient and his family, if not to improve at least to maintain a good health-related quality of life for the patient and his family (104).

The hemophilia nurse has many functions among which the most important are:

- Educating the patient/caregiver in: venous access, administration of factor concentrate, and instructions in the care of CVAD or AVF
- Education in self care
- Participating in the management of pain
- Planning and participating of the regular follow-ups at the hemophilia center
- Administration of factor concentrates and recovery test in the center

- Keeping and updating registries
- Guiding other healthcare providers on hospitalized patients
- Participating in research

Dental care

Regular check up at the dentist is important to prevent damage to the teeth and the mucosa of the mouth and thereby prevent bleeding from the gums and other oral diseases and the need for operations (136-139). The staff at the hemophilia center can provide information to the patient and his dentist about which kind of treatment could be given and which kind of treatment should be given at the department for oral and maxillofacial surgery affiliated with the hemophilia center.

Most patients, both adults and children can have regular check up at their own dentist for caries and cleaning of the teeth. Treatment of caries, root canal treatment, tooth prosthesis and orthodontic tooth regulation could also be done at the local dentist in most cases. All treatments which do not cause bleeding can be performed at the patient's own local dentist. Especially inhibitor patients should be treated in close collaboration between the dental clinic and the hemophilia center since they have a special hemostatic treatment and increased risk of bleeding.

Patients with inflammation in the gums often have problem with bleeding and should be offered treatment by dental hygienist.

Surgical operations should always be performed at an oral and maxillofacial surgical department connected with the hemophilia center as this kind of procedure requires experience in treatment of PWHs and collaboration regarding the need of medication.

Tooth extractions, implantations and jaw surgery should be performed at the department for oral maxillofacial surgery and in some cases prophylaxis with antibiotics is needed.

Hemostatic treatment

Prophylactic treatment with factor concentrate may be necessary for some patients depending on the severity of hemophilia and the character of the procedure at the dentist. The treatment at the dentist/ surgeon could be planned on one of the days when the patient receives prophylactic treatment with factor concentrate. The procedure at the dentist should be done as soon as possible after the infusion of factor concentrate within one to two hours. Tooth extraction can often be managed by a single dose of factor concentrate combined with tranexamic acid tablets and mouth wash for 7 days. Compression of the wound with swaps containing tranexamic

acid and topical hemostatics like fibrin glue can be useful. After tooth extraction cold liquid food is recommended for one to two days.

In more advanced jaw or oral surgery repeated doses of factor concentrate might be necessary for hemostasis.

Desmopressin (Octostim®) can be used in patients with mild hemophilia A who have an adequate rise in factor VIII. Desmopressin should be administered one hour before dental procedure regardless of route of administration. The dosage for subcutaneous administration is 0.3 µg/kg bodyweight.

Besides the treatment with factor concentrate tranexamic acid is very useful in dental surgery as oral suspension of tranexamic acid 5% and/or as tablets and sometimes in combination with desmopressin. Mouthwash with 10 mL 5% oral suspension of tranexamic acid 4 times a day is an efficient adjuvant treatment after dental surgery or minor dental procedures for adults After mouthwash the patient should avoid eating or drinking for 30 minutes. Suspension of tranexamic acid for mouthwash is in some places produced by the hospital pharmacy. Suspension of tranexamic acid could be made by mixing one tablet containing 500 mg tranexamic acid and 10 mL lukewarm water or one soluble tablet containing one gram tranexamic acid in 20 mL lukewarm water. Tablets can also be chewed and the mouth can then be rinsed with a small amount of water keepting that for a couple of minutes in the mouth and then spit the liquid out.

Treatment with tranexamic acid tablets is started before dental treatment in the dosage up to 15-25 mg/kg 3-4 times a day already 1-3 days prior to surgery, as repeated dosing will raise the tissue concentration of tranexamic acid. Treatment with tranexamic acid should continue until wound healing or in the case of tooth extraction most often for seven days. Wounds can be treated with local hemostatic agents as fibrin glue and suturing.

Eruption or exfoliation of teeth in children can be treated with tranexamic acid. Extraction of an exfoliating tooth might be necessary if there is continuous bleeding.

Depending on the severity of hemophilia the following medication can be used alone or in combination:

- Tranexamic acid tablets 15-25 mg/kg 3-4 times daily
- Tranexamic acid mouthwash 10 mL 5% suspension 4 times daily
- Desmopressin in mild hemophilia A or
- Factor concentrate
- Local hemostatic agents

Anesthesia

Anesthetic injections in the bottom of the mouth and mandibular injection (intramuscular) should be avoided unless prophylactic treatment to increase the level of the missing coagulation factor is given. Intra-ligamental injection or infiltration-anesthesia can be used without treatment with factor concentrate. Local anesthetics with or without adrenaline can be used.

The Hemophilia Societies

National hemophilia patient organizations exist in all Nordic countries. The members are persons with bleeding disorders, parents, relatives and persons with interest in hemostasis and bleeding disorders.

Bleeding disorders are rare diseases. Half a century ago all Nordic countries experienced a need to establish forums, where patients could meet and exchange information and find support in times, when treatment was only beginning to appear.

The outcome of modern hemophilia care depends on involvement and active participation of patients having a thorough understanding of how to manage the disease. The activities of hemophilia societies therefore create an important framework for peer to peer learning, empowerment and appropriation of relevant knowledge and coping strategies.

The formalized hemophilia societies have some common goals:

- To improve the quality of life for persons with bleeding disorders
- To work for the improvement of treatment for persons with bleeding disorders, e.g. through centralized comprehensive care in highly specialized units
- To offer guidance and support to their members
- To organize events, where members are able to get information on their disease and to exchange experience
- To inform about bleeding disorders to health care providers, schools, other stakeholders, and the public in general

Membership is mostly lifelong. This reflects into the various activities the societies arrange annually for members and their families in different phases of life, e.g. family seminars, summer camps, youth activities, seminars for 50+, etc.

In order for the hemophilia societies to achieve their goals it is very important to have and maintain a good communication and collaboration between the society and clinicians and nurses of the Hemophilia Treatment Centers (HCCC's). The dialogue should be open and transparent. There should be regular meetings between societies and the centers, to inform about activities, news and changes and topics of common interest and planning of joint initiatives.

Regular surveys on quality of life should be performed in collaboration between the centers and the societies to document the outcome and importance of a high quality of treatment.

The Nordic societies have a strong tradition for collaboration and meet annually in order to discuss common topics and challenges of interest for PWHs in the Nordic region.

Comments to the 1st version of the Nordic Hemophilia Guidelines, December, 2014

The Nordic National Hemophilia Societies continuously strive to support the improvement of treatment for persons with bleeding disorders. The societies recognize the Nordic Hemophilia Guidelines as an important step to unify and streamline the treatment in the Nordic countries and by the recommendations to lift up the treatment to a “golden standard”.

In order to achieve this goal, the societies recommend that the Nordic Hemophilia Guidelines are clarified and developed in some important ways:

EHCCC

The Nordic Hemophilia Guidelines are referring to the EUHANET criteria. As these criteria are covering all of Europe it is important to specify as a minimum that the Hemophilia Treatment Centers in the Nordic countries should live up to the criteria for the comprehensive care centers (EHCCC). Especially it is of high importance to incorporate the medical on call service 24/7 and the curriculum of hemophilia experts (doctors as well as nurses) in the guidelines.

Home treatment

Patients and families are taking a large responsibility for their daily treatment. For the patients and families to carry out this task the framework conditions for home treatment have to be in place, e.g. access to support 24/7, training in IV therapy, delivery of medicine locally, social service, etc.

The importance of the haemophilia nurse

Coping with hemophilia through all stages of life requires professional help and support from nurses that have the necessary expertise to provide training and counselling, especially to families with children with a chronic disease.

National database registries

The Nordic National Hemophilia Societies urge the centers to establish national database registries to document the patient's status and use of medicine. The data should be comparable and interchangeable between the Nordic countries for comparison.

Cooperation between HCCC's and the National Hemophilia Societies

We would suggest that the guidelines explicitly underline the importance of cooperation between HCCC's and the national hemophilia society for the purpose of supporting adequate management and coping of the disease by the individual patient and his/her family through educational initiatives, collective and peer to peer learning, participation to summer camps etc.

The hemophilia societies support the initiative of establishing common guidelines for treatment of hemophilia across the Nordic region and will follow the implementations of these standards very closely in dialogue with the centers in order to obtain the best treatment for their members at any time.

/ National patient member organizations in the Nordic countries:

Denmark: Danmarks Bløderforening

Norway: Foreningen for blødere i Norge

Finland: Suomen Hemofiliayhdistys ry

Iceland: Blæðarafélag Íslands

Sweden: Förbundet Blödarsjuka i Sverige

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References

1. White GC, 2nd, Rosendaal F, Aledort LM, Lusher JM, Rothschild C, Ingerslev J, et al. Definitions in hemophilia. Recommendation of the scientific subcommittee on factor VIII and factor IX of the scientific and standardization committee of the International Society on Thrombosis and Haemostasis. Thrombosis and haemostasis. 2001;85(3):560.
2. Kasper CK, Lin JC. Prevalence of sporadic and familial haemophilia. Haemophilia : the official journal of the World Federation of Hemophilia. 2007;13(1):90-2.
3. Plug I, Mauser-Bunschoten EP, Brocker-Vriends AH, van Amstel HK, van der Bom JG, van Diemen-Homan JE, et al. Bleeding in carriers of hemophilia. Blood. 2006;108(1):52-6.
4. Bolton-Maggs PH, Pasi KJ. Haemophilias A and B. Lancet. 2003;361(9371):1801-9.
5. Ikkala E. Haemophilia. A study of its laboratory, clinical, genetic and social aspects based on known haemophiliacs in Finland. Le Scalpel. 1960;12(Suppl 46):1-144.
6. Patek AJ, Taylor FH. Hemophilia. Ii. Some Properties of a Substance Obtained from Normal Human Plasma Effective in Accelerating the Coagulation of Hemophilic Blood. The Journal of clinical investigation. 1937;16(1):113-24.
7. Nilsson IM, Blomback M, Jorpes E, Blomback B, Johansson SA. Von Willebrand's disease and its correction with human plasma fraction 1-0. Acta medica Scandinavica. 1957;159(3):179-88.
8. Ahlberg A, Nilsson IM, Bauer GC. Use of Antihemophilic Factor (Plasma Fraction I-O) during Correction of Knee-Joint Deformities in Hemophilia A. Report of Three Cases Including One Osteotomy. The Journal of bone and joint surgery American volume. 1965;47:323-32.
9. Nilsson IM, Berntorp E, Lofqvist T, Pettersson H. Twenty-five years' experience of prophylactic treatment in severe haemophilia A and B. Journal of internal medicine. 1992;232(1):25-32.
10. Manco-Johnson MJ, Abshire TC, Shapiro AD, Riske B, Hacker MR, Kilcoyne R, et al. Prophylaxis versus episodic treatment to prevent joint disease in boys with severe hemophilia. The New England journal of medicine. 2007;357(6):535-44.
11. Khawaji M, Astermark J, Berntorp E. Lifelong prophylaxis in a large cohort of adult patients with severe haemophilia: a beneficial effect on orthopaedic outcome and quality of life. European journal of haematology. 2012;88(4):329-35.
12. Mannucci PM. Back to the future: a recent history of haemophilia treatment. Haemophilia : the official journal of the World Federation of Hemophilia. 2008;14 Suppl 3:10-8.
13. Hermans C, de Moerloose P, Dolan G. Clinical management of older persons with haemophilia. Critical reviews in oncology/hematology. 2014;89(2):197-206.
14. Makris M, Calizzani G, Fischer K, Gilman EA, Hay CR, Lassila R, et al. EUHASS: The European Haemophilia Safety Surveillance system. Thrombosis research. 2011;127 Suppl 2:S22-5.
15. Lassila R, Holme PA, Landorph A, Petrini P, Onundarson PT, Hillarp A. Nordic Haemophilia Council's practical guidelines on diagnosis and management of von Willebrand disease. Seminars in thrombosis and hemostasis. 2011;37(5):495-502.
16. Lassila R, Antovic JP, Armstrong E, Baghaei F, Dalsgaard-Nielsen J, Hillarp A, et al. Practical viewpoints on the diagnosis and management of heparin-induced thrombocytopenia. Seminars in thrombosis and hemostasis. 2011;37(3):328-36.
17. Colvin BT, Astermark J, Fischer K, Gringeri A, Lassila R, Schramm W, et al. European principles of haemophilia care. Haemophilia : the official journal of the World Federation of Hemophilia. 2008;14(2):361-74.
18. Soucie JM, Nuss R, Evatt B, Abdelhak A, Cowan L, Hill H, et al. Mortality among males with hemophilia: relations with source of medical care. The Hemophilia Surveillance System Project Investigators. Blood. 2000;96(2):437-42.

19. Lassila R, Armstrong E. Current challenges of pharmacovigilance in bleeding disorders: converting the burden to benefit. Haemophilia : the official journal of the World Federation of Hemophilia. 2010;16(2):231-7.
20. www.EUHANET.org.
21. Clinical and Laboratory Standards Institute. Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline-Fifth Edition. Pennsylvania 2008.
22. Adcock DM. Sample Integrity and Preanalytical Variables. In: Kitchen SO, J. D., Preston, F. E., editor. Quality in Laboratory Hemostasis and Thrombosis: Blackwell Publishing; 2009. p. 31-42.
23. Kitchen SM, A.; Echenagucia, M. on behalf of the WFH Laboratory Sciences Committee. Diagnosis of Hemophilia and Other Bleeding Disorders: A Laboratory Manual. Second Edition 2010 (http://www.wfh.org).
24. Verbruggen B, Meijer P, Novakova I, Van Heerde W. Diagnosis of factor VIII deficiency. Haemophilia : the official journal of the World Federation of Hemophilia. 2008;14 Suppl 3:76-82.
25. Pavlova A, Delev D, Pezeshkpoor B, Muller J, Oldenburg J. Haemophilia A mutations in patients with non-severe phenotype associated with a discrepancy between one-stage and chromogenic factor VIII activity assays. Thrombosis and haemostasis. 2014;111(5):851-61.
26. Bowyer AE, Van Veen JJ, Goodeve AC, Kitchen S, Makris M. Specific and global coagulation assays in the diagnosis of discrepant mild hemophilia A. Haematologica. 2013;98(12):1980-7.
27. Verbruggen B, Novakova I, Wessels H, Boezeman J, van den Berg M, Mauser-Bunschoten E. The Nijmegen modification of the Bethesda assay for factor VIII:C inhibitors: improved specificity and reliability. Thrombosis and haemostasis. 1995;73(2):247-51.
28. Keeney S, Mitchell, M., Goodeve, A. on behalf of the UK Haemophilia Centre Doctors' Organisation (UKHCDO), the Haemophilia Genetics Laboratory Network and the Clinical Molecular Genetics Society. Practice Guidelines for the Molecular Diagnosis of Haemophilia A. 2010. Available from: http://www.ukhcdo.org/UKHCDOguidelines.htm.
29. Pruthi RK. Hemophilia: a practical approach to genetic testing. Mayo Clinic proceedings. 2005;80(11):1485-99.
30. Goodeve A. Molecular genetic testing of hemophilia A. Seminars in thrombosis and hemostasis. 2008;34(6):491-501.
31. Lakich D, Kazazian HH, Jr., Antonarakis SE, Gitschier J. Inversions disrupting the factor VIII gene are a common cause of severe haemophilia A. Nature genetics. 1993;5(3):236-41.
32. The FVIII Mutation Database (http://www.factorviii-db.org).
33. The Factor IX Mutation Database (http://www.factorix.org).
34. Rallapalli PM, Kemball-Cook G, Tuddenham EG, Gomez K, Perkins SJ. An interactive mutation database for human coagulation factor IX provides novel insights into the phenotypes and genetics of hemophilia B. Journal of thrombosis and haemostasis : JTH. 2013;11(7):1329-40.
35. Lavery S. Preimplantation genetic diagnosis of haemophilia. British journal of haematology. 2009;144(3):303-7.
36. Laurie AD, Hill AM, Harraway JR, Fellowes AP, Phillipson GT, Benny PS, et al. Preimplantation genetic diagnosis for hemophilia A using indirect linkage analysis and direct genotyping approaches. Journal of thrombosis and haemostasis : JTH. 2010;8(4):783-9.
37. Schneppenheim R, Budde U, Krey S, Drewke E, Bergmann F, Lechler E, et al. Results of a screening for von Willebrand disease type 2N in patients with suspected haemophilia A or von Willebrand disease type 1. Thrombosis and haemostasis. 1996;76(4):598-602.
38. Zhukov O, Popov J, Ramos R, Vause C, Ruden S, Sferruzza A, et al. Measurement of von Willebrand factor-FVIII binding activity in patients with suspected von Willebrand disease type 2N: application of an ELISA-based assay in a reference laboratory. Haemophilia : the official journal of the World Federation of Hemophilia. 2009;15(3):788-96.

39. Blanchette VS, Key NS, Ljung LR, Manco-Johnson MJ, van den Berg HM, Srivastava A, et al. Definitions in hemophilia: communication from the SSC of the ISTH. Journal of thrombosis and haemostasis : JTH. 2014;12(11):1935-9.
40. Fischer K, Steen Carlsson K, Petrini P, Holmstrom M, Ljung R, van den Berg HM, et al. Intermediate-dose versus high-dose prophylaxis for severe hemophilia: comparing outcome and costs since the 1970s. Blood. 2013;122(7):1129-36.
41. Steen Carlsson K, Hojgard S, Glomstein A, Lethagen S, Schulman S, Tengborn L, et al. On-demand vs. prophylactic treatment for severe haemophilia in Norway and Sweden: differences in treatment characteristics and outcome. Haemophilia : the official journal of the World Federation of Hemophilia. 2003;9(5):555-66.
42. Berntorp E, Astermark J, Baghaei F, Bergqvist D, Holmstrom M, Ljungberg B, et al. Treatment of haemophilia A and B and von Willebrand's disease: summary and conclusions of a systematic review as part of a Swedish health-technology assessment. Haemophilia : the official journal of the World Federation of Hemophilia. 2012;18(2):158-65.
43. Feldman BM, Funk SM, Bergstrom BM, Zourikian N, Hilliard P, van der Net J, et al. Validation of a new pediatric joint scoring system from the International Hemophilia Prophylaxis Study Group: validity of the hemophilia joint health score. Arthritis care & research. 2011;63(2):223-30.
44. Pettersson H, Ahlberg A, Nilsson IM. A radiologic classification of hemophilic arthropathy. Clinical orthopaedics and related research. 1980(149):153-9.
45. Chan MW, Leckie A, Xavier F, Uleryk E, Tadros S, Blanchette V, et al. A systematic review of MR imaging as a tool for evaluating haemophilic arthropathy in children. Haemophilia : the official journal of the World Federation of Hemophilia. 2013;19(6):e324-34.
46. Lundin B, Manco-Johnson ML, Ignas DM, Moineddin R, Blanchette VS, Dunn AL, et al. An MRI scale for assessment of haemophilic arthropathy from the International Prophylaxis Study Group. Haemophilia : the official journal of the World Federation of Hemophilia. 2012;18(6):962-70.
47. Astermark J, Petrini P, Tengborn L, Schulman S, Ljung R, Berntorp E. Primary prophylaxis in severe haemophilia should be started at an early age but can be individualized. British journal of haematology. 1999;105(4):1109-13.
48. Ljung RC. Intracranial haemorrhage in haemophilia A and B. British journal of haematology. 2008;140(4):378-84.
49. Santagostino E, Mancuso ME, Rocino A, Mancuso G, Mazzucconi MG, Tagliaferri A, et al. Environmental risk factors for inhibitor development in children with haemophilia A: a case-control study. British journal of haematology. 2005;130(3):422-7.
50. Gouw SC, van den Berg HM, Fischer K, Auerswald G, Carcao M, Chalmers E, et al. Intensity of factor VIII treatment and inhibitor development in children with severe hemophilia A: the RODIN study. Blood. 2013;121(20):4046-55.
51. Petrini P. How to start prophylaxis. Haemophilia : the official journal of the World Federation of Hemophilia. 2003;9 Suppl 1:83-5; discussion 6-7.
52. Lindvall K, Astermark J, Bjorkman S, Ljung R, Carlsson KS, Persson S, et al. Daily dosing prophylaxis for haemophilia: a randomized crossover pilot study evaluating feasibility and efficacy. Haemophilia : the official journal of the World Federation of Hemophilia. 2012;18(6):855-9.
53. Ljung R. The risk associated with indwelling catheters in children with haemophilia. British journal of haematology. 2007;138(5):580-6.
54. Mancuso ME, Berardinelli L, Beretta C, Raiteri M, Pozzoli E, Santagostino E. Improved treatment feasibility in children with hemophilia using arteriovenous fistulae: the results after seven years of follow-up. Haematologica. 2009;94(5):687-92.
55. Berntorp E, Shapiro AD. Modern haemophilia care. Lancet. 2012;379(9824):1447-56.
56. Petrini P, Seuser A. Haemophilia care in adolescents--compliance and lifestyle issues. Haemophilia : the official journal of the World Federation of Hemophilia. 2009;15 Suppl 1:15-9.

57. Young G. From boy to man: recommendations for the transition process in haemophilia. Haemophilia : the official journal of the World Federation of Hemophilia. 2012;18 Suppl 5:27-32.
58. Geraghty S, Dunkley T, Harrington C, Lindvall K, Maahs J, Sek J. Practice patterns in haemophilia A therapy -- global progress towards optimal care. Haemophilia : the official journal of the World Federation of Hemophilia. 2006;12(1):75-81.
59. Lindvall K, Colstrup L, Wollter IM, Klemenz G, Loogna K, Gronhaug S, et al. Compliance with treatment and understanding of own disease in patients with severe and moderate haemophilia. Haemophilia : the official journal of the World Federation of Hemophilia. 2006;12(1):47-51.
60. Astermark J, Donfield SM, DiMichele DM, Gringeri A, Gilbert SA, Waters J, et al. A randomized comparison of bypassing agents in hemophilia complicated by an inhibitor: the FEIBA NovoSeven Comparative (FENOC) Study. Blood. 2007;109(2):546-51.
61. Young G, Shafer FE, Rojas P, Seremetis S. Single 270 microg kg(-1)-dose rFVIIa vs. standard 90 microg kg(-1)-dose rFVIIa and APCC for home treatment of joint bleeds in haemophilia patients with inhibitors: a randomized comparison. Haemophilia : the official journal of the World Federation of Hemophilia. 2008;14(2):287-94.
62. Santagostino E, Mancuso ME, Rocino A, Mancuso G, Scaraggi F, Mannucci PM. A prospective randomized trial of high and standard dosages of recombinant factor VIIa for treatment of hemarthroses in hemophiliacs with inhibitors. Journal of thrombosis and haemostasis : JTH. 2006;4(2):367-71.
63. Schneiderman J, Rubin E, Nugent DJ, Young G. Sequential therapy with activated prothrombin complex concentrates and recombinant FVIIa in patients with severe haemophilia and inhibitors: update of our previous experience. Haemophilia : the official journal of the World Federation of Hemophilia. 2007;13(3):244-8.
64. Ingerslev J, Sorensen B. Parallel use of by-passing agents in haemophilia with inhibitors: a critical review. British journal of haematology. 2011;155(2):256-62.
65. Teitel J, Berntorp E, Collins P, D'Oiron R, Ewenstein B, Gomperts E, et al. A systematic approach to controlling problem bleeds in patients with severe congenital haemophilia A and high-titre inhibitors. Haemophilia : the official journal of the World Federation of Hemophilia. 2007;13(3):256-63.
66. Konkle BA, Ebbesen LS, Erhardtsen E, Bianco RP, Lissitchkov T, Rusen L, et al. Randomized, prospective clinical trial of recombinant factor VIIa for secondary prophylaxis in hemophilia patients with inhibitors. Journal of thrombosis and haemostasis : JTH. 2007;5(9):1904-13.
67. Leissinger C, Gringeri A, Antmen B, Berntorp E, Biasoli C, Carpenter S, et al. Anti-inhibitor coagulant complex prophylaxis in hemophilia with inhibitors. The New England journal of medicine. 2011;365(18):1684-92.
68. Hay CR, DiMichele DM, International Immune Tolerance S. The principal results of the International Immune Tolerance Study: a randomized dose comparison. Blood. 2012;119(6):1335-44.
69. Rocino A, Santagostino E, Mancuso ME, Mannucci PM. Immune tolerance induction with recombinant factor VIII in hemophilia A patients with high responding inhibitors. Haematologica. 2006;91(4):558-61.
70. Darby SC, Keeling DM, Spooner RJ, Wan Kan S, Giangrande PL, Collins PW, et al. The incidence of factor VIII and factor IX inhibitors in the hemophilia population of the UK and their effect on subsequent mortality, 1977-99. Journal of thrombosis and haemostasis : JTH. 2004;2(7):1047-54.
71. Kempton CL, Allen G, Hord J, Kruse-Jarres R, Pruthi RK, Walsh C, et al. Eradication of factor VIII inhibitors in patients with mild and moderate hemophilia A. American journal of hematology. 2012;87(9):933-6.
72. Berntorp E, Astermark J, Carlborg E. Immune tolerance induction and the treatment of hemophilia. Malmo protocol update. Haematologica. 2000;85(10 Suppl):48-50; discussion -1.

73. Beutel K, Hauch H, Rischewski J, Kordes U, Schneppenheim J, Schneppenheim R. ITI with high-dose FIX and combined immunosuppressive therapy in a patient with severe haemophilia B and inhibitor. Hamostaseologie. 2009;29(2):155-7.
74. Kulkarni R. Comprehensive care of the patient with haemophilia and inhibitors undergoing surgery: practical aspects. Haemophilia : the official journal of the World Federation of Hemophilia. 2013;19(1):2-10.
75. Ingerslev J, Hvid I. Surgery in hemophilia. The general view: patient selection, timing, and preoperative assessment. Seminars in hematology. 2006;43(1 Suppl 1):S23-6.
76. Srivastava A, Brewer AK, Mauser-Bunschoten EP, Key NS, Kitchen S, Llinas A, et al. Guidelines for the management of hemophilia. Haemophilia : the official journal of the World Federation of Hemophilia. 2013;19(1):e1-47.
77. Ljung RC, Knobe K. How to manage invasive procedures in children with haemophilia. British journal of haematology. 2012;157(5):519-28.
78. Hermans C, Altisent C, Batorova A, Chambost H, De Moerloose P, Karafoulidou A, et al. Replacement therapy for invasive procedures in patients with haemophilia: literature review, European survey and recommendations. Haemophilia : the official journal of the World Federation of Hemophilia. 2009;15(3):639-58.
79. Tjonnfjord GE, Brinch L, Gedde-Dahl T, Brosstad FR. Activated prothrombin complex concentrate (FEIBA) treatment during surgery in patients with inhibitors to FVIII/IX. Haemophilia : the official journal of the World Federation of Hemophilia. 2004;10(2):174-8.
80. Dargaud Y, Lienhart A, Negrier C. Prospective assessment of thrombin generation test for dose monitoring of bypassing therapy in hemophilia patients with inhibitors undergoing elective surgery. Blood. 2010;116(25):5734-7.
81. Holmstrom M, Tran HT, Holme PA. Combined treatment with APCC (FEIBA(R)) and tranexamic acid in patients with haemophilia A with inhibitors and in patients with acquired haemophilia A--a two-centre experience. Haemophilia : the official journal of the World Federation of Hemophilia. 2012;18(4):544-9.
82. Holme PA, Tran HTT, Paus A, Tjonnfjord GE. Surgery in haemophilia patients with inhibitors to FVIII/FIX: the Norwegian single centre study. Thrombosis and haemostasis. 2011;9(Suppl. 2):512.
83. Teitel JM, Carcao M, Lillicrap D, Mulder K, Rivard GE, St-Louis J, et al. Orthopaedic surgery in haemophilia patients with inhibitors: a practical guide to haemostatic, surgical and rehabilitative care. Haemophilia : the official journal of the World Federation of Hemophilia. 2009;15(1):227-39.
84. Rangarajan S, Austin S, Goddard NJ, Negrier C, Rodriguez-Merchan EC, Stephensen D, et al. Consensus recommendations for the use of FEIBA((R)) in haemophilia A patients with inhibitors undergoing elective orthopaedic and non-orthopaedic surgery. Haemophilia : the official journal of the World Federation of Hemophilia. 2013;19(2):294-303.
85. Shapiro AD, Gilchrist GS, Hoots WK, Cooper HA, Gastineau DA. Prospective, randomised trial of two doses of rFVIIa (NovoSeven) in haemophilia patients with inhibitors undergoing surgery. Thrombosis and haemostasis. 1998;80(5):773-8.
86. Pruthi RK, Mathew P, Valentino LA, Sumner MJ, Seremetis S, Hoots WK, et al. Haemostatic efficacy and safety of bolus and continuous infusion of recombinant factor VIIa are comparable in haemophilia patients with inhibitors undergoing major surgery. Results from an open-label, randomized, multicenter trial. Thrombosis and haemostasis. 2007;98(4):726-32.
87. Valentino LA, Cooper DL, Goldstein B. Surgical experience with rFVIIa (NovoSeven) in congenital haemophilia A and B patients with inhibitors to factors VIII or IX. Haemophilia : the official journal of the World Federation of Hemophilia. 2011;17(4):579-89.
88. Makris M, Hay CR, Gringeri A, D'Oiron R. How I treat inhibitors in haemophilia. Haemophilia : the official journal of the World Federation of Hemophilia. 2012;18 Suppl 4:48-53.
89. Holme P, Tran H, Paus A, Tjonnfjord G. Surgery in haemophilia patients with inhibitors to

FVIII/FIX: the Norwegian single centre study. Thromb Haemost. 2011;9(s2):0-WE-028.
90. Franchini M, Mannucci PM. Co-morbidities and quality of life in elderly persons with haemophilia. British journal of haematology. 2010;148(4):522-33.
91. Khleif AA, Rodriguez N, Brown D, Escobar MA. Multiple Comorbid Conditions among Middle-Aged and Elderly Hemophilia Patients: Prevalence Estimates and Implications for Future Care. Journal of aging research. 2011;2011:985703.
92. Stephensen D, Rodriguez-Merchan EC. Orthopaedic co-morbidities in the elderly haemophilia population: a review. Haemophilia : the official journal of the World Federation of Hemophilia. 2013;19(2):166-73.
93. Mannucci PM, Schutgens RE, Santagostino E, Mauser-Bunschoten EP. How I treat age-related morbidities in elderly persons with hemophilia. Blood. 2009;114(26):5256-63.
94. Konkle BA. The aging patient with hemophilia. American journal of hematology. 2012;87 Suppl 1:S27-32.
95. Konkle BA, Kessler C, Aledort L, Andersen J, Fogarty P, Kouides P, et al. Emerging clinical concerns in the ageing haemophilia patient. Haemophilia : the official journal of the World Federation of Hemophilia. 2009;15(6):1197-209.
96. Riley RR, Witkop M, Hellman E, Akins S. Assessment and management of pain in haemophilia patients. Haemophilia : the official journal of the World Federation of Hemophilia. 2011;17(6):839-45.
97. Coppola A, Santoro C, Franchini M, Mannucci C, Mogavero S, Molinari AC, et al. Emerging issues on comprehensive hemophilia care: preventing, identifying, and monitoring age-related comorbidities. Seminars in thrombosis and hemostasis. 2013;39(7):794-802.
98. Dolan G. The challenge of an ageing haemophilic population. Haemophilia : the official journal of the World Federation of Hemophilia. 2010;16 Suppl 5:11-6.
99. Coppola A, Tagliaferri A, Franchini M. The management of cardiovascular diseases in patients with hemophilia. Seminars in thrombosis and hemostasis. 2010;36(1):91-102.
100. Schutgens RE, Tuinenburg A, Roosendaal G, Guyomi SH, Mauser-Bunschoten EP. Treatment of ischaemic heart disease in haemophilia patients: an institutional guideline. Haemophilia : the official journal of the World Federation of Hemophilia. 2009;15(4):952-8.
101. Bar-Chama N, Snyder S, Aledort L. Sexual evaluation and treatment of ageing males with haemophilia. Haemophilia : the official journal of the World Federation of Hemophilia. 2011;17(6):875-83.
102. Franchini M, Lippi G, Montagnana M, Targher G, Zaffanello M, Salvagno GL, et al. Hemophilia and cancer: a new challenge for hemophilia centers. Cancer treatment reviews. 2009;35(4):374-7.
103. Fogarty PF, Kouides P. How we manage prostate biopsy and prostate cancer therapy in men with haemophilia. Haemophilia : the official journal of the World Federation of Hemophilia. 2012;18(3):e88-90.
104. de Moerloose P, Fischer K, Lambert T, Windyga J, Batorova A, Lavigne-Lissalde G, et al. Recommendations for assessment, monitoring and follow-up of patients with haemophilia. Haemophilia : the official journal of the World Federation of Hemophilia. 2012;18(3):319-25.
105. Witkop M, Lambing A, Divine G, Kachalsky E, Rushlow D, Dinnen J. A national study of pain in the bleeding disorders community: a description of haemophilia pain. Haemophilia : the official journal of the World Federation of Hemophilia. 2012;18(3):e115-9.
106. Humphries TJ, Kessler CM. The challenge of pain evaluation in haemophilia: can pain evaluation and quantification be improved by using pain instruments from other clinical situations? Haemophilia : the official journal of the World Federation of Hemophilia. 2013;19(2):181-7.
107. Holstein K, Klamroth R, Richards M, Carvalho M, Perez-Garrido R, Gringeri A, et al. Pain management in patients with haemophilia: a European survey. Haemophilia : the official journal of the World Federation of Hemophilia. 2012;18(5):743-52.

108. Polston GR, Wallace MS. Analgesic agents in rheumatic disease. In: Firestein GS, et al, editor. Kelley's Textbook of Rheumatology. 9th Edition ed. Philadelphia, PA, USA: Elsevier Saunders; 2013. p. 1014-33.
109. Cheng OT, Souzdalnitski D, Vrooman B, Cheng J. Evidence-based knee injections for the management of arthritis. Pain medicine. 2012;13(6):740-53.
110. Shupak R, Teitel J, Garvey MB, Freedman J. Intraarticular methylprednisolone therapy in hemophilic arthropathy. American journal of hematology. 1988;27(1):26-9.
111. Fernandez-Palazzi F, Caviglia HA, Salazar JR, Lopez J, Aoun R. Intraarticular dexamethasone in advanced chronic synovitis in hemophilia. Clinical orthopaedics and related research. 1997(343):25-9.
112. Engelbert RH, Plantinga M, Van der Net J, Van Genderen FR, Van den Berg MH, Helders PJ, et al. Aerobic capacity in children with hemophilia. The Journal of pediatrics. 2008;152(6):833-8, 8 e1.
113. Tlacuilo-Parra A, Morales-Zambrano R, Tostado-Rabago N, Esparza-Flores MA, Lopez-Guido B, Orozco-Alcala J. Inactivity is a risk factor for low bone mineral density among haemophilic children. British journal of haematology. 2008;140(5):562-7.
114. Gomis M, Querol F, Gallach JE, Gonzalez LM, Aznar JA. Exercise and sport in the treatment of haemophilic patients: a systematic review. Haemophilia : the official journal of the World Federation of Hemophilia. 2009;15(1):43-54.
115. Heijnen L. The role of rehabilitation and sports in haemophilia patients with inhibitors. Haemophilia : the official journal of the World Federation of Hemophilia. 2008;14 Suppl 6:45-51.
116. Beeton K, De Kleijn P, Hilliard P, Funk S, Zourikian N, Bergstrom BM, et al. Recent developments in clinimetric instruments. Haemophilia : the official journal of the World Federation of Hemophilia. 2006;12 Suppl 3:102-7.
117. Hilliard P, Funk S, Zourikian N, Bergstrom BM, Bradley CS, McLimont M, et al. Hemophilia joint health score reliability study. Haemophilia : the official journal of the World Federation of Hemophilia. 2006;12(5):518-25.
118. Callahan LF, Brooks RH, Summey JA, Pincus T. Quantitative pain assessment for routine care of rheumatoid arthritis patients, using a pain scale based on activities of daily living and a visual analog pain scale. Arthritis and rheumatism. 1987;30(6):630-6.
119. van Genderen FR, Westers P, Heijnen L, de Kleijn P, van den Berg HM, Helders PJ, et al. Measuring patients' perceptions on their functional abilities: validation of the Haemophilia Activities List. Haemophilia : the official journal of the World Federation of Hemophilia. 2006;12(1):36-46.
120. Poonnoose PM, Manigandan C, Thomas R, Shyamkumar NK, Kavitha ML, Bhattacharji S, et al. Functional Independence Score in Haemophilia: a new performance-based instrument to measure disability. Haemophilia : the official journal of the World Federation of Hemophilia. 2005;11(6):598-602.
121. Gurcay E, Eksioglu E, Ezer U, Cakir B, Cakci A. A prospective series of musculoskeletal system rehabilitation of arthropathic joints in young male hemophilic patients. Rheumatology international. 2008;28(6):541-5.
122. De Kleijn P, Blamey G, Zourikian N, Dalzell R, Lobet S. Physiotherapy following elective orthopaedic procedures. Haemophilia : the official journal of the World Federation of Hemophilia. 2006;12 Suppl 3:108-12.
123. Heijnen L, Buzzard BB. The role of physical therapy and rehabilitation in the management of hemophilia in developing countries. Seminars in thrombosis and hemostasis. 2005;31(5):513-7.
124. Kasper CK, Lin JC. How many carriers are there? Haemophilia : the official journal of the World Federation of Hemophilia. 2010;16(5):842.
125. Lyon MF. Sex chromatin and gene action in the mammalian X-chromosome. American journal of human genetics. 1962;14:135-48.

126. Dunn NF, Miller R, Griffioen A, Lee CA. Carrier testing in haemophilia A and B: adult carriers' and their partners' experiences and their views on the testing of young females. Haemophilia : the official journal of the World Federation of Hemophilia. 2008;14(3):584-92.
127. Street AM, Ljung R, Lavery SA. Management of carriers and babies with haemophilia. Haemophilia : the official journal of the World Federation of Hemophilia. 2008;14 Suppl 3:181-7.
128. Lee CA, Chi C, Pavord SR, Bolton-Maggs PH, Pollard D, Hinchcliffe-Wood A, et al. The obstetric and gynaecological management of women with inherited bleeding disorders--review with guidelines produced by a taskforce of UK Haemophilia Centre Doctors' Organization. Haemophilia : the official journal of the World Federation of Hemophilia. 2006;12(4):301-36.
129. Myrin-Westesson L, Baghaei F, Friberg F. The experience of being a female carrier of haemophilia and the mother of a haemophilic child. Haemophilia : the official journal of the World Federation of Hemophilia. 2013;19(2):219-24.
130. Nicolaides K, Brizot Mde L, Patel F, Snijders R. Comparison of chorionic villus sampling and amniocentesis for fetal karyotyping at 10-13 weeks' gestation. Lancet. 1994;344(8920):435-9.
131. Michaelides K, Tuddenham EG, Turner C, Lavender B, Lavery SA. Live birth following the first mutation specific pre-implantation genetic diagnosis for haemophilia A. Thrombosis and haemostasis. 2006;95(2):373-9.
132. Bremme KA. Haemostatic changes in pregnancy. Best practice & research Clinical haematology. 2003;16(2):153-68.
133. Chi C, Lee CA, Shiltagh N, Khan A, Pollard D, Kadir RA. Pregnancy in carriers of haemophilia. Haemophilia : the official journal of the World Federation of Hemophilia. 2008;14(1):56-64.
134. Kadir RA, Economides DL, Braithwaite J, Goldman E, Lee CA. The obstetric experience of carriers of haemophilia. British journal of obstetrics and gynaecology. 1997;104(7):803-10.
135. Ljung R, Lindgren AC, Petrini P, Tengborn L. Normal vaginal delivery is to be recommended for haemophilia carrier gravidae. Acta paediatrica. 1994;83(6):609-11.
136. Zanon E, Martinelli F, Bacci C, Zerbinati P, Girolami A. Proposal of a standard approach to dental extraction in haemophilia patients. A case-control study with good results. Haemophilia : the official journal of the World Federation of Hemophilia. 2000;6(5):533-6.
137. Franchini M, Rossetti G, Tagliaferri A, Pattacini C, Pozzoli D, Lorenz C, et al. Dental procedures in adult patients with hereditary bleeding disorders: 10 years experience in three Italian Hemophilia Centers. Haemophilia : the official journal of the World Federation of Hemophilia. 2005;11(5):504-9.
138. Kalsi H, Nanayakkara L, Pasi KJ, Bowles L, Hart DP. Access to primary dental care for patients with inherited bleeding disorders. Haemophilia : the official journal of the World Federation of Hemophilia. 2012;18(4):510-5.
139. Lee AP, Boyle CA, Savidge GF, Fiske J. Effectiveness in controlling haemorrhage after dental scaling in people with haemophilia by using tranexamic acid mouthwash. British dental journal. 2005;198(1):33-8; discussion 26.