The past and future of haemophilia: diagnosis, treatments, and its complications

Flora Peyvandi, Isabella Garagiola, Guy Young

Haemophilia A and B are hereditary haemorrhagic disorders characterised by deficiency or dysfunction of coagulation protein factors VIII and IX, respectively. Recurrent joint and muscle bleeds lead to severe and progressive musculoskeletal damage. Existing treatment relies on replacement therapy with clotting factors, either at the time of bleeding (ie, on demand) or as part of a prophylactic schedule. The major complication of such therapy is the development of neutralising antibodies (ie, inhibitors), which is most frequent in haemophilia A. Treatment might improve considerably with the availability of new modified drugs, which might overcome existing prophylaxis limitations by reducing dosing frequency and thereby rendering therapy less distressing for the patient. Subcutaneous administration of some new therapies would also simplify prophylaxis in children with poor venous access. Gene therapy has the potential for a definitive cure, and important results have been obtained in haemophilia B. Despite improvements in haemophilia care, the availability of clotting factor concentrates for all affected individuals worldwide remains the biggest challenge.

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Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy (Prof F Peyvandi MD); **Department of Pathophysiology and Transplantation, University of Milan, Milan, Italy** (Prof F Peyvandi, I Garagiola PhD); **The Saban Research Institute, Children's Hospital Los Angeles, Los Angeles, CA, USA** (G Young MD); **and University of Southern California Keck School of Medicine, Los Angeles, CA, USA** (G Young)

Correspondence to:
Prof Flora Peyvandi, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan 9-20122, Italy
flora.peyvandi@unimi.it

Introduction

Haemophilia is an inherited bleeding disorder caused by deficiency or dysfunction of the coagulation proteins factor VIII, leading to haemophilia A, and factor IX, leading to haemophilia B. Since these plasma glycoproteins have an essential role in coagulation, faults cause decreased and delayed generation of thrombin, giving rise to defects in clot formation that lead to haemorrhagic diathesis. These defects are associated with bleeding episodes affecting soft tissue, joints, and muscles. Repeated haemorrhages result in chronic arthropathy, with loss of joint movement.[1] At present, patients receive intravenous replacement therapy of the deficient coagulation factors, either at the time of bleeding (ie, on-demand therapy) or as a prophylactic regimen. These disorders are inherited as X-linked recessive traits—ie, male individuals are affected and female individuals are asymptomatic or mildly affected carriers. Globally, the prevalence of haemophilia A is around 1 in 5000 male births, and 1 in 30 000 male births for haemophilia B.[1,2] These figures are similar throughout the world regardless of ancestry or ethnic origin. In this Seminar, we provide an overview of the diagnosis of haemophilia and describe the clinical manifestations and treatment (both on-demand and prophylactic). Finally, we discuss inhibitor formation as a complication of replacement therapy and its treatment.

Diagnosis

Coagulation screening tests typically reveal a prolonged activated partial thromboplastin time with a normal prothrombin time.[3] Measurement of factor VIII or IX clotting activity is used for diagnosis. On the basis of the residual coagulant activity in blood (factor VIII C or factor IX C), haemophilia is classified into three main forms: severe, moderate, and mild. Patients with a coagulation factor level of less than 1 IU/dL (ie, <1% of normal) are classified as severe and constitute about half of diagnosed cases. Moderate haemophilia is defined as factor levels of 1–5 IU/dL (ie, 1–5% of normal), and mild disease as 5–40 IU/dL (ie, 5–40% of normal).[4] Around 70% of patients have a positive family history of haemophilia.

Haemophilia A can be diagnosed at an early age, sometimes even by measuring activity of factor VIII in the cord blood directly after birth.[5] The plasma activity level of factor VIII can be assessed with one-stage and two-stage or chromogenic assays. However, these assays are unable to measure factor VIII levels accurately below 1 IU/dL,[6] which precludes prediction of clinical phenotype on the basis of factor VIII activity. New global assays (eg, thrombin generation assay and thromboelastography) can be used to measure factor VIII activity in the range of 0–2 IU/dL, and showed that clinical heterogeneity in individuals with levels less than 1 IU/dL is not associated with their factor VIII activity level.[6–8] In 30% of patients with mild or moderate haemophilia A, a discrepancy exists between the one-stage assay and the two-stage or chromogenic assay. Several genetic defects have been associated with these findings—eg, defects clustered in the A1–A2–A3 domain interfaces are associated with higher factor VIII activity when measured by the one-stage assay than by the two-stage or chromogenic assay.[9,10]

All vitamin K-dependent factors, including factor IX, are reduced at birth and further reduced in preterm infants,[11] so a diagnosis of haemophilia B at birth,

Search strategy and selection criteria

We searched MEDLINE and PubMed for articles published in English between Jan 1, 1980, and March 31, 2015, with the following search terms: "Hemophilia/diagnosis[Majr] OR Hemophilia/hemorrhage[Majr] OR Hemophilia/drug therapy/therapy[Majr] OR Hemophilia [Majr] AND Antibodies[Majr]", and "(haemophilia [TI] OR hemophilia [TI]) AND (diagnos* OR therap* OR treatmen*) AND "2015/03/30"[PDAT]". After excluding articles on the basis of their titles or abstracts, the remaining articles and those identified from reference lists were included in this Seminar.

particularly of mild disease, could be misleading and needs to be confirmed after age 6 months. The one-stage clotting assay is the established and standardised method to measure factor IX activity level in plasma. Chromogenic assays can also be used, but standardised procedures for their use in clinical haemostasis laboratories have not yet been approved.[12]

Genetic analysis in all patients with haemophilia is recommended to establish the causative mutation. A gene defect is thought to contribute to about 40% of the risk of inhibitor formation, a serious treatment complication. Overall, large deletions and nonsense mutations show the highest risk, whereas missense and splicing mutations show the lowest risk.[13] Additionally, identification of the gene mutation is important to recognise female relatives who might be carriers. Accurate knowledge of carrier status can lead to the identification of affected individuals at or before birth by prenatal diagnosis.

In patients with severe haemophilia A, molecular characterisation starts with the detection of inversions of intron 22 (reported in 40–45% of severe patients) and intron 1 (reported in 1–6% of severe patients) of *F8* (which encodes factor VIII).[14,15] If these two frequent genetic variations are not present, full mutation screening of the gene is done by direct Sanger sequencing, covering all exons, intron–exon boundaries, and the promoter region. More than 2000 unique molecular defects in *F8* have been described and are included in the Worldwide Factor VIII Variant Database. Point mutations (missense, nonsense, and splice site mutations) account for 67% of molecular defects described, and small insertions and deletions represent 25% of such defects. Roughly 6% of all mutations are large deletions. A possible method for detection of deletions and duplications is the multiplex ligation-dependent probe amplification assay.[16] Genetic variations have not been identified in about 2–18% of patients with haemophilia A.[17–22] In moderate and mild haemophilia A, because of the absence of common gene defects, full mutation analysis of *F8* by direct Sanger sequencing is necessary.

For more on the **Worldwide Factor VIII Variant Database** see http://www.factorviii-db.org/

Molecular characterisation in haemophilia B is done by sequence analysis of the eight exons, intron–exon boundaries, and the promoter region in *F9* (which encodes factor IX). Causative genetic variations are scattered over the entire length of the gene, and 1095 unique variants have been described so far (see Factor IX Variant Database). Missense, nonsense, and splice site mutations are the most common, accounting for around 70% of mutations, followed by frameshift mutations (roughly 17%). Deletions and duplications in *F9* can be detected with a multiplex ligation-dependent probe amplification kit.[23] Large deletions in *F9* and mutations in the promoter region are relatively rare, accounting for roughly 3% and 2% of the total reported gene variations, respectively. Mutations in the short region of the proximal promoter (Human Genome Variation Society c.-50 to c.-18; legacy –27 to +13) can give rise to the unique haemophilia B Leyden phenotype, which might arise from mutations that spare the androgen-responsive element while affecting other transcription factor binding sites (HNF4α, C/EBPα, and HNF6).[24,25] These mutations result in a severe phenotype at birth that resolves at puberty, producing normal levels of factor IX in adulthood.[26] No causative gene variation was identified in a small proportion (<3%) of patients with haemophilia B.[27–29] Unlike in haemophilia A, in haemophilia B no common frequent genetic variation, such as intron 22 inversion, has been identified. However, 20–30% of cases of mild haemophilia B are caused by roughly ten founder gene variants.[30,31] Next-generation sequencing provides enhanced opportunities to characterise molecular defects in patients with haemophilia, particularly in those whose molecular defect has not been determined yet.[32]

For more on the **Factor IX Variant Database** see http://www.factorix.org

Existing methods of prenatal diagnosis all require cells of fetal origin by chorionic villus sampling at 11–14 weeks of pregnancy or amniocentesis after the 15th week. These procedures are invasive and have a small but significant risk of pregnancy loss (0·5–1·0%).[33] Free fetal DNA in maternal blood, first recognised in 1997,[34] offers a method for fetal sex determination at early gestational age (after the 9th week of gestation) for X-linked disorders by a normal venepuncture, avoiding the procedure-related risks of invasive methods.[35,36] An alternative to conventional prenatal diagnosis is preimplantation genetic diagnosis, which provides the opportunity for couples who have a known genetically transmittable disease to start a pregnancy with the knowledge that their child is unaffected by the specific disorder tested.[37,38]

Clinical manifestations

The clinical pictures of patients with haemophilia A and B are largely similar, and conflicting data are reported in the literature. Some evidence suggests that patients with haemophilia B have a lower bleeding frequency and better long-term outcomes than those with haemophilia A,[39,40] although a 2014 study[41] suggested similar severity and variation in bleeding phenotype.

The risk of haemorrhage depends both on the severity of clotting factor deficiency and on the age of the patient. The earliest and most serious complication in neonates with severe haemophilia is intracranial haemorrhage, which occurs in 1–4% of cases and can lead to permanent neurological sequelae.[42] Extracranial haemorrhage, including both subgaleal bleeding and cephalhaematoma formation, occurs either alone or in conjunction with an intracranial haemorrhage, and can be life threatening. Most cases of intracranial and extracranial haemorrhage are caused by traumatic deliveries, particularly with the use of forceps, vacuum extraction, or fetal scalp monitors.[42,43] Bleeding after surgery is an important complication in patients affected by all severities of

haemophilia. Circumcision of male babies is a common operative procedure in some regions of the world. Bleeding from circumcision has an incidence of 0·1–35·0%,[42] and was reported in 27·4% of 404 neonates and toddlers in a prospective study.[44] In severe haemophilia, spontaneous muscle haemorrhage occurs in the lower legs, buttocks, iliopsoas muscle, and forearms.[45] Almost half of children with severe haemophilia have a muscle bleed or haematoma at age 6–8 months when physical activity increases.[46]

Another common and frequent manifestation of severe haemophilia A and B is non-traumatic (spontaneous) intra-articular bleeding (ie, haemarthrosis). With increasing age, the occurrence of joint bleeding increases from a mean of 21% of all haemorrhages in children aged 1–6 years, to 50% in those aged 10–17 years, and 60% in patients aged 18–65 years.[47] The most frequent site of bleeding in both children and adults is the ankle, followed by the elbow and the knee.[48] Recurrent haemorrhages in the same joint (ie, a target joint) cause inflammation of the synovial tissue (ie, synovitis), with progressive damage of the tissue and the development of haemophilic arthropathy.[49] The final stage of haemophilic arthropathy is chronic joint deformity, pain, muscle arthropathy, and functional impairment.[50] Prophylactic factor replacement therapy helps to decrease the occurrence of joint bleeding in young patients (younger than 2 years).[51] Arthropathy is the main comorbidity in older patients who have not had the benefit of early prophylactic treatment. In the past 25 years, joint replacement has shown a substantial improvement in measurable outcome parameters, such as reduction in spontaneous haemarthroses, and in quality of life. Nonetheless, the risk and benefits of these procedures should be considered carefully, taking into account patient age, life expectancy, and immunological status.[52]

In patients with moderate haemophilia, the diagnosis is made later and spontaneous bleeds are infrequent; haemorrhages tend to occur after injury, trauma, or surgery. Clinical phenotypes in moderate haemophilia are heterogeneous, with roughly 25% of patients having several joint bleeds similar to severe haemophilia, and a similar proportion without any joint bleeds over long follow-up.[53] There are scant data, particularly from prospective and long-term studies, for the prevalence and severity of arthropathy in patients with moderate haemophilia, but it is well recognised that a considerable number have joint disease, with subsequent musculoskeletal pain, disability, and need of orthopaedic aids or surgery.[53]

Mild haemophilia is not generally associated with spontaneous bleeding, with nearly all incidents caused by trauma or surgery. These patients are frequently diagnosed later in life when routine coagulation screening before elective surgery shows abnormalities. Haemophilia carriers have a tendency to bleed, including heavy menstrual bleeding, atraumatic haemarthrosis, cutaneous bruising, haematomas, and post-surgical and peri-partum haemorrhage.[54]

Treatment strategies

The main treatment is replacement therapy (also known as substitution therapy)—ie, administration of the deficient clotting factor to achieve adequate haemostasis. On-demand treatment is infusion of the deficient clotting factor at the time of bleeding. The appropriate dose, frequency, and number of concentrate infusions depend on the type and severity of the bleed. In 2013, the World Federation of Haemophilia Treatment Guidelines Working Group published detailed recommendations on disease management (appendix).[55]

For more on the **World Federation of Haemophilia Treatment Guidelines Working Group** see http//:www.wfh.org

See **Online** for appendix

Prophylaxis in haemophilia is treatment with intravenous injection of factor concentrate to prevent bleeding and joint destruction, with the objective of preserving normal musculoskeletal function. It was first applied after the finding that patients with moderate haemophilia (clotting factor level >1 IU/dL) seldom bleed spontaneously and have much better preservation of joint function than those with severe haemophilia.[56] Primary prophylaxis in haemophilia is long term and requires treatment 2–3 times per week, starting at a very young age (≤2 years) before joint disease develops, whereas secondary prophylaxis begins after the onset of joint disease.[57,58] Many approaches to prophylaxis exist,[59,60] even within the same country, and the optimum regimen remains to be defined. Prophylactic therapy is usually given as a dose of 25–40 IU/kg 2–3 times per week. A low-dose prophylactic regimen of 10–20 IU/kg twice per week has been proposed in some countries with limited supply of concentrates, and seems to be effective.[61]

The challenge with early initiation (age 1–2 years), which is important to prevent future joint disease,[51,62] is venous access. Central venous access device is often necessary to facilitate home infusions by parents, but these devices carry a risk of infection, sepsis, and thrombosis.[63] To overcome this drawback, several investigators have recommended initiating therapy with one infusion per week; although this regimen is fairly effective, the need for a central venous access device is not always avoided and haemarthrosis can still occur. In the past, most prophylactic regimens used fixed doses; however, a present movement is to adapt dosing regimens to individual patient needs. Methods to tailor regimens have included individual pharmacokinetics with computer-simulated doses and intervals to achieve a predetermined trough activity level.[64] Although researchers generally agree that prophylaxis should be started early, there is less consensus regarding whether or when it should be stopped.[65]

In conclusion, although prophylaxis has clearly become the standard approach in countries with a plentiful supply of factor concentrates, many uncertainties remain regarding the ideal age to begin treatment; the ideal dosing regimen both at initiation and later in life, taking

into account physical activity; individualised tailoring of dosing regimens, which involves laboratory monitoring; and long-term outcomes. Additionally, concerns such as quality of life and pharmacoeconomics still need to be addressed. These issues will become even more important with the introduction of products with extended half-lives, which have already become available in some countries.

In mild forms of haemophilia A, the synthetic vasopressin analogue desmopressin acetate can be used to increase plasma concentrations of factor VIII and von Willebrand factor.[66] Desmopressin can be administered intravenously, as an intranasal spray, or subcutaneously. A single dose of 0·3 µg/kg bodyweight, either intravenously or subcutaneously, can boost the concentration of factor VIII by 3–6 times.[67] The peak response is seen around 1 h after administration and generally depends on the patient's baseline factor VIII activity. Not all patients achieve an acceptable haemostatic activity level with this drug.

Development of treatment

The first treatment for haemophilia consisted of direct blood transfusion in 1840. In the 1950s and much of the 1960s, bleeding episodes were treated with fresh frozen plasma. Modern treatment started in 1965 with identification of the cryoprecipitate fraction of fresh frozen plasma by Judith Pool.[68] Subsequently, technologies to separate factor VIII or IX from large pools of donor plasma resulted in freeze-dried, lyophilised factor VIII or IX concentrates,[69] which made home therapy possible and improved patients' quality of life considerably. Unfortunately, in the 1970s and 1980s, thousands of patients with haemophilia worldwide were infected with HIV and hepatitis C virus from contaminated plasma-derived products.[70] Although safer products have emerged, the theoretical risk of contamination of blood products with known and unknown infectious agents (ie, parvovirus B19 and prion-associated disease, such as variant Creutzfeldt-Jakob disease) still remains.[71] The first commercially available recombinant protein products, free of blood-borne pathogen transmission, became available in 1992 for haemophilia A and 1997 for haemophilia B.[70]

Since then, various plasma-derived and recombinant products have been developed. However, the main limitation with existing standard products is their short half-lives (8–12 h for factor VIII and 18–24 h for factor IX), and frequent administrations are thus necessary. The most serious complication of replacement therapy is the development of inhibitors—ie, antibodies directed against infused concentrates after such therapy. New products have extended half-lives or act through different routes—eg, by enhancing thrombin generation through reduction of natural anticoagulant activity—rather than replacing the deficient protein, thereby diminishing the frequency of injections and potentially reducing their immunogenicity.

One bioengineering technology that has increased the half-lives of recombinant clotting factors is PEGylation, which is widely used in drug modification and consists of the covalent binding of polyethylene glycol (PEG) polymers to therapeutic agents, either through site-specific binding to free cysteine residues or via protein engineering (ie, site-specific PEGylation and glycoPEGylation).[72–77] Another strategy to extend the half-lives of proteins is to fuse them to another protein with a much longer half-life, such as the fragment crystallisable (Fc) region of IgG or human albumin. Both approaches take advantage of neonatal Fc receptor (FcRn)-mediated recycling, which delays lysosomal degradation of the fusion proteins and recycles them back into circulation.[78–81] These strategies have shown an improvement in half-life for both products, although the increase is greater for recombinant factor IX (3–6 times) than for recombinant factor VIII (roughly 1·5–1·6 times; table 1).

	Half-life (h)	Half-life improvement	Clinical trials in adults	Paediatric trials	Clinical trials in previously untreated patients
Factor VIII					
Site-specific PEGylation (PEG 60 kDa in A3 domain)	18·4 (13·7–28·1)	1·5–1·6 times	Phase 3 trial completed (NCT01580293)	Ongoing (NCT01775618)	None
Site-specific PEGylation (PEG 40 kDa in B domain linker region)	19·0 (11·6–27·3)	1·5–1·6 times	Phase 3 trial completed (NCT01480180)	Ongoing (NCT01731600)	Ongoing (NCT02137850)
PEGylation (PEG molecule of 20 kDa)	18	1·5–1·6 times	Approved by FDA in 2015	Completed (NCT02210091)	Ongoing (NCT02615691)
Fc fusion	18·8 (14·3–24·5)	1·5 times	Approved by FDA in 2014	Completed (NCT01458106)	Ongoing (NCT02234323)
Factor IX					
Site-directed glycoPEGylation	93 (85–111)	6 times	Phase 3 trial completed (NCT01333111)	Ongoing (NCT01467427)	Ongoing (NCT02141074)
Fc fusion	82·1 (71·4–94·5)	3–5 times	Approved by FDA in 2014	Completed (NCT01440946)	Completed (NCT02234310)
Albumin fusion	91·57	5 times	Phase 3 trial completed (NCT01496274)	Completed (NCT01662531)	Ongoing (NCT02053792)

Data are mean (range). PEG=polyethylene glycol. Fc=fragment crystallisable. FDA=US Food and Drug Administration.

Table 1: **Clinical trials of therapeutics with extended half-lives**

	Product	Dosing schedule	Route of administration	Status of clinical trials
Inhibition of natural anticoagulants				
Antibody against tissue factor pathway inhibitor	Monoclonal antibody 2021	Once weekly	Subcutaneous injection	Phase 1 trial ongoing (NCT02490787)
RNA interference agent against antithrombin	ALN-AT3	Once weekly	Subcutaneous injection	Phase 1 trial ongoing (NCT02035605); phase 1/2 trial ongoing (NCT02554773)
Promotion of thrombin generation by mimicking the cofactor activity of factor VIII				
Bispecific antibody to factors IXa and X	ACE910	Once weekly	Subcutaneous injection	Phase 1/2 trial completed (JapicCTI-121934)

Table 2: **Clinical trials of novel non-replacement products**

	Viral vector	Route of administration	Status	Sponsor
NCT00979238 (phase 1)	Self-complementary adeno-associated virus (scAAV 2/8-LP1-hFIXco)	Peripheral vein infusion	Recruiting	St Jude Children's Research Hospital, Memphis, TN, USA
NCT01620801 (phase 1/2)	Single-stranded, adeno-associated pseudotype 8 virus (AAV8-hFIX19 vector)	Intravenous	Active, not recruiting	Spark Therapeutics
NCT00515710 (phase 1)	Adeno-associated virus (AAV2-hFIX16)	Intrahepatic	Active, not recruiting	Spark Therapeutics
NCT02484092 (phase 1/2)	Novel recombinant adeno-associated virus (SPK-9001)	Intravenous	Recruiting	Spark Therapeutics
NCT01687608 (phase 1/2)	Self-complementing optimised adeno-associated virus serotype 8 (BAX 335)	Intravenous	Recruiting	Baxalta US
NCT02396342 (phase 1/2)	Adeno-associated viral vector containing a codon-optimised human *F9* gene (AAV5-hFIX)	Intravenous	Recruiting	UniQure Biopharma

Table 3: **Clinical trials of gene therapy in haemophilia B**

Additionally, other technologies attempt to improve haemostasis by reducing the effect of natural anticoagulants rather than replacing the missing factor (ie, monoclonal antibody 2021, which targets tissue factor pathway inhibitor, and ALN-AT3, a small RNA interference agent designed against antithrombin; table 2).[82–84] Yet another approach is the use of a bispecific antibody (ACE910) that promotes thrombin generation by binding to factors IXa and X, thus mimicking the cofactor activity of factor VIII.[85] Preliminary data suggest that these approaches have the potential to be effective in patients with inhibitors, who otherwise have very few options for management of their bleeding. In a phase 1/2 clinical trial,[86] prophylactic treatment with weekly subcutaneous administration of ACE910 was well tolerated and decreased the number of bleeding episodes in patients with and without factor VIII inhibitors. These non-replacement products might overcome existing issues of prophylaxis (ie, dosing frequency and patient distress) and potentially reduce treatment-related immunogenicity. Moreover, subcutaneous administration would also simplify prophylaxis in children with poor venous access. Clinical studies of all of these compounds are underway; if they show favourable safety and suitable efficacy profiles, they could make prophylactic regimens more straightforward, thus reducing dosage frequency and extending protection from bleeding.

Gene therapy

Present treatment is prophylactic rather than curative. Gene therapy has the potential to lessen disease severity from a severe phenotype to a moderate or mild phenotype through continuous production of factor VIII or IX after one administration of a gene vector, especially since a small rise in circulating coagulant proteins to at least 1% of normal levels can substantially ameliorate the bleeding phenotype. Nathwani and colleagues[87] have reported an important milestone in a gene therapy trial for haemophilia B using a self-complementary adeno-associated virus serotype 8. A consistent increase in factor IX activity to 5–7% was seen in patients followed up for 1·0–4·5 years.[88] Severe side-effects have not been reported, with the exception of increased liver enzymes, which resolved with prednisolone, in four patients treated with the high vector dose. Clinical trials in haemophilia B based on different strategies are ongoing (table 3).

Gene therapy in haemophilia A showed a substantial limitation in the generation of efficient viral gene delivery systems—the large size of the *F8* cDNA, which at 7·0 kb far exceeds the normal packaging capacity of adeno-associated viral vectors.[89] The development of codon-optimised human *F8* cDNAs showed that shorter *F8* constructs can result in increased efficacy and might give rise to effective gene therapy.[90] Several groups are attempting to overcome the packaging limitation with

Panel: Risk factors for inhibitor development

Patient-related factors

- Severity of haemophilia
- *F8* gene mutation
- Family history of inhibitors
- Being of black ethnic origin
- Polymorphisms of immune-response genes (*IL-10*, *CTLA4*, *TNFA*, and *FCGR*)

Treatment-related factors

- Number of exposure days
- Intensity of exposure
- Product type—plasma derived versus recombinant
- Age at first exposure
- Prophylaxis with factor replacement therapy

the production of dual recombinant adeno-associated viral vectors for *F8* delivery[91] or by using lentiviral vectors for gene transfer.[92]

Inhibitors

An inhibitor is a polyclonal high-affinity IgG antibody that specifically neutralises the procoagulant activity of the relevant clotting factor, rendering management of bleeds difficult. Inhibitors are characterised in two ways—by the titre and by the anamnestic response. The titre refers to the inhibitory capacity of the patient's plasma to neutralise clotting factor in normal plasma. The Nijmegen Bethesda assay should be adopted to quantify inhibitors, as recommended by the FVIII/IX Subcommittee of Scientific Standardization Committee of the International Society of Thrombosis and Haemostasis.[93] A high-titre inhibitor is defined as having 5 Bethesda units (BU) or higher, and a low-titre one as between a cutoff value (usually 0·6 BU) and 5 BU. Patients whose titre is less than 5 BU are divided into those in whom a rapid anamnestic response to factor infusion occurs (ie, high responders) and those in whom such a response does not occur (ie, low responders). This characterisation is important because patients with a low titre and low responding inhibitors can be treated with standard replacement therapy, albeit at increased doses to overwhelm the inhibitor. Patients with a high titre or high responding inhibitors can be treated effectively only with bypassing agents, unless the inhibitor is eradicated.[94]

In severe haemophilia A, factor VIII inhibitors form in 30% of patients, usually during the first 20–30 days of exposure.[95] The immunology of inhibitor development is complex and not completely understood. Such development results from a multicausal immune response involving both patient-related and treatment-related factors (panel). Studies on genetic determinants of inhibitor development have shown the involvement of genetic markers such as the type of causative *F8* mutation,[13,96] single-nucleotide polymorphisms in the *HLA* locus and other immune regulatory genes,[97–100] and ethnic background.[101] The most common acquired risk factor is the intensity of exposure to concentrate.[102] Although the type of concentrate (plasma derived *vs* recombinant[103–107]) has been implicated in several studies, controversies remain. The SIPPET randomised trial[108] showed that recombinant factor VIII concentrates were associated with an 87% higher incidence of inhibitor formation than did plasma-derived products in previously untreated or minimally treated patients. With respect to the risk of inhibitor development among the presently available recombinant factor VIII concentrates, three recent studies[105–107] showed that second-generation recombinant products lead to an increased rate of inhibitor formation, although a further study[109] did not confirm these findings. However, these studies each had different data collection systems and designs.

Inhibitors in non-severe forms of haemophilia A usually arise when the immune system is under intense stimulation or when exposure to concentrates is unusually high (eg, in the postoperative period).[110] Mutations that lead to a stable abnormal conformation of factor VIII are associated with a high risk of inhibitor formation in mild haemophilia,[111] particularly those clustered in the A2 and C2 domains (ie, Arg593Cys and Arg2150His).[112,113]

In severe haemophilia B, development of inhibitors occurs at a lower frequency than in severe haemophilia A, although the cumulative incidence can be as high as 4–5%. More than 80% of such patients are high responders. Patients with non-severe disease very rarely develop inhibitors (0·05 per 100 treatment years reported in one study[114]). Inhibitors in haemophilia B also appear after a median of only 9–11 exposure days, and the incidence with recombinant factor IX is equivalent to that with plasma-derived factor IX.[115] A unique feature of inhibitors to factor IX is the propensity for patients to develop anaphylactic reactions at the time of inhibitor formation. For this reason, direct medical supervision is recommended for the first ten factor infusions.

Reports on mortality in severe and non-severe patients with and without inhibitors are available from both Europe and the USA.[95,116,117] An increased mortality was seen in patients with inhibitors compared with those without in both non-severe (five times increased mortality rate)[116] and severe (odds of death 70% higher)[117] haemophilia. Patients with inhibitors also have worse morbidity[118] and more disability than those without.[119]

Eradication of inhibitors

Immune tolerance induction is used to eradicate inhibitors and involves frequent injections of concentrates over many months (table 4).[120–124] In haemophilia A, this approach is effective in about 65–70% of patients. The International Immune Tolerance study[120] showed that although both a high dose (200 IU/kg) per day and a low dose (50 IU/kg) every other day are equally effective at inducing tolerance, patients

	Dose	Comments
Recombinant factor VIII	50 IU/kg every other day, 100 IU/kg per day, or 200 IU/kg per day	All doses are equally effective at inducing tolerance. The 50 IU/kg dose is the least costly, but resulted in increased bleeding compared with the 200 IU/kg dose during early phase of treatment. The 100 IU/kg dose has not been assessed in a randomised study but could be considered
Plasma-derived factor VIII concentrate containing von Willebrand factor	50 IU/kg per day, 100 IU/kg per day, or 200 IU/kg per day	Several non-controlled, non-randomised studies have suggested that this drug can result in successful immune tolerance induction after failure with recombinant factor VIII. It can be considered for first-line therapy for high-risk patients (historical titre >200 Bethesda units)

Table 4: **Suggested treatment regimens for immune tolerance induction for patients with haemophilia A**[119–123]

on the low-dose treatment had more bleeding events in the first 5 months of therapy. For the remaining 30–35% of patients in whom induction does not eradicate the inhibitor, options are few and none has clearly shown effectiveness. Approaches for such patients include attempting immune tolerance induction with a factor VIII concentrate containing von Willebrand factor[121] or with rituximab.[125] Immune tolerance induction can be attempted in patients with mild haemophilia A, although no studies on dosing, efficacy, and safety have been done in these patients.

This therapeutic approach is less common in haemophilia B, since inhibitors occur much less frequently than in haemophilia A. Even if immune tolerance induction is attempted in these patients without anaphylaxis, no studies exist to support a specific regimen. However, the use of the immunosuppressive strategies in the tolerisation of factor IX inhibitors could be important for patients who develop anaphylaxis or severe allergic reactions.[126]

Treatment of patients with inhibitors

The management of acute bleeding in patients with inhibitors depends on the inhibitor titre. A minority of patients with a low titre and low responding inhibitors can be treated with standard replacement therapy, albeit at increased doses to overcome the neutralising effects of the antibody. For patients with a high titre, the only effective therapies are bypassing agents, which essentially circumvent the need for factors VIII and IX by generating thrombin through other mechanisms. Two available bypassing agents are activated prothrombin complex concentrate (APCC; FEIBA NF) and recombinant activated factor VII. Both agents have shown efficacy and safety in clinical trials.[127,128] Two comparative studies suggest that their efficacy and safety are equivalent,[129,130] although some patients seem to have a better response to one agent than the other.[130] In view of the equivalent efficacy and safety, the choice of product depends on patient-specific factors. In newly diagnosed patients with inhibitors, the general approach is to use recombinant activated factor VII, because APCC, which contains some factor VIII, has been reported to boost the inhibitor titre in an anamnestic response.[131,132] However, for patients with poor venous access, the use of APCC might be preferred, since recombinant activated factor VII has a shorter duration of action and requires more frequent infusions. Differences exist between the products regarding their content, properties, dosing, and infusion schedules.[133–135] The two drugs have been also used in combination both sequentially and simultaneously in patients with refractory bleeds, taking advantage of their different mechanisms of action.[135,136] However, this approach carries an increased risk of thrombosis and should be reserved for situations in which each of the drugs tried alone did not resolve the bleeding.[137]

In the past few years, several case series and reports regarding the use of prophylactic factor replacement in patients with inhibitors have led to the completion of clinical trials for both APCC and recombinant activated factor VII.[138–140] Both drugs have shown efficacy as prophylactic agents, but it is clear that they are not as effective as prophylaxis in patients who do not have inhibitors.[138–140] Moreover, although prophylaxis in non-inhibitor patients is universally effective, in patients with inhibitors it seems to be effective in some but not in others. A case series[141] suggests that if prophylaxis with bypassing agents (APCC in this report) is started before the onset of joint disease, it has the potential to prevent most bleeds and is nearly as effective as prophylaxis in non-inhibitor patients, but it should be cautioned that this case series is the only report of this finding so far. Novel non-replacement products using a bispecific antibody could be another therapeutic approach in patients with inhibitors.[86]

Conclusion

Over the past 20 years, the diagnosis and treatment of haemophilia have improved considerably. The production of novel therapeutic proteins has been a topic of great interest to biopharmaceutical companies, and several methods have been developed to optimise and ensure the safety and effectiveness of these products. The half-life limitations of haemostatic drugs have led to the redesign of therapeutic proteins to increase their clinical potential. Although the half-lives of recombinant factor IX products have been extended by 3–6 times, the prolongation of recombinant factor VIII's half-life remains only partly successful, with an increase of roughly 1·5–1·6 times. These novel drugs could therefore mainly simplify the prophylactic regimens for patients with haemophilia B, reducing the dosage frequency and extending the

protection from bleeding, thus improving adherence to treatment and rendering this therapy less distressing to the patient. Novel recombinant factor VIII products with extended half-lives might prove beneficial without substantially affecting the therapeutic intervals. The use of smaller amounts of products with slightly less frequent infusions can probably attain increased trough levels, thus protecting patients from breakthrough bleeding. However, proper investigation of these strategies is necessary, and reasonable pricing needs to be maintained. The use of novel non-replacement products could be an alternative strategy in patients both with and without inhibitors. Finally, gene therapy could offer a definitive cure, and an important milestone has already been reached for haemophilia B—patients treated with gene therapy had a stable expression of factor IX for almost 5 years without serious side-effects.[88]

The most important future advance in the management of haemophilia will be to treat the bleeding tendency without the risk of inhibitor development and to develop novel therapeutic options for patients with inhibitors. In many parts of the world, increased availability of treatment options would be a large step forward. A range of new treatment modes are being developed, and adequate and harmonised post-registration surveillance systems need to be in place to assess their efficacy and safety.

Contributors

All authors contributed to the design of the Seminar, identified priorities and needs of the scientific community, wrote and critically reviewed the manuscript, and approved the final for publication. FP coordinated the writing of the Seminar.

Declaration of interests

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