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The Evolution of Recombinant Factors for Hemophilia: Making Therapeutic Choices

Guest Editor: Louis M. Aledort

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The origin and power of a name

Ancient Greek

αιμα [aima] = blood;
αιματος [aimatos] = of blood,
λογος [logos]= reasoning

Scientific Latin

haematologicus (adjective) = related to blood

Scientific Latin

haematologica (adjective, plural and neuter, used as a noun) = hematological subjects

Modern English

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The Evolution of Recombinant Factors for Hemophilia: Making Therapeutic Choices

Guest Editor: Louis M. Aledort

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A dedication to Jeanne M. Lusher

I would like to dedicate this supplement to Jeanne M. Lusher, who, through her dedication to the hemophilia research and patient communities, has greatly expanded the field of hemophilia medicine. Jeanne's successes can be likened to producing a fine wine–planting the vineyard seedlings and dedicating time to cultivating the grape vines, managing good and bad conditions that may arise, until the vines bear their fruit. Jeanne's medical training can be equated with those seedlings, and her early academic experiences until she found her niche are analogous to the vines growing taller and spreading their branches. Her commitment in the area of pediatric hemophilia and our early cooperative study on prothrombin complex concentrates versus albumin for inhibitor patient bleeding, can be considered the time the vintner knows the wine grapes are maturing and will make a satisfactory wine. As she gained years of experience through research, travel, and bedside care, Jeanne also nurtured and inspired others in the hemophilia community with her teachings and countless publications. This era, including her intimate involvement in important multicenter trials, can be considered to parallel the use of vine cuttings to cultivate new vineyards, and the blending of excellent grapes to produce superior wines. Such wines receive international recognition and awards, as has Jeanne because of her fundamental belief in excellence, key contributions to the hemophilia knowledge base, and compassionate patient care. As the vineyard weathers seasonal changes, the sturdiest vines see productivity return in the right conditions of sun and rain. Jeanne, too, has continued to flourish, and like a world-recognized wine, gets better with age.

Louis M. Aledort

The Evolution of Recombinant Factors for Hemophilia: Making Therapeutic Choices

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In this supplement to *Haematologica*, a distinguished panel reviews the development, safety, and efficacy of factor VIII (FVIII) products, plasma-derived and recombinant, which have reversed the previously poor prognosis of children born with hemophilia A. Fifty years ago, most children with hemophilia had approximately 20 to 30 annual bleeding episodes, which occurred spontaneously or after minor trauma. The result was a significantly shortened life of pain and disability. The only known treatment, acute transfusion of whole blood, carried the risk of fluid overload. The subsequent introduction of fresh frozen plasma significantly reduced that risk, and by the 1970s, it was obviated by the removal of nonessential proteins from the cryoprecipitate used to create plasma-derived FVIII concentrates. This advance extended life span; however, many patients had severe disabilities caused by cumulative effects on young joints of the brief spontaneous bleeds that occurred before treatment could be administered.

Widespread adoption of home-administered replacement therapy allowed earlier control of hemorrhages, thus reducing the crippling arthropathy characteristic of untreated patients. Concentrates manufactured from pooled plasma obtained from thousands of donors, however, carried hepatitis B or C virus contamination, resulting in post-transfusion hepatitis in practically all treated patients. Even so, treatment benefits seemed to outweigh risks until the early 1980s, when the human immunodeficiency virus (HIV) was introduced into the donor pool, and the majority of severe hemophilia patients in Western Europe and the United States became HIV-infected.

By the mid to late 1980s, widespread adoption of virucidal methods had reduced the incidence of new HIV infections to only a few well-documented cases, most of which were related to inadequate processing of heavily contaminated plasma pools. Improved donor screening, mandatory testing for HIV and hepatitis C, and viral inactivation procedures have resulted in a remarkably unblemished safety record for the plasma-derived products during the past decade.

Finally, identification of the FVIII gene structure and development of recombinant FVIII products all but abolished the possibility of viral transmission. Despite this improved outlook, some hemophilia patients remain at risk for development of antibodies to factor VIII and, in turn, for uncontrollable bleeding. Inhibitor patients can be treated with modified FVIII regimens or with so-called by-passing agents, including factor VII. However, induction of immune tolerance to the antibodies is considered the ideal goal for this complication.

The paper by Erik Berntorp reviews viral safety measures of FVIII products, beginning with the plasma-derived concentrates, which are still used today to treat many hemophilia patients in the developed world. Concerns that remain regarding viral contamination of these preparations should be considered more theoretical and psychological than real, as stated by Dr. Berntorp. Safety measures in the production of each recombinant product are discussed, as are future developments in factor VIII replacement, including via the gene itself.

H. Marijke van den Berg surveys the issues involved in making therapeutic choices for hemophilia patients, with a focus on early prophylactic treatment with recombinant factor VIII. The features of a strict prophylaxis regimen initiated before any bleeding occurs versus a regimen tailored to an individual's bleeding pattern are discussed. Other considerations include venous access, particularly in patients undergoing prophylactic therapy; cost, an ever-present concern with prophylactic factor use; and evaluation of affected joints to monitor disease status and therapeutic outcome. Finally, inhibitor development is discussed with reference to both previously treated and untreated patients.

Victor S. Blanchette expands on the benefits of prophylaxis compared with acute,

on-demand treatment of bleeding episodes in hemophilia. He presents data showing that even dedicated and skilled follow-up of severe hemophilia patients receiving on-demand treatment cannot prevent the development of significant musculoskeletal disease, and presents other studies examining various factor doses and individualized programs used for prophylaxis. Preliminary results of the ongoing Canadian study of so-called escalation prophylaxis are outlined. Consensus, or lack thereof, regarding when to start prophylaxis, as well as when (or if) prophylaxis can be discontinued are also discussed. Some findings suggest that the answer to the latter question may lie in an individual's response to therapy, with a possible role for on-demand treatment in older patients.

S. W. Pipe and R. J. Kaufman discuss the structure of the factor VIII gene and the functional roles of its three domains, with a particular focus on the B domain. Comparison of the currently available B domain-deleted (BDD) recombinant factor VIII (rFVIII) product and full length rFVIII products show the former to have comparable ability to participate as a cofactor in the coagulation cascade. In detailed studies, removal of the B domain improved the FVIII yield by no more than two-fold. Studies of several B domain mutants with variably sized B domain segments showed increased FVIII yield compared with that of BDD-rFVIII, suggesting that the B domain may not be as dispensable as originally thought. Meta-analysis of published studies suggests that BDD-rFVIII may have a shorter plasma half-life than full-length rFVIII, the clinical significance of which is unknown. However, Pipe and Kaufman noted that in the same meta-analysis, patients receiving routine FVIII prophylaxis with BDD-rFVIII had a two-fold higher bleeding incidence than those receiving full-length preparations.

W. Keith Hoots focuses on issues specific to young hemophilia patients, opening with a brief discussion of imparting the diagnosis to both the family and the patient. Although acute, on-demand treatment remains the most common approach worldwide, early prophylaxis has become a standard treatment in many European hemophilia centers and is gaining ground in North America. The inadequacy of joint scoring systems for infants and young children is emphasized, with specific suggestions for improvement. A Swedish program leading to home- and, eventually, self-treatment is presented. Evidence that very early treatment may be conducive to inhibitor development is tempered by a finding of no antibodies in children beginning prophylaxis after the age of 18 months. Finally, the correlation of academic achievement with severity of disease speaks for early prophylaxis against progressive arthropathy.

Claude Negrier discusses two trends involving primarily adult patients: the diagnosis and treatment of established arthropathy and HIV infection. While the precise role of MRI has yet to be established, one study showed it to be superior to plain radiography in both early and late hemophilic arthropathy. Management approaches for hemophilic arthropathy include radiosynovectomy, joint debridement, arthroplasty, and others depending on the affected area and severity. Because of advances in treatments of both hemophilia and HIV, deaths among HIV-infected hemophilia patients have declined significantly since the late 1990s. Furthermore, prognosis of this cohort continues to follow the chronic HIV infection pattern seen in the non-hemophilia community. HIV viral load is seen as an independent factor in determining need for prophylaxis, and disease progression seems to be influenced by genotype.

I want to thank the authors for their fine contributions, which reflect the optimistic prognosis for hemophilia patients afforded by effective and safe factor replacement therapies available today, particularly the recombinant factor VIII products. We hope these presentations will help practicing clinicians in making optimal therapeutic choices for their patients.

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Viral Safety Measures of Recombinant Factor VIII Products

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The era of modern hemophilia therapy began in the mid 1960s with the introduction for clinical use of human plasma cryoprecipitate containing the coagulation protein factor VIII (FVIII).[1] Continued purification measures resulted in the removal of most contaminating proteins, and by the 1970s, the increased availability of plasma-derived concentrates led to early control of hemorrhage and the resulting musculoskeletal damage. In Sweden, the initiation of prophylactic therapy prevented most bleeding episodes and further minimized the impact of arthropathy. Patients with even severe hemophilia A could finally look forward to relatively normal lives and life spans.

Plasma-derived FVIII preparations

The source of the life-saving factor, pooled human plasma from thousands of donors, contained silent blood-borne hepatitis B and C viruses, however. Thus, for most patients with hemophilia, the price of treatment included chronic hepatitis infection, albeit generally mild and non-progressive enough to be acceptable when compared to the risks of no treatment.[2] But a few years later, in the early 1980s, the equally silent but deadlier human immunodeficiency virus (HIV) invaded the plasma pool to infect 60% to 80% of North American and European patients.

The past two decades have seen substantially improved replacement therapy. Screening of blood and plasmapheresis donors with mandatory testing for HIV-1 and -2 and hepatitis C virus seropositivity as well as for hepatitis B surface antigen greatly reduces the viral burden of the starting material from which FVIII preparations are made.[1] Moreover, in most countries, the use of virucidal methods in the preparation of licensed FVIII concentrates is mandatory.

Virucidal methods in current use include terminal heating of the lyophilized products at 80°C (dry heating), heating in solution at 60°C in the presence of stabilizers (pasteurization), heating with hot vapor under high pressure, and adding a detergent-solvent mixture during manufacture.[3] The latter process is widely used because it effectively inactivates hepatitis B and C and HIV viruses, all of which have lipid envelopes, but it has no effect on non-enveloped viruses. Therefore, concentrate manufacturers may need to use more than one virucidal method in order to inactivate non-enveloped viruses such as hepatitis A, which caused a hepatitis outbreak in several countries in the early 1990s.[4] To assess the viral load, pooled plasma or single units of plasma are screened with assays involving the amplification of nucleic acids, a procedure that has become obligatory in the United States and Europe.[3]

No significant transmission of hepatitis B and C and HIV viruses has been unequivocally documented since adoption of these measures.[5] However, the non-enveloped but highly thermoresistant B19 parvovirus and the transfusion-transmitted virus (TTV) may still be transmitted by plasma concentrates.[6-8] Infection with parvovirus is of little significance in normal subjects, and in persons with hemophilia the clinical consequences are rare and limited in severity. Nevertheless, a few clinically significant events have been reported.[6] Transfusion-transmitted virus may be transmitted parenterally through transfusion of blood products, by the fecal-oral route, and by pregnant women to fetuses.[7] It may have little or no pathogenicity but has been reported in 67% of hemophiliac patients treated with virus-inactivated concentrates.[8]

The more recently documented transmission of West Nile virus through transfusion and organ transplantation once again demonstrates the need for continued vigilance for and response to new pathogens.[9] However, the West Nile virus is lipid-enveloped and has features that make reasonable the expectation that even if present in source plasma, it would be inacti-

vated by virucidal methods currently employed in the manufacturing of coagulation factors.[10]

Another perceived threat is that of a new variant of Creutzfeldt-Jakob (vCJD) disease that can affect humans. Several studies carried out in multitransfused hemophilic patients have shown that classical, sporadic CJD is not transmitted by blood or its derivatives,[11-14] but these data cannot be extrapolated to the new variant form. The fractionation processes used to purify plasma proteins, including albumin and coagulation factors, contribute significantly to clearing abnormal prions, making it unlikely that these agents, even if present in plasma, would be carried into the final products at concentrations capable of causing clinical disease.[15] Thus, the risk of new viral or prion infection of plasma-derived products is probably more theoretical and psychological than real, a result of the HIV epidemic and its dire consequences among the hemophiliac population. Of course, the absence of a test to screen donors for live virus supports the theoretical possibility of transmission and infection through transmission and should encourage strict surveillance.[16]

Safety issues with recombinant FVIII products

The fears of viral/prion transmission have carried over into the new state-of-the-art treatments for hemophilia A, recombinant factor VIII (rFVIII) products, which do not inherit a potential risk for viral or prion transmission.[17] Nevertheless, the theoretical and psychological problems remain. For example, the recently published guidelines on the selection and use of therapeutic products to treat hemophilia and other hereditary bleeding disorders of the United Kingdom Haemophilia Centre Doctors' Organisation (UKHCDO) cite a case of viral infection of cell culture lines used to produce recombinant concentrates.[18-20] Thus, a viral inactivation step in the manufacturing process is seen as necessary to enhance safety.[19] This step (in some cases, two such steps) is indeed included in the manufacture of all available preparations. Nevertheless, the current generation of rFVIII products has even reduced or abolished the use of plasma-derived human or animal proteins in the manufacturing process and in the final formulation.[21] A second hypothetical situation proposed by the guidelines concerns the possible occurrence of new FVIII mutations during cell culture, resulting, in turn, in a higher incidence of inhibitors.[18] Neither of these theoretical problems has been seen during the more than ten years rFVIII preparations have been in use.

A review of the safety profiles, particularly viral removal measures, involved in the currently available second- and third-generation rFVIII preparations may provide the most effective way to reassure prescribing physicians and, in turn, patients with hemophilia. All recombinant products undergo similar developmental steps including cell line development, with cloning of the human gene and its transfer to a suitable mammalian host cell; preparation of master and working cell banks, development of serum-free culture media that can support cell growth and ensure product stability; development of a technical culture system for large-scale mammalian cell culture; a purification process, for removal of host cell- and process-derived impurities and removal or inactivation of viruses; and formulation.[1]

Recombinant products now available in the United States include one first-generation, three second-generation, and one third-generation products. The nomenclature of generations is used here for practical purposes and is not based on any official consensus. As shown in Table 1, first-generation products contain added human or animal proteins in the cell culture and final formulation, while more current generations have no added protein in the final formulation (second-generation), or no added protein in the cell culture or final formulation (third-generation). The first-generation product is Recombinate (Baxter). Second-generation products are Helixate FS/NexGen (Aventis Behring) and Kogenate FS/Bayer (Bayer), which are identical formulations, and ReFacto (Wyeth). Advate (Baxter) is a third-generation preparation. All the second- and third-generation products include a dedicated, viral inactivation solvent-detergent treatment step and a purification procedure that does not involve albumin as a stabilizer. In addition, these preparations are formulated using sucrose as a final stabilizer, replacing the albumin used in first-generation rFVIII products (when administered intravenously, the disaccharide sucrose bypasses hydrolysis by gut glycosidases to its components glucose and fructose and is excreted unchanged in the urine without affecting blood glucose levels).[22,23]

Recombinate

The purification process for first-generation Recombinate begins with 7000 L of harvest, which is first run through a depth filter to remove cells and cell debris.[24] Three column chromatography steps are then carried out, the first of which is an immunoaffinity chromatography using immobilized monoclonal antibodies against rFVIII (Table 2). This is followed by two ion-exchange columns with an anion and a cation exchanger in series for the removal of additional host cell impurities, media impurities, and contaminating IgG derived from the immunoaffinity chromatography. Recombinate is formulated using human serum albumin, polyethyleneglycol 3350, histidine, and calcium.

Table 1. Key features of the rFVIII generations.

First generation	*Second generation*	*Third generation*
Culture medium supplemented with HSA	Culture medium supplemented with HSA	No human-or animal- derived raw material in culture medium
	Specific viral reducing step	Specific viral reducing step
Purification using Mabs against rFVIII	Purification using Mabs against rFVIII	Purification using Mabs against rFVIII
Final formulation stabilized with HSA	Final formulation stabilized without HSA	Final formulation stabilized without HSA

HSA, human serum albumin; Mabs, monoclonal antibodies.

Table 2. Production process: Recombinate.

Cell line	Chinese hamster ovary (CHO)
Expression system	Full-length factor VIII molecule
Culture medium additives	Bovine insulin, bovine albumin, bovine aprotinin
Fermentation system	
Mode of operation	Batch reefed process
Large fermentor size	2500 L
Run length	55 days with 15 growth and harvesting cycles
Purification	
Column chromatographies	3 steps (immunoaffinity-anion exchange-cation exchange)
Virus inactivation	None
Formulation	Human albumin containing

Adapted from Boedeker,[24] with permission.

Helixate FS/NexGen and Kogenate FS/Bayer

These second-generation products combine the advantages of the natural full-length FVIII molecule with an albumin-free formulation and improved virus safety profile by incorporation of solvent-detergent viral inactivation methods (Table 3).[24,25] Improvements on the first-generation product include a faster purification procedure, consisting of a six-step column chromatography process and viral inactivation.[26] The filtered cell-free fermentation harvest is first purified using anion-exchange chromatography, followed by the solvent-detergent step and immunoaffinity chromatography, using immobilized monoclonal antibodies to remove most of the host cell protein and remaining DNA contaminants.

Table 3. Production process: Helixate NexGen/ Kogenate FS.

Cell line	Baby hamster kidney BHK-21
Expression system	Full-length factor VIII
Culture medium additives	Human albumin fraction, recombinant insulin
Fermentation system	
Mode of operation	Continuous perfusion culture with cell retention
Large fermentor size	100, 200, and 500 L
Run length	185 days
Purification	
Column chromatographies	6 steps (anion exchange–immunoaffinity–metal chelate affinity–gel filtration–cation exchange–anion exchange)
Virus inactivation	Solvent detergent treatment
Formulation	Albumin-free, synthetic formulation with sucrose, glycine, histidine, calcium

Adapted from Boedeker,[24] with permission.

The rFVIII eluate from the immunoaffinity chromatography column is subjected to high salt conditions as a further viral-inactivation measure. The purification process has been further improved with the addition of immobilized metal affinity chromatography; a column containing immobilized copper binds rFVIII by building chelates and is subsequently eluted by an imidazole buffer that acts as a competing chelating agent. This step removes additional trace levels of residual host cell proteins.

The final steps are gel filtration, cation-exchange chromatography, and flow-through anion-exchange chromatography in which trace impurities specifically bind to the column matrix and are screened out. Finally, the purified product is diafiltered and formulated in an albumin-free preparation consisting of sucrose, glycine, histidine, and calcium as stabilizers and buffer, yielding a stable formulation without added excipients from human or animal sources.[27]

ReFacto

In plasma, FVIII occurs as a heterodimer consisting of a light chain (domains A3, C1, and C2) and various heavy chain derivatives (domains A1, A2, and B).[24] Because the heavily glycosylated B-domain appears to be dispensable for the hemostatic activity of FVIII, the rFVIII preparation ReFacto is constructed without the central part of the B-region (Table 4).

The purification process for ReFacto consists of a five-step chromatography process with the addition of

Table 4. Production process: ReFacto.

Cell line	Chinese hamster ovary (CHO)
Expression system	B-deleted, truncated factor VIII molecule with peptide linker (r-VIII SQ)
Culture medium additives	Human serum albumin, recombinant insulin
Fermentation system	
Mode of operation	Continuous perfusion culture with cell retention, separate growth and production phase
Large fermentor size	500 L
Run length	Unknown
Purification	
Column chromatographies	5 steps (cation exchange-immonoaffinity-anion exchange -hydrophobic-gel filtration)
Virus inactivation	Solvent detergent treatment
Formulation	Albumin free, synthetic formulation with sucrose, polysorbate 80, histidine, calcium

Adapted from Boedeker,[22] with permission.

a virus-inactivation step using an active solvent detergent for selective removal of potential enveloped viruses.[18,24,27] The five steps include cation-exchange chromatography followed by virus inactivation, immunoaffinity chromatography, anion-exchange chromatography, a hydrophobic chromatography using butyl-sepharose, and gel filtration. ReFacto is formulated using a human serum albumin-free formulation containing sucrose, polysorbate 80, histidine, and calcium for stabilization or as buffers.[24] An improved product, ReFacto AF, manufactured and formulated without human or animal protein, is currently under clinical trial.[18]

Advate

The cornerstone of the Advate purification process is an immunoaffinity chromatography step in which a monoclonal antibody directed against factor VIII is employed to selectively isolate it.[28] The production process also includes a dedicated viral-inactivation solvent-detergent treatment step. The major difference of Advate compared with previous preparations is replacement of human albumin in the cell culture.[21]

A fourth generation of rFVIII products

Generation four of rFVIIIs may well have longer half-lives and less immunogenicity (Table 5). The production of recombinant FVIII in a human cell line may overcome the disadvantages of FVIII expression in the nonhuman mammalian cell lines used until now; specifically, low FVIII secretion levels, the result of differences in intracellular pathways of protein translation and posttranslational modification (which might also affect FVIII biological activity) and potential contamination of FVIII purified from nonhuman cell lines with cellular components that may induce antigenic reactions.[17] Current routes of administration for rFVIII infusion include a number of novel devices for venous access, external and implantable central venous catheters, and portable or implantable minipumps (which allow patients to participate in activities such as swimming), all of which are associated with some risk of infection, sepsis, or thrombosis.[29] One mind-challenging vision of future therapy scraps both replacement FVIII preparations and intravenous administration, focusing on development of an oral compound, peptide, or peptidomimetic agent with the capacity to activate the coagulation cascade in a controllable way.[29,30]

Table 5. Key features of the rFVIII fourth generation?

- FVIII molecule with a longer half-life
- FVIII molecule with less immunogenicity
- Use of human cell line to overcome low FVIII secretion levels
- Improved routes of administration

Finally, a new promise for patients with hemophilia A and B lies in gene therapy, aimed at correcting the physiologic defect at the gene rather than the protein level.[17,24] Gene therapy is already in development by at least six different companies at the preclinical or phase I clinical stage.[24]

Conclusions

A variety of both plasma-derived and recombinant FVIII preparations is now available. They differ in virus inactivation methods used and in purity. The most important criterion in the choice of which to use is viral safety. Other aspects to consider are efficacy, availability, purity, cost, and convenience in handling.

In the United States, approximately 60% to 70% of patients with severe hemophilia currently use recombinant products and the proportion is increasing.[2] In Europe, the numbers are generally smaller. For example, given a dramatic recent shortage of recombinant factors, priority guidelines were developed in Italy for treatment with recombinant FVIII: first, newly diagnosed, previously untreated patients; and then those who have been spared from blood-borne infections

despite previous exposure to plasma-derived factors.[2] However, there seems little doubt that replacement therapy will eventually be dominated by the recombinant preparations (ie, in resource-rich countries).

Recombinant factors cost from 20% to 50% more than plasma-derived products, which are still used in a significant number of patients worldwide and are the only foreseeable option for 80% of those who have no or limited access to any replacement material. Patients currently using plasma-derived preparations should be reassured that the infection risks are more theoretical and psychological than real. In an ideal world, however, the recombinant FVIII preparations — products of high technology perceived as guaranteeing superior safety — would be the treatment of choice for all patients with hemophilia A.

A fourth generation of rFVIII products may have longer half-lives, less immunogenicity, and given the possibility of a human cell line, higher secretion levels and purer concentrate. None of these considerations may be relevant given the future development of oral compounds that take control of the coagulation cascade.[29]

Finally, the fourth, or even the third, generation of recombinant FVIII product may be the last for replacement therapy. Gene transfer therapy is already in clinical trial and may well be the therapy of choice for future patients with hemophilia A. Despite this exciting picture, however, the greatest challenge in hemophilia therapy has still to be faced: how to spread modern hemophilia care to the large areas of the world where it has still to be implemented.

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Structural and Functional Role of the Factor VIII B Domain

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The cloning of the human factor VIII (FVIII) complementary DNA (cDNA) allowed the study of FVIII expression, the genotyping of molecular defects causing hemophilia A, and the production of recombinant FVIII (rFVIII) ultimately available as a commercial product for the treatment or prevention of bleeding episodes. Recombinant DNA technology has allowed the design of modified rFVIII molecules that aid in studying the consequences of FVIII gene defects and to investigate FVIII structure and function through detailed examination of FVIII subdomains and critical residues. Recently, FVIII molecules have been bioengineered to improve on its functional properties and to adapt FVIII for improved expression in gene therapy studies (reviewed in Saenko *et al.*, 2003).[1] These bioengineering strategies have built on insights gained from such structure and function analysis.

Several biochemical qualities of FVIII help to account for the high cost and, in turn, limited universal availability of rFVIII commercial preparations. Expression of rFVIII in heterologous mammalian systems is two to three orders of magnitude lower than that of other comparably sized proteins, thus compromising rFVIII production and gene therapy strategies. The reasons for this limitation are multiple and inherent for FVIII, and much insight has been gained from structure and function analysis of FVIII. This review will focus on insights gained from the study and comparison of B domain-deleted (BDD) forms of FVIII compared to full-length FVIII.

FVIII structure and activation

FVIII is synthesized as a 2351 amino acid monomer with the domain structure A1-*a1*-A2-*a2*-B-*a3*-A3-C1-C2.[2] Domains *a1*, *a2* and *a3* represent acidic amino acid rich regions between the major structural domains and contain sulfated tyrosine residues.[3] Upon secretion, the FVIII precursor molecule is processed to a heterodimeric complex formed by a heavy chain (A1-*a1*-A2-*a2*-B) and light chain (*a3*-A3-C1-C2) associated through a cation. This dimer is stabilized by non-covalent interaction with von Willebrand factor (vWF) within the plasma. FVIII is activated by thrombin through a series of proteolytic cleavages during which the heavy chain is bisected between the *a1* and A2 domains, the B domain is removed and the light chain acidic region (*a3*) is cleaved from the light chain.[4-6] This facilitates dissociation from vWF and results in an active FVIII heterotrimer (A1-*a1*/A2-*a2*/A3-C1-C2). Thrombin-activated FVIII (FVIIIa) is then able to associate with the protease, activated factor IX (FIXa), and its substrate, factor X (FX). FVIIIa provides cofactor activity to FIXa, increasing its catalytic efficiency by four to five orders of magnitude.[7]

Interestingly, the domain structure and procoagulant functions of FVIII are very similar to that of factor V (FV).[8] FVIII and FV are both activated by thrombin and the activated cofactors form complexes with their respective enzyme partners, FIXa and FXa, to activate FX and prothrombin. The A domains of FVIII share ~40% amino acid identity with each other and to the A domains of FV. The FVIII C domains in turn also exhibit ~40% amino acid identity to each other and to the C domains of FV and proteins that bind negatively charged phospholipids, suggesting a role in phospholipid interaction. The B domains of both cofactors are encoded by single exons and do not share homology with each other or with any other presently known gene. However, both B domains contain by far the largest clustering of asparagine (*N*)-linked oligosaccharides suggestive of a functional significance of this structural component.[9,10]

The functional roles of the FVIII A and C domains have been extensively investigated, aided by insights gained from analysis of structural models of FVIII. The first structural insights followed analysis of scanning transmission electron microscopy of human

and porcine FVIII and FVIIIa, which demonstrated that the A1, A2, and A3 domains of the heavy chain and the light chain were closely associated and formed a globular core structure, with the B domain forming a peripheral satellite appendage.[11,12] Modeling of the triplicated A domains based on their homology to copper-binding proteins[13,14] suggests that each A domain comprises two highly conserved β-barrel core structures, with the domains arranged concentrically to form a heterotrimer with a tightly packed hydrophobic core at a pseudo-threefold A1-A2-A3 interface. Recent studies have now provided some insights into C domain structure and function. A crystal structure for the C2 domain[15] and of the C1-C2 bound to a phospholipid membrane,[16] demonstrate the C2 domain as a β-sandwich core, with two pairs of hydrophobic residues extending from adjacent loops forming a major lipid binding surface, and may also contribute to vWF binding.[17] In another structural study, two-dimensional crystals of FVIII lacking the B domain were prepared on phospholipid monolayers.[18] The hydrophobic loops of the C2 were shown to be embedded in the lipid monolayer, with the C1 domain almost forming a right angle with the C2 such that its long axis nearly paralleled the membrane.

Until recently, there has not been extensive characterization of the structural and functional role of the FVIII B domain. However, the presence of the B domain and a similar predominance of *N*-linked oligosaccharides has been conserved amongst a number of species' FVIII analyzed thus far.[19-21] This review will summarize recent insights into the functional role of the B domain gained from analysis of BDD-FVIII, variants of FVIII with modified B domains and comparison with the FV B domain.

Role of the FVIII B domain within the secretion pathway

The study of FVIII expression within heterologous mammalian expression systems has identified several inherent limitations (reviewed in Saenko *et al.*, 2003, and Kaufman *et al.*, 1997).[1,22] The mRNA is inefficiently expressed, a significant portion of the primary translation product is misfolded and ultimately degraded, and FVIII is retained within the endoplasmic reticulum (ER) through interaction with various ER chaperones including immunoglobulin binding protein (BiP), calnexin (CNX) and calreticulin (CRT). Properly folded FVIII requires a facilitated transport mechanism for efficient transport from the ER to the Golgi via interaction with the mannose-binding lectin LMAN1 (previously identified as ERGIC-53). The FVIII B domain contributes in part to all of these observations within the secretion pathway.

Early on in the study of rFVIII expression, it was demonstrated that the B domain of FVIII could be removed from the cDNA without loss of FVIII procoagulant activity. Removal of the B domain, the equivalent of approximately 38% of the primary cDNA sequence, significantly improved the yield of FVIII.[19] The increased expression resulted from markedly increased levels of mRNA and increased translation.[23] However, detailed studies on the expression of B domain deleted forms of FVIII (BDD-FVIII) indicated that despite an increase in mRNA approaching 20-fold, the yield of secreted BDD-FVIII was improved by no more than 2-fold.[24]

A significant portion of the FVIII primary translation product is misfolded, resulting in its retention within the endoplasmic reticulum (ER). FVIII is co-translationally translocated into the lumen of the ER where it folds and assembles into its tertiary structure. Enzymes and molecular chaperones facilitate these reactions by interacting with FVIII folding intermediates. Molecular chaperones assist in folding by inhibiting alternative assembly pathways that produce non-functional structures. Within the ER, FVIII acquires *N*-linked oligosaccharide structures. Of the 25 potential *N*-linked glycosylation sites, 19 are located within the B domain. Productive secretion of FVIII requires interaction and subsequent release from several ER chaperones including BiP, CNX, and CRT. BiP binds FVIII at a hydrophobic site within the A1 domain.[22,25,26] BiP has a peptide-dependent ATPase activity and FVIII release from BiP and transport out of the ER requires high levels of intracellular ATP.[27-29] CNX and CRT both display substrate specificity for glycoproteins containing partially glucosylated *N*-linked core oligosaccharides. Interaction of FVIII with CNX and CRT is mediated in part by interaction with *N*-linked oligosaccharides within the B domain.[30] Properly folded FVIII is released from these chaperones but requires interaction with the mannose-binding lectin LMAN1 for efficient transport from the ER to the Golgi apparatus. Recent work has also demonstrated that LMAN1 directly interacts with FVIII and that high mannose-containing oligosaccharides, mostly clustered within the B domain, provide a significant contribution to this interaction.[31,32] Thus, the *N*-linked oligosaccharides within the B domain can participate in the folding interactions within the ER as well as potentially facilitate ER-Golgi transport.

BDD-FVIII variants

A number of BDD-FVIII variants have undergone biochemical characterization, which has yielded important insights.[24,33-35] Previously described mutants have all been designed to reduce the size of the FVIII construct to improve FVIII mRNA expression, yet retain functional biochemical characteristics. However, insights from analysis of the role of the B domain within the secretion pathway suggested that complete FVIII B domain

deletions may be compromising the efficiency of intracellular trafficking and reducing potential protein yield.[23,24] Recently, FVIII B domain mutants were constructed with variably sized B domain segments in an effort to retain proper intracellular chaperone interactions.[36,37] Oligonucleotide site-directed mutagenesis was used to prepare variants with variably sized B domains, each of which included one or two additional consensus sites for *N*-linked glycosylation. Amino-terminal B domain sequence was used, with size ranging from 29 amino acids (aa) to 269 aa, beginning with residue 741. The number of consensus sites for N-linked glycosylation ranged from one to eight. The relative efficiency of FVIII secretion was measured by a one-stage clotting assay and a FVIII-specific ELISA (enzyme-linked immunoabsorbent assay) and compared to expression of a complete BDD-FVIII.

A stepwise incremental increase in the amount of FVIII measured in the cell media from transiently transfected COS-1 monkey kidney cells correlated with each B domain size increase and the addition of each additional consensus site for *N*-linked glycosylation. A variant with 226 amino acids of B domain sequence and 6 potential *N*-linked oligosaccharides (226aa/N6) was expressed with an approximately 10-fold increase by one-stage clotting activity assay compared to BDD-FVIII. The secretion advantage of these mutants was also confirmed with pulse-chase analysis of metabolically labeled cells expressing the B domain variants. A similar pattern of results was observed in transiently transfected Chinese hamster ovary (CHO) cells, indicating that the observations were not cell-line specific.

To elucidate further the source of these B domain variants' superior secretion efficiency, an alternate FVIII B domain variant was prepared that encoded for the same 226 amino acids of B domain sequence as the 226aa/N6 construct, but with Asn→Gln point mutations at the consensus sites for *N*-linked glycosylation at residues 757, 784, 828, 900, and 963. Although the remaining Asn at residue 943 is a potential consensus site for *N*-linked glycosylation, previous detailed study of B domain glycosylation indicates that this site is not used.[38] Thus, this construct should be completely devoid of *N*-linked oligosaccharides within the B domain segment. After being transfected into COS-1 cells, this alternative B domain variant was analyzed for secretion efficiency compared to BDD-FVIII and the 226aa/N6 variant. This new construct, 226aa/N1, was secreted 4.4-fold more efficiently than BDD-FVIII, as determined by one-stage clotting activity assay, but significantly less efficiently than 226aa/N6 (11-fold more efficient than BDD-FVIII), which retained all consensus sites for *N*-linked glycosylation. Antigen determination by ELISA was also significantly lower for the 226aa/N1 than for the 226aa/N6 variant indicating that this was not attributable simply to a decrease in specific activity. Thus, the B domain, at least in part, increases FVIII secretion efficiency through the *N*-linked oligosaccharide content. Nevertheless, 226aa/N1 was still secreted more efficiently than BDD-FVIII, suggesting a residual benefit of the B domain primary amino acid sequence as well.

To test whether the secretion improvements observed *in vitro* within the COS-1 and CHO cells would also be observed in an *in vivo* heterologous expression system, the authors utilized hydrodynamic tail vein injection of DNA to obtain transient expression of the FVIII variants in the liver of hemophilia A mice.[36] The animals were injected with 100 µg of plasmid DNA containing the 226aa/N6 construct. Expression of the variant was analyzed from mouse plasma harvested at 24 and 48 hours post-transfection. Results were compared to littermate controls injected with BDD-FVIII, and activity determined by two-stage chromogenic activity assay. The 226aa/N6 variant was expressed 5-fold higher than BDD-FVIII, suggesting that the chaperone interactions and inherent limitations to FVIII expression in tissue culture systems are relevant to *in vivo* expression and can be overcome with similar strategies. These results are important because they support that information gained from the analysis of FVIII expression in tissue culture systems is likely relevant to FVIII expression in hepatocytes *in vivo*.

Role of the B domain in intermolecular interactions

The first and only bioengineered FVIII molecule to come to commercial production so far is B domain deleted recombinant FVIII (BDD-rFVIII, ReFacto, Wyeth).[34] This product lacks residues 744 to 1637 of the B domain, resulting in the fusion of Ser 743 to Gln 1638, creating a 14 residue B domain linker between the A2 and A3 domains. This portion of B domain contained no consensus sites for *N*-linked glycosylation. It was observed that with this B domain deletion, the protein was less prone to proteolytic degradation. Therefore, no addition of plasma-derived albumin was needed for stabilization of the final product.[39] Comparison studies with full-length FVIII have shown BDD-rFVIII to have comparable ability to participate as a cofactor in the coagulation cascade: in interactions with thrombin and activated protein C; in FXa generation in a mixture of FIXa, FX, phospholipid, and calcium; and in binding capacity for phospholipid vesicles and vWF.[34]

Despite the B domain's apparent dispensability for FVIII procoagulant function and its unique advantages, some biological differences between BDD-rFVIII and the full-length protein remain. For example, unacti-

vated BDD-rFVIII binds to activated platelets with higher affinity than native FVIII, with thrombin activation further increasing binding affinity.[40] These results demonstrate that the binding of FVIII to platelets increases with each activation step largely through release of the B domain and is consistent with the multistate binding described for FVIII.[41]

Moreover, FVIII assay discrepancies exist in which one-stage clotting assays of BDD-rFVIII activity, using commercial activated partial thromboplastin time (APTT) reagents, are consistently about 50% lower than that measured by the chromogenic assay.[42,43] This assay discrepancy occurs *in vitro* as well as *ex vivo* after plasma analysis from treated patients. At low levels of phospholipid, the one-stage activity of BDD-rFVIII exceeds the chromogenic result. However, when mixtures of phosphatidylserine (PS) and phosphatidylcholine were used as the source of phospholipid, the one-stage activity results were in agreement with the chromogenic results as long as the content of PS was maintained below 10%.[32] This is an unexpected observation following deletion of the B domain, and the mechanism has not yet been explained. BDD-rFVIII also has a high specific activity (~15,000 U/mg protein) as measured by the chromogenic assay compared to full-length FVIII (~4000 U/mg).[39] The mechanism for this is also not determined; however, several studies indicate that BDD-FVIII has increased sensitivity to thrombin cleavage.[24,33,44] Therefore, the B domain may provide a buffer to protect against cleavage and activation. This feature may be attributed to the negative charge of sialic acid residues in the carbohydrate structures of the B domain that reduce interactions with thrombin and FXa.

Despite these observed biochemical differences, clinical studies demonstrate that the bioengineered BDD-rFVIII is safe, well-tolerated and an effective treatment for hemophilia whether given as on-demand therapy for hemorrhagic complications, administered in routine or intermittent prophylaxis, or for surgical management.[45] Most significantly, despite concerns regarding potential neoantigenicity of the truncated FVIII molecule, rates of inhibitor formation in previously untreated patients with hemophilia A were similar to that observed with full-length rFVIII concentrates.[46] However, ReFacto has only been directly compared with a full-length rFVIII protein in a clinical trial by pharmacokinetic analysis. In a randomized, single-blind (patient blind) cross-over study, the volume of distribution at steady state and during elimination phase as well as the clearance were higher for ReFacto than for a full-length plasma-derived FVIII.[39] Although this did not result in an observable difference in plasma half-life in this analysis, data from comparison with other clinical trials,[45,47] and a meta-analysis of published studies have provided some evidence that the plasma half-life may be shorter than that of full-length FVIII.[48] It is not clear if this is clinically significant although the same meta-analysis of studies reporting patients under routine FVIII prophylaxis indicated that the bleeding incidence was more than two-fold higher in patients receiving ReFacto than in those receiving other full-length FVIII products.[48]

Functional role of the FV B domain

The high homology between the functional domains of FVIII and FV has provided additional insights into their structure and function. The A domains of FV are also predicted to form a heterotrimeric structure[49] and provide sites for functional interaction with FXa and prothrombin. Similarly, the crystal structure for the FV C2 domain[50] also predicts two hydrophobic spikes at the tips of protruding β-hairpin turns that are hypothesized to penetrate the hydrophobic core of the phospholipid bilayer. Although the B domains of FVIII and FV share no apparent amino acid homology, they are both heavily glycosylated with *N*-linked oligosaccharides.

Studies suggest a similar role for the FV B domain, and in particular *N*-linked and *O*-linked oligosaccharides, in interaction with molecular chaperones within the ER. However, CNX and CRT display different preferences for FVIII and FV interaction, as FV does not require CNX interaction for efficient secretion.[30] FV also exhibits a requirement for a facilitated transport mechanism for efficient transport from the ER to the Golgi via interaction with the mannose binding lectin LMAN1, and this interaction is also facilitated by *N*-linked oligosaccharides primarily within the B domain.[31]

The FV B domain also exhibits important functional roles in intermolecular interactions following FV secretion. The B domain is required for proper thrombin activation of FV[51] and regulates exposure of the FXa binding site,[52] preventing the association of FXa with intact FV which may avoid premature thrombin generation. The importance of the FV B domain for expression of FV-activated protein C cofactor function has also been investigated.[53] These studies demonstrated that the carboxy terminal portion of the B domain was crucial for this anticoagulant activity.

Conclusions

The inefficient expression of FVIII in heterologous mammalian systems has compromised rFVIII production and may be contributing to reduced expression following gene transfer strategies. Expression is limited by unstable mRNA, interaction with ER chaperones, and a requirement for facilitated ER to Golgi apparatus transport through interaction with the mannose-binding lectin LMAN1. Insights into the role of the FVIII B

domain have aided bioengineering strategies designed to overcome each of these limitations. FVIII bioengineered for improved secretion will significantly improve rFVIII production in cell culture manufacturing or transgenic animals, as well as increase the potential for success in gene therapy strategies for hemophilia A. The functional role of the FVIII B domain for intermolecular interactions following secretion is less well characterized than the role of the FV B domain. However, there are important biochemical differences, and observations from clinical experiences with BDD-rFVIII should not be overlooked. Studies over the last 20 years, since the cloning of the FVIII cDNA, have provided insights into the functional role of the FVIII B domain, suggesting it may not be as dispensable as once thought.

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Issues Surrounding Making Therapeutic Choices for Hemophilia Patients

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Of the several clinical issues surrounding therapeutic choices for hemophilia patients, prophylactic therapy stands out as the most positive. The first study comparing prophylactic and on-demand treatment, which involved 22 years of follow-up, found that the primarily prophylactic treatment strategy led to better outcome at equal treatment costs in young adults with severe hemophilia.[1]

In contrast, one of the most challenging issues in hemophilia treatment is development of inhibitors to factor VIII (FVIII). Previous reports of inhibitor development risk have varied widely, ranging from approximately <5% to 40%.[2-5] This variability may stem from patient-related, therapy-related, and assay-related influences on inhibitor development and detection, as reported by Wight and Paisley in a current review.[2] Their systematic review concluded that, based on large-scale prevalence studies and hemophilia registry data, 5% to 7% of all hemophilia patients have antibodies to FVIII, with a substantially higher prevalence of approximately 13% among those with severe disease (with prevalence referring to the proportion of the patient population with inhibitors at a given time).[2] On the other hand, the cumulative risk of inhibitor development (number of new cases over a prolonged period adjusted for different patient follow-up durations) varied from 0%[6] to 39%.[7] In any case, inhibitor development complicates patient management and may require immune tolerance induction. Other important issues attendant on FVIII therapy, whether preventive or acute, include cost, venous access, FVIII dosage and dosing intervals, and joint scoring systems.

Prophylaxis or on-demand therapy?

The rationale for prophylactic treatment of hemophilia is based on observations that patients with moderate hemophilia (FVIII/FIX >0.01-0.05 IU/mL) rarely develop chronic arthropathy.[8] Moreover, many studies have shown that, even at high doses, on-demand therapy is not effective in preventing arthropathy.[9,10]

The possibility of changing the clinical phenotype of patients with severe hemophilia to a moderate phenotype has been a challenge. Without adequate therapy, patients with severe hemophilia (FVIII/FIX < 0.01 IU/mL) have a life expectancy of about 20 years, during which they suffer from severe bleeds, spontaneous or from minor trauma, and early, crippling arthropathy.[11] Those with moderate disease experience only traumatic bleeds and, in turn, develop far less arthropathy. It follows, therefore, that increasing the level of clotting factor activity to at least 1% with prophylactic therapy should prevent bleeding in patients with severe hemophilia.

As defined by the European Paediatric Network for Haemophilia Management, primary prophylaxis is started before the age of 2 years, either before or after the first joint bleed.[12] Classic treatment consists of thrice-weekly doses for hemophilia A, to achieve permanent minimum factor VIII levels of >1%. Another option is one dose every 2 days. Dosage varies between 20 and 50 IU/kg of weight, depending on the pharmacokinetic properties of a particular product in each patient and dosing intervals. The program is continued until the end of the growth period, when the patient has the option of suspending continuous prophylaxis and changing to on-demand treatment interspersed with periods of prophylaxis if appropriate.

Prophylaxis has been practiced for many years in Sweden and The Netherlands, as well as other European countries.[1,8,13-18] A number of early studies demonstrated that long-term prophylaxis can prevent arthropathy. The first study to compare on-demand with primary prophylactic treatment involved 49 Dutch (prophylaxis) and 106 French (on-demand) patients.[1] All were born between January 1970 and January 1981; none had a history of antibodies to FVIII or FIX. On-demand therapy was given per

Table 1. Rate of infection in hemophilia patients using central venous lines.

Study	*Number of patients*	*Rate of infection per 1000 patient days*	*Comment*
Blanchette *et al*., 1996[25]	19	0.7	3 patients with inhibitors, 3 HIV+
Perkins *et al*., 1997[22]	35	1.2 (central) 0.7 (peripheral device)	7/32 inhibitors, 2/32 vWD
Ljung *et al*., 1998[24]	53	0.19	11 patients with inhibitors
Santagostino *et al*., 1998[26]	15	0.3	2 inhibitor patients, 13 on prophylaxis
Miller *et al*., 1998[27]	41	0.14	Includes external
McMahon *et al*., 2000[28]	58	1.6 (without inhibitor) 4.3 (with inhibitor)	77/86 devices Port-A-Cath; 37/58 patients hemophilia
Tusell [*personal communication, 2002*]	35	0.28 (prophylaxis) 0.68 (ITI)	Port-A-Caths used for prophylaxis/on demand or ITI

ITI, immune tolerance induction; vWD, von Willebrand disease. Adapted from Ljung,[23] with permission.

bleeding episode; prophylaxis was started at an early age according to each patient's bleeding pattern, in most, after several joint bleeds. For prophylaxis, intermediate doses of 15 to 25 IU/kg were administered twice or three times a week, with doses adjusted in cases of breakthrough bleeds. Patients with very mild bleeding patterns received only episodic prophylactic treatment, and some discontinued prophylaxis in adulthood.[19] Compared with those primarily treated with prophylaxis, on-demand patients had more joint bleeds, higher clinical scores, and higher Pettersson scores.[1]

In the United States, the Orthopedic Outcome Study, a 6-year prospective, cross-national follow-up study of clinical outcomes associated with different patterns of factor VIII utilization, confirmed the beneficial effects of prophylaxis compared with on-demand therapy.[10] On the basis of these positive data, the Medical and Scientific Advisory Council of the National Hemophilia Foundation recommended prophylaxis as optimal therapy for individuals with severe hemophilia A and B.[20]

Among the concerns raised about prophylactic therapy is the potential increased exposure to blood-borne infectious agents with large donor pooled plasma products. This concern has been obviated by modern donor screening, plasma-derived FVIII concentrate purification and virucidal procedures, and the introduction of recombinant products.[21]

Venous access

Regimens of primary prophylaxis beginning in the first year of life can prevent hemophilic arthropathy. However, reliable venous access is needed for these treatments and repeated peripheral venipuncture can be difficult or impossible in very young children. Thus, central venous catheters (CVCs) are commonly used in these patients, with the attendant risks of infection and deep venous thrombosis (DVT).

Most studies with implantable venous access devices (IVADs) have been conducted using the Port-A-Cath system. However, peripheral ports have been associated with a higher frequency of thrombophlebitis and thrombosis. In a study of central and peripheral ports in 35 children, the rates of local infection and bacteremia with central devices were 3% and 33%, respectively, compared with rates of local infection of 25% and bacteremia of 25% with peripheral ports.[22] One patient required removal of a central port due to thrombosis. The majority of infections were cleared with antibiotics, and ports remained intact. Both types of IVADs were associated with high patient and parent satisfaction.

Infection is the most frequent complication when using an IVAD. Several recent, large studies are listed in Table 1.[23-28] A 1998 review reported that 50% to 83% of patients with inhibitors can be expected to get an infection.[29] One possible reason for this is that the patients have small hemorrhages around the port post-injection, which can stimulate bacterial growth in subcutaneous tissue.

For patients without inhibitors, the need for a port has to be considered together with risk of complications. Whether the infection frequency in these children is acceptable depends on individual patient factors and treatment regimens.[24] A recent case of catheter-associated *Staphylococcus aureus* septicemia in a hemophilic child (eradicated with antibiotics injected via the catheter) prompted a warning to clinicians.[30] In another study of CVCs in 23 children with

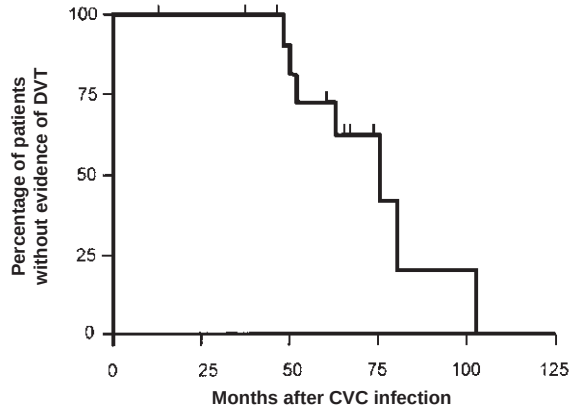

Figure 1. The probability of hemophilia patients remaining free of deep venous thrombosis (DVT) at various intervals after insertion of a central venous catheter (CVC). No patient whose catheter was in place for < 48 months had an abnormal venogram, whereas all those with catheters in place for >73 months had venographic evidence of DVT. Adapted from Journeycake *et al.*,[32] with permission.

severe congenital coagulopathy, despite 13 documented catheter infections (five children had inhibitors), both clinicians and parents believed the potential hazards of the devices to be acceptable given the considerable benefits.[31]

Thrombosis in patients with bleeding disorders is seemingly paradoxical. Nevertheless, thrombi do occur, albeit more slowly, perhaps because hemostasis is only intermittently normalized by factor infusions. Figure 1 depicts the probability of a patient remaining free of DVT after insertion of a CVC.[32] Among 15 boys with severe hemophilia, eight had evidence of DVT on contrast venograms. However, these children had had CVCs in place for at least 4 years. The investigators concluded that removal of catheters within 4 years might prevent thrombosis, and screening venography may be warranted for patients who require the devices for longer periods.

Others have reported little or no infection and no DVT associated with implantable catheters; rather, their use has permitted optimal prophylactic home treatment by parents,[33] low risk of infection and other complications,[27] and overwhelming enthusiasm by parents and children with no major complications.[34]

Cost

Cost is the main reason why prophylaxis is not implemented on a larger scale. Several studies have attempted to measure the cost-effectiveness of this approach.[35–37] One major cost analysis was conducted using data from the Orthopedic Outcomes Study.[35] A total of 831 patients with severe hemophilia aged 1 to 31 years from 19 centers were included. Patients were categorized into three groups according to the number of weeks in which they received prophylactic regimens, and costs of hospitalization, surgery, days lost from school or work, and factor VIII utilization were estimated. Patients who received factor VIII on demand incurred substantially greater disability-related costs (most accounted for by hospitalization for hemophilia-related conditions) than those who received prophylaxis for some or all of the study period. Reductions in non-factor healthcare costs and disability associated with prophylactic therapy helped to offset the much higher costs of the prophylactic regimen. Although frequent on-demand treatment may be more expensive than full-time prophylaxis for certain patient subgroups, total healthcare expenditures were highest among patients receiving prophylaxis, given the high cost of year-round factor VIII use.

Several groups have tried to reduce cost by modifying strict prophylactic regimens, including using early but progressive, escalating-dose, or individualized regimens.[38,39] Treatment is started equally early, before 2 years of age, but the interval between doses is adjusted according to each patient's clinical behavior. These and other studies suggest it is possible to select patients for prophylaxis based on clinical factors. Using the date of the first joint bleed as a parameter of clinical severity, one group found the age to range from 0.4 to 7.7 years (mean 2.4 years).[40] Whereas prophylaxis would have been routinely started at 1 year of age, in this study population, 50% of the patients would have been treated a minimum of 1.5 years before experiencing their first joint bleed. These investigators have also shown that waiting for the first joint bleed before starting prophylaxis does not increase the risk of arthropathy.[41]

Dosing and dose interval are important issues in efforts to optimize hemophilia care (primarily orthopedic outcomes)[10] and treatment costs. Low doses at frequent intervals and ideally, as continuous infusion, will probably give the best cost efficacy of prophylaxis.[18] Prophylaxis can be targeted at preventing spontaneous joint bleeds (intermediate-dose regimen), or at maintaining minimum clotting factor activity levels (high-dose regimen).[42] In young adults, clotting factor consumption for intermediate dose prophylaxis is similar to consumption for on-demand treatment, whereas outcome is more favorable. Clotting factor consumption for high-dose prophylaxis is two-fold higher, but outcome is only slightly better than that achieved with intermediate-dose prophylaxis.[42]

One group suggested prophylaxis as a standard treatment until the age of 18 years,[43] and recently a cohort study in 49 patients suggested that 22% of patients with severe hemophilia could safely stop taking prophylaxis in adulthood.[19] Apparently, these patients were

all treated with early prophylaxis, but were characterized by a milder bleeding pattern than the patients who continued prophylaxis. However, the long-term effects of discontinuing prophylaxis in patients with milder bleeding patterns should be assessed, preferably in a prospective study, before becoming standard treatment.

Evaluation of joints

A main goal of prophylaxis is to prevent not only joint bleeds but also the development of arthropathy, which is independently associated with the age of prophylaxis initiation.[44] However, neither the orthopedic nor the radiologic (Pettersson) joints score, both of which are approved by the World Foundation of Hemophilia (WFH),[45,46] detects very early joint changes in young children. The advent of magnetic resonance imaging (MRI) has opened up new possibilities of precise evaluation of small joints,[18] resulting in more consistent assessment of changes and more targeted treatment.[47] Comparison of findings from clinical examination (including bleeding scores, pain scores, and physical examination scores) and MRI assessments of blood, synovia, and cartilage in 21 joints of 16 hemophilia patients showed little correlation.[48] Clinical examination revealed evidence of a bleeding episode in 12 joints, whereas MRI identified blood or blood products in 15 joints. Given the MRI findings, therapeutic management was changed from on-demand to prophylactic therapy in six study patients. MRI is difficult to perform in young children, however, who require general anesthesia for the procedure. It is also time-consuming and costly.

FVIII inhibitors

Development of inhibitors is a primary concern of physicians with current use of highly purified blood products and recombinant FVIII preparations. The immune systems of patients with severe hemophilia A recognize administered FVIII as foreign, and in some patients, mount an immune response. The resulting antibodies rapidly inactivate FVIII, dramatically decreasing treatment efficacy.

Inhibitor development appears to relate to defects in the factor VIII gene rather than to concentrate infusion.[3] Mutations leading to the absence of endogenous factor VIII protein (for example, large multidomain deletions, nonsense mutations, or intron 22 inversions) are associated with the highest risk of inhibitor development.[49,50] It has been confirmed that other factors also influence inhibitor development. For example, severity of disease seems to be an important risk factor, whereas few patients with mild disease acquire the antibodies.[5] Some families seem more likely to develop inhibitors,[5] as do children of African and Hispanic descent.[48] Recently, study results demonstrated that age at first exposure was associated with inhibitor development.[5,51] Patients who received their first exposure very early had a higher probability of developing an inhibitor. Other studies are necessary to confirm these results.

Patients with FVIII antibodies are generally categorized into two groups: low responders (inhibitor titer $\leq$ 5 BU) and high responders (>5 BU), based on the Bethesda assay.[52] Development of a high titer inhibitor is the strongest challenge in the field of hemophilia therapy.

Previously treated patients (PTPs) seem to be at lower risk for inhibitor formation than those previously untreated (PUPs), although this has not been definitively established. For example, the Cooperative Inhibitor Study sponsored by the National Heart, Lung, and Blood Institute reported an incidence of new inhibitor formation of 8 cases per 1,000 patient years of observation, but based these findings on a patient population of PTPs.[53] In prospective trials with rFVIII preparations (both full length and B-domain deleted), the percentage of PUPs with severe hemophilia A who developed FVIII inhibitors has varied between 28.3% and 30.6%.[54] Many of the inhibitors were transient, however, disappearing while the patient was receiving on-demand treatment, others responded to immune tolerance induction regimens with rFVIII alone, while other inhibitors persisted. Moreover, in trials with rFVIII preparations in PTPs, no or only one subject per trial developed an inhibitor.

Although immune tolerance induction is generally seen as the therapeutic goal for patients with inhibitors, opinions differ regarding how to perform induction, and cost remains a deterring factor. Several regimens of FVIII products have been described, involving low, moderate, and high doses. Another, termed the Malmö regimen, combines factor VIII infusions with immunomodulating treatment with cyclophosphamide and high-dose intravenous gamma globulin followed by a regular prophylactic program of factor VIII therapy.[55] For patients who are resistant to immune tolerance induction, or for whom it is impossible for economic or availability reasons, treatment of acute bleeding has been possible with so-called bypassing agents. Recombinant activated factor VII is reported to induce hemostasis in many patients,[56] and prophylaxis with activated prothrombin complex concentrate has successfully controlled bleeding episodes in patients with high-titer inhibitors.[57]

Induction of early immune tolerance (already tested in animal models)[58] or use of recombinant factors that lack immunogenic regions of factors VIII or IX to prevent inhibitors from developing in the first place,[59] are both potential solutions to a problem that continues to jeopardize outcome of hemophilia patients.

Conclusions

The hemophilia community generally agrees that factor prophylaxis is the 21st-century method-of-choice for treating severe hemophilia A or B. A number of prophylactic regimens are currently in use, all of which markedly reduce/prevent bleeding episodes and prevent arthropathy. Some concerns remain, however, including the high cost of such therapy and its requirement for long-term venous access in young patients.

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Considerations in Pediatric Patients With Hemophilia

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Before factor concentrates became readily available, children with hemophilia lived with chronic pain from hemarthropathy. With currently available care, the average child with hemophilia can enjoy a relatively normal quality of life, and clinicians can focus more attention on issues of growth and development, as well as other conventional pediatric concerns. Dr. van den Berg discusses the major issues involved in making therapeutic choices for all patients with hemophilia elsewhere in this supplement.[1] This brief review will approach some of these issues as they relate to the youngest patients, as well as other considerations specific to childhood.

Pediatric care of children with hemophilia should focus on the health of the child, not on the disorder. The information first given a family with a child diagnosed with hemophilia is of crucial importance, since it influences how this family and later the child will cope with the disease in their daily lives.[2] Also, the patient, once he is old enough to understand, is in need of different information from that provided to parents.

The optimal approach to hemophilia treatment is the use of factor VIII (FVIII) preparations, plasma-derived or recombinant, in such a way that bleeds and chronic joint damage are prevented, short- and long-term complications of treatment are avoided, and the patient is fully integrated into society. This goal can best be achieved by early home treatment and primary prophylaxis.[3,4]

Care for young children with hemophilia is evolving rapidly, with a particular emphasis on the prevention of joint disease.[5] Current standard measures of joint function are inadequate for evaluation of children's joints, however.[6] Three new instruments, revisions of the standard World Federation of Haemophilia Physical Joint Examination scale, have now been designed to detect the subtle abnormalities in the developing gait and coordination of children. Radiographic scoring using conventional radiography has also been useful in monitoring the progress of hemophilic arthropathy, but it, too, is inadequate for the identification and monitoring of early changes and minor progress.[7] Several systems based on magnetic resonance imaging (MRI) promise to improve the visualization of these early arthropathic changes.[8] Additional MRI data from hemophilic cohorts are needed to confirm the reliability and validity of this technology before it can be considered the standard assessment tool for hemarthropathy.

Finally, antibodies to FVIII usually develop during the first few administrations of clotting factor concentrates and thus, are frequently seen early in a patient's life.[4] Treatment of bleeding episodes and immune tolerance induction in patients with inhibitors are similar in both children and adult patients.

Introducing the diagnosis

The word hemophilia and its description can have a great impact on how it is perceived by the young patient's family.[2] This initial information should ideally be given to both parents and the patient's older siblings. All family members, particularly the parents, are likely to be in a state of shock during this first talk and may remember only fragments of what they are told about the child's diagnosis; thus, information should be repeated in subsequent meetings.

The goal of this first talk should be that the family understands it is possible for the patient to live a practically normal life with normal life expectancy. Vital questions that must be answered in the initial interview include: Will the patient survive into adulthood? Will he be able to play like normal children? Will he be able to attend school? The mother who might be a genetic carrier is obviously at risk for feeling guilty, and families may also harbor the belief that the child's illness resulted from totally irrelevant past events or things they may have done wrong. Once the child is old enough

to understand, he, too, must be informed about his chronic disorder. He should be approached differently, however, since small children are more involved with immediate than with the existential worries of his parents. He will be involved more with the necessity of current blood sampling and hospital stays. It is important that he not feel guilt for having caused inconvenience and/or distress to his parents.

Prophylaxis

In much of the world, on-demand treatment of bleeding episodes is still the main approach to hemophilia care of patients of any age. Training patients' caregivers in infusion techniques permits intervention at an early stage, thereby enhancing control of bleeds and minimizing both their immediate impact and the likelihood of complications. Optimally, hemarthroses should be treated with a combination of factor replacement, rest, ice, and supervised rehabilitation. In developing countries, however, factor concentrates are usually unavailable, necessitating the treatment of bleeding episodes in hemophilic children with physical means alone or with cryoprecipitate or fresh frozen plasma (see Berntorp[9] and van den Berg,[1] this issue). In the developed world, prophylactic regimens have an established role in the management of severe hemophilia. Popularized in Europe, where prophylaxis is now standard pediatric treatment at many hemophilia centers,[10] these regimens are being used increasingly in the United States and Canada. Prophylactic regimens have been shown to be effective in preventing not only joint bleeding but also the later development of arthropathy when started early in children with severe hemophilia.[11] There is some discrepancy in published estimates of the proportion of patients with severe (<1 U/dL) and mild (>5 U/dL) hemophilia, however. A survey of 30 hemophilia centers in Europe found that 52% of patients had severe hemophilia and 29% had mild disease,[10] whereas epidemiologic studies showed that 50% to 55% of the total hemophilia population had mild hemophilia.[12] Thus, either mild hemophilia is underdiagnosed in some countries or, more likely, many children with mild disease are not being treated at hemophilia centers.[2] The obvious answer is the registration and regular attendance of all children with hemophilia at specialty centers, whether diagnosed as severe, moderate, or mild, thus ensuring that all receive the same information, general treatment, and choice of concentrate.

The child with moderate hemophilia (1-5 U/dL) presents another interesting issue.[2] Some of these children have the same clinical manifestations as those with severe disease but rarely bleed. On the other hand, approximately 10% to 15% of boys with severe involvement also have a low bleeding tendency.[13] The effect of this low bleeding tendency on joint function later in life is unknown. However, an early radiologic study showed joint changes at the start of prophylaxis despite no clinically recognized joint bleeds.[7] This suggests that subclinical bleeds may trigger the development of arthropathy in children with only isolated clinical bleeds, and that better instruments to monitor joint status, both clinical and radiologic, are needed.

Joint scoring systems for children

Current orthopedic scoring systems, which were originally devised to monitor adult patients, are not sensitive enough for follow-up of children with hemophilia who today are being treated more intensively. New scoring systems have been proposed,[6] which expand on the World Federation of Hemophilia (WFH) Physical Joint Examination (see Blanchette,[14] this issue). The WFH instrument contains many tasks that cannot be performed by young children due to their developmental immaturity and was not designed to detect abnormalities in normal childhood activities such as walking, skipping, hopping, jumping, galloping, running, and stair-climbing. Nor does it provide an adequate evaluation of adults with mild hemophilic arthropathy. Two of the new scoring systems can detect subtle abnormalities of joint structure and function in children.[6] These systems are called the Colorado physical examination (PE) 1 and PE-0-5 instruments. The actual assessments are the same for these two instruments but are scored differently, with the half-point scale of PE-0-5 reflecting the lesser importance of very early abnormalities. As with the WFH instrument, healthy preschool children were developmentally incapable of completing all of the tasks on the Colorado PE instruments. Therefore, a third scale, Child PE, is specifically tailored to the dynamic growth and gait development of young children (Table 1).[6]

Comparison of the WFH instrument with all three new scales showed the three to have better correlation with the WFH pain scale. Preliminary findings suggest that all three are more indicative of early joint dysfunction than the WFH instrument. According to the developers of the instrument, if future analyses validate these findings, it may be reasonable to replace the WFH instrument with the Colorado PE-0-5 for children at and above the age of 7 years and the Child PE scale for those aged 12 months to 6 years.

Radiographic scoring systems for children

Assessment of hemophilic arthropathy using conventional radiography has been useful, particularly in adults with advanced disease, but does not reveal early and minor progressive changes.[7] Furthermore, many terms used in current Pettersson or Arnold-Hilgartner schemes need clarification.[8] For example, in the former,

Table 1. Child Instrument.

Physical finding	*Score*	*Scoring key*
Swelling	0-3	0 = none 1 = joint looks slightly "*puffy*"; there is slight palpable swelling present; may not be any measurable difference between the joints; bony landmarks clearly visible. 2 = joint looks swollen and the swollen area feels firm on palpation; may also feel boggy; there is measurable difference between the joints; bony landmarks are palpable but not visible. 3 = swollen and are tense on palpation; there is measurable difference between the joints and the bony landmarks are difficult to palpate.
Muscle atrophy	0-3	0 = none 1 = muscle has slightly less contour than the contralateral side. 2 = flattening of the muscle belly. 3 = severe muscle wasting and depression.
Axial deformity:		
Knee	0-2	0 = normal; 0-7° valgus 1 = 8-15° valgus or 0-5° varus 2 = > 15° valgus or >5° varus
Ankle	0-2	0 = no deformity 1 = up to 10° valgus or 1-5° varus 2 = > 10° valgus, or > 5° varus
Crepitus with motion	0-3	0 = none 1 = barely detectable audible or palpable sensation during joint motion 2 = more pronounced cracking and/or rough sensation during joint motion 3 = audible and palpable grinding and crunching during joint motion
Range of motion	0-3	0 = no loss 1 = loss of < 10% of total FROM 2 = loss of 10-33% of total FROM 3 = loss of >33% of total FROM
Flexion contracture: measured at hip, knee, ankle and elbow	0-3	0 = normal 1 = 0-7° 2 = 8-15° 3 = >15°
Instability		
New additions:		Deleted
Pain with activity	0-3	Uses Faces Pain Rating Scale (Wong-Baker) 0 = Face is very happy 1 = Wong-Baker faces 1 & 2: hurts a little bit or a little bit more 2 = Wong-Baker face 3: hurts even more 3 = Wong-Baker faces 4 & 5: hurts a whole lot and as much as you can imagine
Pain without activity	0-3	Uses Faces Pain Rating Scale (Wong-Baker) 0 = Face is very happy 1 = Wong-Baker faces 1 & 2: hurts a little bit or a little bit more 2 = Wong-Baker face 3: hurts even more 3 = Wong-Baker faces 4 & 5: hurts a whole lot and as much as you can imagine
Gait	0-3	0 = normal walking, running, skipping, galloping, stairs 1 = normal walking, one or more other gait abnormality 2 = abnormal walking and ≤ 2 other gait abnormalities 3 = abnormal walking and > 2 gait abnormalities
Strength	0-3	0 = moves easily through full ROM against gravity without observable/measurable atrophy and can take additional resistance 1 = moves through available ROM, easily against gravity, may have observable/measurable atrophy and can take some additional muscle resistance 2 = moves through full or available ROM against gravity, cannot take resistance 3 = unable to move through full or available ROM against gravity due to weakness
Splinting/Orthotics	0-3	0 = no use of splinting/orthotics 1 = splinting/orthotic use required as needed after an acute hemarthrosis or for occasional support 2 = splinting/orthotic use required regularly for high activity sports or to prevent recurrent hemarthrosis 3 = splinting/orthotic use required continuously
Total	0-31 0-29	Ankle or knee Elbow

FROM, free range of motion; ROM, range of motion. Reprinted from Manco-Johnson et al.,[6] with permission.

Table 2. Denver MRI Scale.

	Finding	*Score*
Effusion	Absent	0
	Small	1
	Moderate	2
	Large	3
Hemarthrosis	Absent	0
	Small	1
	Moderate	2
	Large	3
Synovial hyperplasia	Absent	0
	Small	4
	Moderate	5
	Large	6
Hemosiderin	Absent	0
	Small	4
	Moderate	5
	Large	6
Erosion	Absent	0
	Partial surface erosion	7
	Full surface erosion	8
Subchondral cyst	Absent	0
	1 cyst	7
	> 1 cyst	8
Cartilage loss	Absent	0
	< 50% loss	9
	≥ 50% loss	10
Ancillary findings on Denver MRI Scale		
Pseudotumor	Absent	
	Present	
Osteonecrosis	Absent	
	Present	
Fibrocartilage tear (applies to knee)	Absent	
	Present	
Ligament tear	Absent	
	Present	
Loose body	Absent	
	Present	

Reprinted from Kilcoyne et al.,[8] with permission.

narrowing of joint space uses the terms *greater than* or *less than 1 mm,* numbers not applicable in a young child. In the latter, *cartilage space narrowed* may be easy to tell in adults, but is far more difficult in children, particularly if comparison films are unavailable.

Radionuclide studies including bone scans, bone mineral density studies with quantitative computed tomography or dual-energy x-ray scanning[15,16] and sonography[17,18] have all been used to evaluate early and late changes. However, neither spatial nor tissue resolution is as good as that achieved by magnetic resonance imaging (MRI), which is capable of delineating all of the soft tissue findings long before they are evident on plain radiographs.

The key to successful early treatment of hemophilic joint disease is the recognition of synovial hyperplasia, which can develop after only one or a few bleeding episodes. Quantification of synovial hypertrophy usually involves drawing a region of interest measurement on a given MRI slice, summing the areas on contiguous slices to form a volume estimate.[19]

Alternatively, the semiquantitative Denver MRI scale has been developed to describe the various components of hemophilic joint disease (Table 2).[20] However, this scale also needs further clarification.[8] *Effusion* and *synovial hyperplasia* are critical MRI findings. It is important to ascertain whether these findings can be quantified more precisely. Other outstanding questions include whether it is important to distinguish *hemarthrosis* from *effusion*, whether hemosiderin deposition should be counted as a finding separate from synovial hyperplasia, whether cartilage loss is easy to detect in adults, and whether, in young children, one can differentiate articular cartilage from immature growth cartilage.

In short, MRI is a powerful tool in the diagnosis, staging, and treatment of patients with hemophilic joint disease. It is much more sensitive than radiologic assessment because it identifies early soft tissue changes and can differentiate between blood and nonhemorrhagic synovial fluid in joints.[8] Yet this technology awaits further standardization with regard to these elements before it can be established as the standard for quantifying hemarthropathy.

Home treatment

Home therapy programs have been expanding over more than three decades. For example, in The Netherlands, the proportion of hemophilia patients in such programs increased from 4% to 53% from 1972 to 1985.[21] In addition, prophylaxis has been initiated at earlier ages than was the traditional practice: Karolinska Hospital in Sweden has a standard protocol for initiating home care of children between 1 and 2 years of age.[3] Prophylaxis may be initiated with one injection of clotting factor concentrate weekly (made less painful by the use of cream containing 2.5% each of lidocaine and prilocaine). As soon as possible, the frequency of injections is raised to two to three injections per week. Before the age of 2 years, and before bleeding symptoms are occurring frequently, patients are on home treatment with full prophylaxis given by their parents every day. When treatment was initiated this way, only 4 of 34 children needed an implanted port to make regular injections feasible.[22]

Another advantage of introducing preventive therapy with small doses at greater intervals may make the frequent treatments more acceptable to children and parents because patients are likely to experience joint hemorrhage before starting the full prophylactic regimen. However, the question of when to start treatment remains controversial given findings that suggest early primary prophylaxis regimens in infants may predispose them to the development of FVIII antibodies. Also, individualized prophylaxis regimens, including those in which treatment is not begun until after one or more joint bleeds, apparently do not increase the risk of arthropathy.[1]

In general, safety issues are infrequent with all forms of home therapy for hemophilia whereas patient satisfaction is high.[3,23,24] An early study of the impact of prophylactic treatment on children with severe hemophilia outlines how treatment was given in 27 children, aged 1.3 to 15.9 years.[24] Nine children required the insertion of a right atrial indwelling catheter. In all families, parents were taught to use the catheter aseptically and were able to give the factor VIII or IX regularly at home. Eight of the older children were able to administer the intravenous injection themselves. Many children will require the reliable venous access afforded by implantable venous access devices for long-term prophylactic therapy. These devices are discussed by van den Berg in this issue.[1]

FVIII inhibitors

According to an early study of recombinant factor VIII for the treatment of previously untreated children with hemophilia A, transient or low levels of inhibitor may represent part of the natural history of hemophilia in infants.[25] Others have found antibodies to be associated with specific mutations in the factor VIII gene, with severity of disease, with ethnicity, and with family disposition. It has also been suggested that early treatment with FVIII may be associated with increased risk of inhibitor development. In one study of 62 previously untreated patients, the cumulative incidence of inhibitors was 41% in patients treated before the age of 6 months, 29% in those treated between 6 and 12 months, and 12% after the age of 1 year.[26] Similar findings were reported in a study of 81 previously untreated (PUPs) Dutch patients.[27] Incidence of inhibitors was 31% among infants first treated during their first 6 months, 17% in those treated between 6 and 12 months, and 11%, between 12 and 18 months. None of the children first treated after age 18 months developed inhibitors. Controlled, prospective studies may be required to determine age effects on inhibitor development risk. Nonetheless, optional age at prophylaxis initiation in children is still an open question.

An ongoing prospective, multicenter study of PUPs will compare different types of concentrates with regard to their propensity to induce inhibitor development.[28] Immune tolerance induction (ITI) is the only methodology at present with the potential of eliminating inhibitors. Various therapeutic regimens have been attempted (see van den Berg).[1] One small longitudinal study of the influence of the type of concentrate used for ITI showed success rates as high as 90% with concentrates containing high amounts of von Willebrand factor,[28] compared with success rates of 70% to 80% reported in other published studies.[29,30]

Academic achievement

A study of the association between academic achievement and coordination and gait abnormalities in children with hemophilia found that lowered achievement was related to the functional severity of hemophilia.[31] Another investigation among school-aged children with severe hemophilia explored the association between bleeding episodes experienced during the year before study enrollment and academic achievement.[32] Results showed better total achievement among those with fewer bleeding episodes after adjusting for IQ and parents' education level. Moreover, those with fewer bleeds scored higher on a Physical Summary measure, which captured limitations in physical activity and in the kind or amount of schoolwork or social activities the child engaged in, and the presence of pain or discomfort. According to the investigators, these data support the assertion that therapeutic care programs in young children must not be evaluated exclusively in terms of financial cost to achieve adequate musculoskeletal outcomes. Other quality-of-life assessment tools for hemophilia are being developed to broaden outcome assessment.

Conclusions

With the advent of safe, effective replacement therapy, a relatively normal childhood and a normal life span have become possible for children with severe hemophilia A. One of the first challenges for physicians and other health professionals caring for these children lies in the diagnosis itself: learning to inform parents, and eventually the child himself, that despite this seemingly devastating disease, he can live a long and fruitful life. With registration at a specialized hemophilia clinic, treatment can usually be centered at home and can eventually be self-administered. Clinical considerations include the need for improved visualization modalities to monitor joints in infants and children, and the identification and possible elimination of inhibitors to factor VIII concentrates. A major issue for all children with chronic disorders who are now living essentially normal lives is the impact of the

associated physical or emotional disabilities on their daily lives, particularly their academic achievement.

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Considerations in Adult Patients With Hemophilia

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The treatment of adult patients with hemophilia is influenced by two very different historical trends. One is the dwindling cohort of patients with human immunodeficiency virus (HIV) infection: advances in care appear to have reduced the mortality rate from HIV-related disease in this population, while the principal source of new HIV infections in hemophiliacs was long ago eliminated through safety improvements in the production of plasma-derived factor concentrate and in the manufacture of recombinant factor concentrate (see Berntorp,[1] in this issue). Second is the dwindling cohort of patients with more severe complications of hemophilia—in particular, arthropathy: with improvements in delivery of on-demand treatment and the growing use of prophylactic regimens, increasing numbers of hemophilic patients are reaching adulthood with lesser disability than that suffered by earlier generations of patients. Along with these trends, one constant remains: the need for regular factor replacement.

Choice of factor concentrate

Several considerations influence the choice of factor concentrate in adult patients with hemophilia. Availability and cost are important. Personal preference may be involved: a patient with a long-term history of successful use of a particular product understandably may wish to continue using it. Treatment history — in particular, past transmission of blood-borne infections with untreated plasma-derived factor concentrates — may also play a role in factor choice. Concern about potential transmission of infectious agents via factor concentrate may favor use of recombinant products due to the perception of greater safety. Of particular concern in this regard was the emergence in the 1990s of a new variant of Creutzfeldt-Jakob disease (vCJD) in the United Kingdom.[2] Emergence of vCJD has suggested the theoretical potential for contamination of plasma-derived concentrates with the prions implicated in transmissible spongiform encephalopathies. Although to date there is no evidence implicating factor concentrates in the transmission of prion disease,[3] the possibility of such transmission has been the driving force behind the progressive switch to recombinant products in the United Kingdom, where blood donors may have consumed products from cattle with bovine spongiform encephalopathy. New recombinant factor VIII products, which are free of human or animal proteins, are unlikely to have any risk of viral or prion transmission,[4] and are now considered the treatment of choice for hemophilia.

For patients with high-titer inhibitors that have not responded to an immune tolerance protocol, or in whom such a protocol cannot be undertaken, treatment with by-passing agents such as recombinant factor VIIa or activated prothrombin complex concentrates may achieve hemostasis. It should be emphasized, however, that clinical efficacy achieved with those products is usually not as high as that achieved with factor VIII concentrates, and that a real difficulty still remains with regard to laboratory monitoring.

Factor use is especially high with patients on prophylaxis programs. Although the best age at which to initiate such programs remains uncertain, prophylaxis is typically begun at an early age to minimize the development of arthropathy. It has been suggested that when patients on prophylaxis reach adulthood, it may sometimes be possible to switch to treatment on demand while still maintaining a low rate of bleeding.[5] Alternatively, prophylaxis might be discontinued only when the patient becomes elderly, or not at all. Further research is needed to resolve this question.[6]

Treatment of arthropathy

Most bleeding episodes in hemophilic patients involve the joints, particularly in those with severe disease. The knee is most

commonly affected, followed by the elbow, ankle, hip, and glenohumeral joint. In an individual hemophiliac, the number of joints involved has usually stabilized by age 20; often, however, progressive damage to involved joints from repeated intra-articular bleeds results in arthropathy. Initially, affected joints exhibit only synovial hyperplasia. Early arthropathy is marked by subsynovial fibrosis; as the arthropathy progresses, both intra-articular and capsular fibrosis develop, and joint contracture may ensue.[7] Periarticular bone is involved as well: osteoporosis is a relatively early finding, followed by the formation of subchondral cysts. Collapse of these cysts can lead to loss of joint surface congruity, mechanical instability, and degradation of cartilage.[7]

The precise role of magnetic resonance imaging (MRI) in patient follow-up remains to be established. MRI may be considered as more sensitive than x-rays in identifying cartilage damage and subchondral lesions, but its use is not yet widely accepted as standard practice. In both early and late hemophilic arthropathy, MRI was, however, found superior to plain radiography.[8] Hemophilic synovitis is easily seen on MRI because of the presence of hemosiderin deposits in much of the inflamed synovium. Bone and joint damage can be documented using classification scales for hemophilic joints.[9,10]

For patients with chronic hemophilic synovitis, radiosynovectomy with intra-articular injection of ^{32}P chromic phosphate, yttrium and rhenium has proved effective, with one study reporting a 75% to 100% reduction in hemarthrosis in 79% of patients 6 months to 8 years later.[11] Advanced arthropathy, marked by irreversible bone changes, may be an indication for surgery.

In patients disabled by advanced arthropathy — especially around the elbow or ankle — joint debridement may effectively restore function while minimizing risk to the patient.[12] In patients who have painful genu varum but have maintained mobility of the joint, proximal tibial valgus osteotomy is reliably effective. Total knee arthroplasty has been shown to improve physical activity and quality of life, although arthropathy in other joints and intercurrent diseases seem gradually to reduce these benefits.[13] In one series, survival rate for knee prostheses was 90% after 5 years; the most common cause of failure was infection.[14] Postoperative infection is especially problematic in HIV-positive patients; the infection rate after arthroplasty may be 10-fold higher in this population.[12]

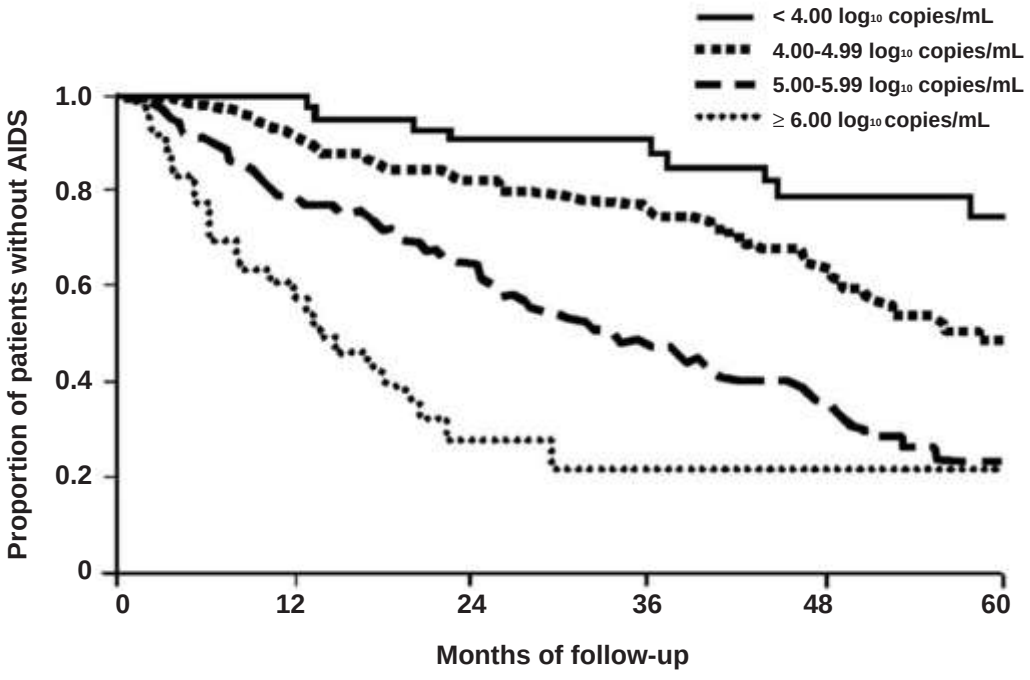

Figure 1. Kaplan-Meier curves showing time to AIDS-related illness for strata defined by baseline HIV viral load. As seen here, viral load immediately affected disease progression. Illness-free survival for patients with high baseline viral loads diverged rapidly from that for patients with lower viral loads. Over time, this divergence was less apparent, however, implying attenuation of the effect of baseline viral load. In a survival model with an interaction term between log_{10} viral load and time since baseline, baseline viral load remained important, but the magnitude of its effect decreased about 20% a year. Adapted from Engels *et al.*,[16] with permission.

HIV infection and prognosis

In the last two decades of the 20th century, HIV infection devastated the hemophilia community. Approximately 70% of hemophiliacs who received pooled, untreated factor VIII concentrates became HIV positive. In the United States, HIV-related disease was responsible for almost half of deaths in persons with hemophilia A.[15] Mortality in patients with hemophilia A increased markedly in the late 1980s. In the last half of the 1990s, however, deaths in hemophilia A patients with HIV-related disease decreased by 78%. This decrease appears to reflect advances in care for HIV-related disease, and parallels a decline in HIV mortality seen in the general population.[15]

Previous studies have shown that in the first 3 years after HIV seroconversion, HIV viral load is predictive of long-term prognosis: patients with viral loads of 10,000 copies/mL or higher were at 16-fold higher risk for AIDS-related illness than were patients with viral loads of less than 1,000 copies/mL. More recent studies have shown that in hemophiliacs with late-stage HIV disease, viral load continues to predict disease progression, and does so independently of CD4 cell counts.[16] Viral load apparently reflects the patient's current level of immunosuppression, in that it most strongly predicts progression in the immediate future (Figure 1). Engels *et al.*[16] have suggested that viral load could be incorporated as an independent measure when determining the need for prophylaxis. For example, among patients with CD4 cell counts below 200 cells/mm^3, risk of *Pneumocystis carinii* pneumonia was low when viral load was less than 5.00 $\log_{10}$ copies/mL.

In hemophiliacs with HIV infection, progression of disease appears to be influenced by genotype. A prospective study of 207 HIV-infected hemophilic patients has shown lower levels of HIV RNA in plasma and higher levels of CD4$^+$ T cells in patients with the chemokine receptors CCR2b, and to a lesser extent CCR5. The risk of progression to AIDS and of AIDS-related death tended to be lower in patients with the CCR2b mutant allele compared with those who had the wild type allele; this effect is incompletely explained by viral load or CD4$^+$ T cell count.[17]

Many hemophiliacs with HIV infection are also infected with hepatitis C virus (HVC). Coinfection with HIV and HCV has been associated with a reduced likelihood of HCV clearance, and higher levels of HCV RNA are associated with increased hepatic inflammation.[18] A different picture emerges in hemophiliacs who have been infected with hepatitis G, a distant flavivirus relative of HCV that apparently does not cause chronic disease. For unknown reasons, hemophiliacs with either past or current infection with hepatitis G have been found to have higher CD4 cell counts and better AIDS-free survival rates.[19]

Conclusions

Before the advent of virus-inactivation procedures, most hemophilia patients who were treated with plasma factors became chronically infected with the hepatitis B virus, the hepatitis C virus, and during its disastrous introduction in the 1980s, with HIV. Given the advances in treatment of hemophilia and HIV infection, most patients with both diseases live out their lives. Such advances, primarily early prophylactic therapy in hemophilia, have also seen a significant decrease in the number of adult hemophilic patients who have painful arthropathy and physical disability.

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Prophylaxis in Hemophilia: A Comprehensive Perspective

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Hemophilia A and B are inherited hemorrhagic disorders caused by deficiencies of factor VIII (hemophilia A) and factor IX (hemophilia B), respectively.[1] The clinical hallmark of the hemophilias is bleeding into muscles and joints, especially the ankles, knees and elbows.[2] The frequency and severity of bleeding is greatest in boys with the severe form of the disorder, defined by circulating factor VIII or IX levels of 1% or less; boys with moderate and mild hemophilia (factor levels of 2% to 5% and 6% to 30%, respectively) bleed less frequently.

The consequence of repeated bleeding into joints is the development of hemophilic arthropathy. This unwanted complication of hemophilia can be prevented by early institution of a program of prophylaxis defined as *treatment by intravenous injection of factor concentrates in anticipation of and in order to prevent bleeding*.[3] The *gold* standard prophylaxis regimen is that pioneered by Professor Inga Marie Nilsson and her colleagues in Sweden, starting in the late 1950s.[4] In this regimen (*the Malmö protocol*), prophylaxis is usually started when boys with severe hemophilia are 1 to 3 years of age and continued at least until the age of 20 years but usually for longer. The infusion dose is 25 to 40 factor VIII Units/kilogram (U/kg) on alternate days (minimum $\times$ 3/week) for hemophilia A cases and 25 to 40 factor IX U/kg $\times$ 2 per week for hemophilia B cases.

If compliance is good, this regimen is associated with very few joint bleeds and a high percentage of cases with perfect musculoskeletal status.[4,5] However, prophylaxis programs are very expensive and a number of important questions remain unanswered, including: 1) when should prophylaxis be started in boys with severe hemophilia; 2) what is the optimal prophylaxis regimen, and should this regimen be the same for all age groups; 3) when should prophylaxis be stopped, if at all; and 4) what are the barriers to prophylaxis? To set the stage for a discussion of these questions it is useful to first summarize key outcome measures relevant to prophylaxis, as well as the evidence that supports prophylaxis as the optimal treatment strategy for prevention of musculoskeletal disease in persons with hemophilia.

Outcome measures

Key outcome measures relevant to prophylaxis programs are musculoskeletal status as assessed by physical examination and plain radiographs. Widely used scoring systems are the orthopedic joint score as recommended by the Orthopaedic Advisory Committee of the World Federation of Hemophilia[6] and radiologic joint scores as described by Pettersson *et al.*[7] For the orthopedic assessment each joint is scored on a 15-point scale (Table 1); the sum of the elbows, knees, and ankles is the patient's joint score with a maximum possible score of 90. For the radiologic assessment, each joint is rated on a 13-point scale (Table 2); the sum for the elbows, knees, and ankles is the patient's radiologic score with a maximum possible score of 78. The two assessments are generally performed in parallel with a perfect musculoskeletal score rated as *0/0*.

A limitation of the WFH orthopedic joint scoring system is its insensitivity to early hemophilic arthropathy plus the fact that the measurement tool contains certain tasks that cannot be performed by young children due to their developmental immaturity (see Hoots,[8] this issue). To address these limitations, Manco-Johnson and coworkers have reported modified joint scoring systems tailored to the dynamic growth and gait development in children.[9] In like fashion, plain radiographs may not be the optimal measure for detection of early joint damage; magnetic resonance imaging (MRI) may be better, and studies are now required to assess the role of this powerful imaging tool in the assessment of early hemophilic arthropathy in boys with hemophilia.

Table 1. Orthopedic Joint Score.

	Score			
Item	*0*	*1*	*2*	*3*
Chronic pain	None	Mild	Moderate	Severe
Axial deformity				
Elbow	None	≤ 10° varus or valgus	>10° varus or valgus	–
Knee	No deformity (0-7° valgus)	8-15° valgus or 0-5° varus	>15° valgus or > 5° varus	–
Ankle	No deformity	≤10° valgus or ≤ 5° varus	>10° valgus or > 5° varus	–
Contracture				
Flexion	< 15°	–	≥ 15°	–
Equinus	< 15°	–	≥ 15°	
Joint physical findings				
Instability	None	Slight	Severe	–
Range of motion*	0–10%	11–33%	33–100%	–
Pronation and supination*	0–33%	–	> 33%	–
Chronic swelling	None	–	Present	–
Atrophy	None/minimal	Present	–	–
Crepitus on motion	None	Present	–	–

**Expressed as percentage loss of full range of motion. Possible joint score 0-15 points. Reproduced from Löfqvist et al.,[5] with permission.*

On-demand versus prophylaxis therapy

On-demand therapy refers to the administration of clotting factor concentrates following the occurrence of bleeding. Although generally very effective in the short-term, this management approach is suboptimal when considered over the long-term. The report of Molho and colleagues for a group of 116 males with severe hemophilia treated from birth using an on-demand strategy is instructive in this regard.[10] The mean age of the study cohort was 23 years with replacement therapy started on average at the age of 2.2 years in boys with hemophilia A and 1.7 years in boys with hemophilia B. At the time of assessment, only 16% of patients had all joints normal on physical examination and only 3.7% on radiologic examination. Sixty-three subjects (54.3%) had a history of orthopedic surgery (chiefly synovectomy) or rheumatology procedures since birth, and 26 subjects (22.4%) were hospitalized because of their orthopedic status. The frequency of bleeding into the knees, elbows, and ankles averaged 16.3 during the one year period before study entry, and the annual factor consumption was 1634 factor U/kg.

Our experience with an on-demand treatment regimen is similar.[11] Forty-one percent (14/34) of boys with severe hemophilia, aged 13 years or younger, required at least one short-term (3 to 6 months) course of secondary prophylaxis. Five boys received intraarticular corticosteroid therapy into at least one joint, and ankle synovectomy was performed in three patients. Median annual factor use was 1450 U/kg (range, 129 to 4800 U/kg/year). Perfect orthopedic and radiologic joint scores (0/0) were present in only 45% (14/31) of patients. For the group of 34 boys orthopedic scores ranged from 0 to 6 and radiologic joint scores, from 0 to 5.

Comparable data for 20 older boys with severe hemophilia, ages 14 to 18, showed progression of musculoskeletal disease. Ninety percent (18/20) of boys in this older age group had received at least one course of short-term factor prophylaxis, 40% (8/20) had received at least one corticosteroid injection into a target joint, and 50% (10/20) had undergone surgical synovectomy in at least one joint. None of the older group had perfect, 0/0, joint scores. Orthopedic joint scores ranged from 1 to 23 (median, 7) and radiologic scores from 3 to 19 (median, 9). Annual factor use for this group of boys ranged from 468 to 1800 U/kg (median value, 994 U/kg/year).

In summary, in patients 18 years of age or younger with severe hemophilia treated with on-demand therapy, joint disease develops early and is present in approximately one-half of cases by age 13. Moreover, joint disease is progressive over time. Disturbingly, severe joint disease, assessed by the need for surgical synovectomy in at least one joint, occurred in 24% (13/54) of the entire cohort. Based on our clinical experience over two decades, we conclude that conventional on-demand factor replacement therapy fails to prevent the development of significant musculoskeletal disease in boys with severe hemophilia.[10]

The experience of other groups is confirmatory. Manco-Johnson and colleagues reported the outcome of

Table 2. Radiologic Joint Score.

Radiologic Change	*Finding*	*Score (Points)*
Osteoporosis	Absent	0
	Present	1
Enlargement of epiphysis	Absent	0
	Present	1
Irregularity of subchondral surface	Absent	0
	Present	1
	Pronounced	2
Narrowing of joint space	Absent	0
	< 50%	1
	> 50%	2
Subchondral cyst formation	Absent	0
	1 cyst	1
	> 1 cyst	2
Erosions at joint margins	Absent	0
	Present	1
Incongruence between joint surfaces	Absent	0
	Slight	1
	Pronounced	2
Deformity (angulation and/or displacement of articulating bones)	Absent	0
	Slight	1
	Pronounced	2

Possible joint score: 0-13 points. Adapted from Pettersson et al.[7]

secondary prophylaxis in a group of 13 boys. Despite the institution of prophylaxis, 6 boys had progressive joint disease and 4 required synovectomy.[12] It is clear, therefore, that on-demand therapy, even if delivered by a dedicated and expert comprehensive care hemophilia team, cannot prevent the development of clinically significant arthropathy that becomes evident at a relatively early age of life. Once established, musculoskeletal damage is progressive.[13]

Fischer and colleagues have reported a comparison of costs and long-term outcome in a multicenter cohort of 49 Dutch and 106 French patients with severe hemophilia born from 1970 through 1980.[14] Treatment in France (on-demand group) was primarily given per bleeding episode. Prophylaxis was the primary treatment strategy in The Netherlands (prophylaxis group). Prophylaxis was started at an early age and was aimed at preventing joint bleeds, with the intensity of prophylaxis adjusted in case of breakthrough bleeds. The regimen of prophylaxis was intermediate in intensity: 15 to 25 factor U/kg 2 or 3 times per week for hemophilia A and 30 to 50 U/kg once or twice per week for hemophilia B cases. Clotting factor consumption was similar for patients receiving primarily on-demand treatment and those on intermediate-dose prophylaxis (median, 1260 and 1550 U/kg/year, respectively). Patients treated with prophylaxis had fewer joint bleeds per year than the on-demand group (2.8 versus 11.5, respectively), a better joint status (25% with *0/0* scores versus 11%), and a more favorable quality of life. The investigators concluded that a primary prophylactic strategy leads to a better outcome at equal treatment costs in young adults with severe hemophilia and recommended, therefore, that prophylaxis be offered to all children with severe hemophilia.

The Dutch group has also reported a comparison of their intermediate dose prophylaxis regimen with the Swedish high-dose Malmö prophylaxis protocol.[15] A total of 128 patients (86 Dutch; 42 Swedish) with severe hemophilia, born between 1970 and 1990, were studied. The Swedish prophylaxis regimen involved the infusion of factor VIII, 25 to 40 U/kg three times a week for hemophilia A patients and 25 to 40 U/kg twice a week for hemophilia B patients. Prophylaxis was generally aimed at keeping trough factor VIII/IX levels above 1%. Patients treated with high-dose prophylaxis had fewer joint bleeds than those on the intermediate dose (median, 0.3 and 3.3/year, respectively). Moreover, the proportion of patients without arthropathy as measured by the Pettersson radiologic joint score was higher in the high-dose as compared to the intermediate-dose group (69% and 32%, respectively). However, the reduction in arthropathy at the 17 year follow-up timepoint was only slightly greater for the full-dose as compared to the intermediate-dose prophylaxis regimen, raising the question as to whether the two-fold increase in clotting factor consumption associated with the high-dose prophylaxis regimen is cost-effective when compared to the intermediate-dose regimen. Future prospective studies are needed to address this question.

When to start

Recommendations regarding the optimal time to start factor prophylaxis in boys with severe hemophilia are, in the main, based on retrospective studies and are opinion driven. Currently there is consensus that, in order to achieve maximal benefit, prophylaxis should be started before the onset of recurrent joint bleeding and early arthropathy. However, recommendations range from starting prophylaxis before or following the first joint bleed[16] to waiting until boys have experienced a total of three joint bleeds or two successive bleeds into the same joint.[17] In the context of this discussion it is instructive to review the data presented in support of these divergent recommendations.

The recommendation that effective prophylaxis should be started before or at least after the first joint bleed in boys with severe hemophilia was made by Kreuz and colleagues in a study of 21 patients, aged 7.35 to 27.75 years, of whom 18 had hemophilia A and

Table 3. Orthopedic and radiologic joint scores in boys with hemophilia related to the age of onset of prophylaxis.

	Group I	*Group II*	*Group III*
Number of cases	8	6	7
Median age (yr) at start of prophylaxis	1.75	4.25	8.75
Median number of joint bleeds before start of prophylaxis	1	6	>10
Orthopedic joint score*			
1993	0	0	4
1997	0	4	8
Radiologic joint score*			
1993	0	0	11
1997	0	0	19.5

*Reproduced from Kreuz et al.,[16] with permission. *Orthopedic joint score as recommended by the Orthopedic Advisory Committee of the World Federation of Hemophilia,[6] and radiologic joint scores as described by Pettersson et al.[7]*

three hemophilia B.[16] Patients received factor prophylaxis of 30 to 50 U/kg three times per week or on alternate days for those with hemophilia A, twice a week or every 3 days for hemophilia B. A significant correlation was found between the presence of hemophilic arthropathy, as assessed radiologically, with the absolute number of joint hemorrhages before starting prophylaxis, and in boys who started prophylaxis at 3 years of age or older, musculoskeletal status showed deterioration (groups II and III, Table 3). A perfect joint score (*0/0*) was preserved in boys who experienced no or only one joint hemorrhage before starting factor prophylaxis. Although these data clearly support starting prophylaxis early in life before significant joint bleeding and the development of early hemophilic arthropathy have occurred, they fall short of providing irrefutable evidence-based support for the investigators' conclusions, *our data strongly indicate that prophylaxis treatment in severe hemophilia A and B should be instituted at the latest after the first joint bleeding.*[16]

The recommendation that prophylaxis be deferred until boys with severe hemophilia have experienced three joint bleeds or two successive bleeds into the same joint comes from Liesner and colleagues.[17] The investigators base their recommendations on the need to ensure that each new *severe* hemophiliac, in terms of factor level, behaves as a clinically severe hemophiliac before embarking on an expensive and demanding program of factor prophylaxis.

How can these two approaches best be reconciled? A study by Astermark and colleagues of 121 boys with severe hemophilia (108 with hemophilia A; 13 hemophilia B) is helpful.[18] Study subjects had started factor prophylaxis at least once weekly before the age of 10 years and had no history of inhibitor development. In 75 patients, prophylaxis was started before age 3 years; in 31, between ages 3 to 5 years; and in 15, between 6 to 9 years. Patients received 25 to 40 U/kg three times per week for those with hemophilia A and twice a week for those with hemophilia B. A key finding was the observation that boys who started prophylaxis before the age of 3 years had a better clinical outcome (ie, significantly more subjects had an orthopedic joint score of 0 as compared with those starting prophylaxis at a later age (Figure 1).[18] Also important was the finding of no increase in number of joint bleeds or in severity of arthropathy among subjects given only one infusion each week during the first year(s) of life followed by two (hemophilia B) or three (hemophilia A) infusions per week at the age of 3 to 5 years when compared with patients on the more intensive prophylaxis regimen from the time of diagnosis. Based on these observations, the investigators suggested it might be possible to further individualize treatment by following the patient's bleeding pattern during the first year(s) with weekly infusions and thereafter shortening the interval, with or without the use of a central venous access system, thus possibly reducing the need for a surgical implant as well.[18]

The potential benefit of an individualized program of prophylaxis was emphasized by van den Berg and colleagues.[19] In a study of 75 boys with severe hemophilia (70 hemophilia A, 5 hemophilia B), prophylaxis was initiated and intensified based on the frequency of joint bleeding. The investigators concluded that the low-dose prophylactic regimen used in The Netherlands can prevent arthropathy to a large extent. Use of an individualized program in which the patient's bleeding pattern drives any increase in treatment intensity or frequency resulted in significantly lower factor consumption as compared with programs designed to maintain pre-infusion factor levels above 1% irrespective of the patient's bleeding pattern. The Netherlands group has also reported the effect of postponing prophylaxis treatment on long-term musculoskeletal outcome in 76 patients with severe hemophilia.[20] Their data suggest that postponing prophylactic treatment has a negative effect on outcome and that prophylaxis started before the third joint bleed may prevent joint damage. After two decades of follow-up, the radiologic joint score (Pettersson) was 8% higher (95% confidence interval, 1-16%) for every year prophylaxis was postponed after the first joint bleed.

Several conclusions can be drawn from these studies. The goal of preventing hemophilic arthropathy in boys with severe hemophilia, while avoiding excessive use of expensive factor concentrates and the need for central venous access devices, can best be achieved by 1) delaying the start of prophylaxis until at least one definite joint hemorrhage has occurred but starting before or at the third joint bleed, and 2) using a prophylaxis regimen

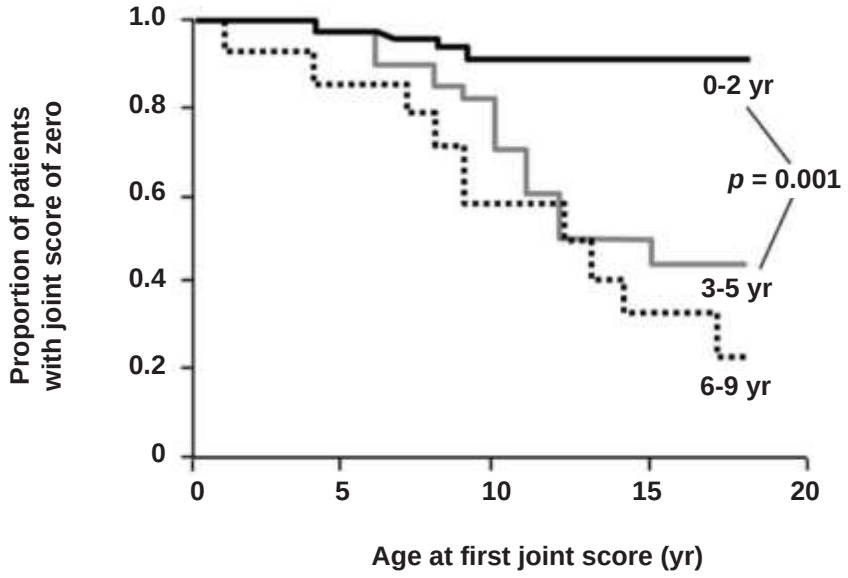

Figure 1. Kaplan-Meier plot showing development of arthropathy (ie, orthopedic joint score above zero) in patients starting prophylactic treatment before age 3, at ages 3 to 5, and at ages 6 to 9 years. The difference between the latter two subgroups was not significant (p = 0.275). Reproduced from Astermark *et al.*,[18] with permission.

in which the intensity (dose/frequency) is adjusted based on an individual's bleeding pattern rather than an arbitrary pre-infusion factor level above 1%.

The Canadian experience

A novel prophylaxis program is being used in an ongoing prospective Canadian study. In this study, boys with severe hemophilia A (factor VIII levels < 2%) between the ages of 1 and 2.5 years who have no current or past history of a circulating inhibitor are started on once weekly prophylaxis at a dose of 50 U/kg.[21] The frequency of prophylaxis is escalated to 30 U/kg twice weekly and finally to 25 U/kg on alternate days (minimum three times a week) based on any of the following bleeding patterns: more than three bleeds into any single joint in a consecutive 3-month period (the arbitrary study definition for *target* joint bleeding); more than four significant soft tissue or joint bleeds into any number of joints in a consecutive 3-month period; and more than five bleeds into any single joint over any time period.

A key component of this study is the use of an enhanced episodic treatment protocol for breakthrough bleeds: 40 U/kg on the day of the bleed, 20 U/kg on the following day, and 20 U/kg after a gap of one day. Treatment is continued until full resolution of the bleed, at which time the prophylaxis regimen is resumed.

The Canadian study was started in 1997 and has just completed its first 5 years, for which preliminary results have been reported.[21] Briefly, 48% (12/25) of boys have remained on once weekly prophylaxis and a further 36% (9/25) on twice weekly treatment. The orthopedic outcome, based on serial assessment of orthopedic joint and radiologic scores, appears very favorable with minimal joint disease present in the cohort.[21] Exit MRIs are planned.

Our experience raises the intriguing question of whether enhanced on-demand treatment of breakthrough bleeds on a background of low-dose prophylaxis in the first 6 years of life affords a musculoskeletal outcome comparable to full-dose prophylaxis. The advantages of a low-dose prophylaxis program include a reduction in the use of factor concentrates and a decrease in the need for central venous access devices. Answers to these questions must await the full report of the Canadian dose escalation study plus the anticipated results of a prospective, randomized trial of full-dose prophylaxis versus enhanced on-demand therapy that is now ongoing in the United States. The final results of the US study, which recruits young boys (ages 1-2.5 years) with severe hemophilia and no history of an inhibitor, are anticipated in the fall of 2005. The US study includes entry and exit MRIs of the ankles, knees, and elbows, and will provide data regarding the onset and severity of early hemophilic arthropathy as assessed by serial physical examination plus radiographs and MRI studies of the ankles, knees, and elbows.

Prophylaxis: When to stop

The issue of discontinuation of factor prophylaxis is important and currently unresolved. In a recent study of

49 patients with severe hemophilia treated with factor prophylaxis from an early age (median, 5.5 years), Fischer and associates reported that approximately one third of subjects permanently discontinued prophylaxis in early adulthood (median, 20.4 years).[22] This subgroup appeared to have milder bleeding patterns than those who temporarily discontinued prophylaxis, as evidenced by an older age at the start of prophylaxis, a lower number of joint bleeds, and a lower prophylaxis dose per kilogram body weight. Although the radiologic joint scores were lower in the 15 subjects who continued prophylaxis compared to the 34 who temporarily or permanently stopped (4.0 and 8.0, respectively), the data suggest that a significant subgroup of patients can discontinue prophylaxis permanently while maintaining a low frequency of joint bleeding. The question of when to stop factor prophylaxis deserves further study and may be an area where an enhanced on-demand treatment program could be of value in preventing significant deterioration in joint status.

Prophylaxis: Barriers

Since the musculoskeletal benefits of long-term prophylaxis started from an early age are now clear, it might be assumed that the majority of boys with severe hemophilia in North America are on such treatment. This is not the case, however. In a 2002 survey of factor prophylaxis use in Canadian and US hemophilia treatment centers, only 33% (177) of 533 boys 5 years of age or older with severe hemophilia were receiving full-dose prophylaxis, defined as infusion of 25 to 40 U/kg of factor VIII on alternate days (minimum three times per week) or 25 to 40 U/kg of factor IX twice weekly.[23] Reasons for this relatively low frequency of prophylaxis use include fear of clotting factor concentrate-transmitted viral infections, the need for central venous access devices, and cost.

The fear of clotting factor concentrate-transmitted viral infection is understandable given the tragedy of the HIV and HCV epidemics in the 1980s, but is likely to abate with the availability of very high purity, virus-inactivated, plasma-derived and recombinant factor concentrates. However, the need for central venous access devices to assure reliable venous access for full-dose prophylaxis started at a very early age in life, and the high cost of factor concentrates are real ongoing barriers even in countries with significant health care resources.

The complications of long-term central venous access devices placed from a very early age in boys with severe hemophilia, in particular, systemic infection and thrombosis, are now better appreciated. The frequency of central venous line (CVL)-associated thrombosis was not appreciated until recently. We first reported this complication in 1999.[24] In a follow-up study, which included repeat ultrasound examinations of the jugular venous system and bilateral arm venograms, 81% of 16 boys with hemophilia who had a Port-a-Cath placed were found to have evidence of catheter-related deep vein thrombosis.[25] Although this complication is often silent and non–life-threatening, occasional patients manifest severe and potentially life-threatening symptoms such as superior venocaval occlusion.[26] Other investigators have reported a similar high prevalence of Port-a-Cath-associated thrombosis in boys with hemophilia, leading to a recommendation that such devices be removed whenever possible by 4 years after initial placement.[27] To minimize the need for central venous access devices in boys with severe hemophilia, Petrini and colleagues start factor prophylaxis using a once weekly regimen with an increase to full-dose prophylaxis over the subsequent 18 months to 2 years.[28]

The high cost of factor concentrates is undoubtedly the single largest barrier to more widespread use of factor prophylaxis in both well-resourced and under-resourced countries. In a 1996 publication, Smith and colleagues, using US data, published estimated costs for three factor VIII regimens: on-demand therapy, $2,890,180; prophylaxis from age 3 to 20 years followed by on-demand therapy to age 50 years, $3,357,320; and prophylaxis from ages 3 to 50 years, $4,955,780.[29] Factor concentrate cost accounted for more than 90% of the total healthcare costs of both on-demand therapy and prophylaxis. Although prophylaxis significantly reduced the number of joint bleeds as compared with on-demand therapy, the cost was clearly substantial. Nonetheless, these investigators concluded, *as expensive as this improvement is, its potential for markedly restoring the quality of a child's life may provide excellent value*. Similar findings and recommendations were made by Bohn and colleagues following an economic evaluation of factor VIII prophylaxis.[30] The investigators found that because of the very high cost of year-round prophylaxis, total health care expenditures were highest among patients receiving this therapeutic regimen. They concluded, however, that since prophylaxis clearly offers important clinical benefits, this approach may be warranted on medical rather than economic grounds. The benefits of prophylaxis are such that Schramm and colleagues have recommended that clinicians and health policy decision makers should consider the advantages of prophylactic therapy for hemophilia patients in formulating treatment protocols and allocating health resources.[31]

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